Synthetic lethality: the road to novel therapies for breast cancer

Kiranjit K Dhillon[1,*], Ilirjana Bajrami[2,*], Toshiyasu Taniguchi[1] and Christopher J Lord[2]

[1]Fred Hutchinson Cancer Research Center, Seattle, Washington, USA
[2]The CRUK Gene Function Laboratory and Breast Cancer Now Toby Robins Research Centre, The Institute of Cancer Research, London, UK
*(K K Dhillon and I Bajrami contributed equally to this work)

Correspondence should be addressed to C J Lord or T Taniguchi
Email
chris.lord@icr.ac.uk or ttaniguc@fredhutch.org

Abstract

When the *BRCA1* and *BRCA2* tumour suppressor genes were identified in the early 1990s, the immediate implications of mapping, cloning and delineating the sequence of these genes were that individuals in families with a *BRCA* gene mutation could be tested for the presence of a mutation and their risk of developing cancer could be predicted. Over time though, the discovery of *BRCA1* and *BRCA2* has had a much greater influence than many might have imagined. In this review, we discuss how the discovery of *BRCA1* and *BRCA2* has not only provided an understanding of the molecular processes that drive tumourigenesis but also reignited an interest in therapeutically exploiting loss-of-function alterations in tumour suppressor genes.

Key Words
- BRCA1
- BRCA2
- breast

Endocrine-Related Cancer (2016) **23**, T39–T55

BRCA1 and BRCA2: discovery, genetics and biology

Globally, breast cancer is the most common cancer in women. Its familial form constitutes 5–10% of all breast cancers and has a dominant mode of inheritance and is characterised by earlier onset of disease, relative to breast cancer in the general population. Heterozygous germ-line mutations in either the *BRCA1* or *BRCA2* tumour suppressor genes are the most common genetic cause of familial breast cancer and were identified as breast cancer susceptibility genes in the 1990s through linkage analysis in families with the disease (Futreal *et al.* 1994, Miki *et al.* 1994, Wooster *et al.* 1995, Tavtigian *et al.* 1996, King 2014). *BRCA1* and *BRCA2* mutations are found in 25–28% of familial breast cancers, and mutation carriers have a lifetime risk of 40–87% for developing breast cancer by the age of 70 years. Mutation carriers also have a lifetime risk of 45–60% (*BRCA1* mutation carriers) or 11–35% (*BRCA2* mutation carriers) for developing ovarian cancer (Ford *et al.* 1998, King *et al.* 2003). Other types of cancers also found in *BRCA1* and *BRCA2* mutation carriers include pancreatic and prostate cancers (Ozcelik *et al.* 1997, Breast Cancer Linkage Consortium 1999, Antoniou *et al.* 2003, Edwards *et al.* 2003, King *et al.* 2003, van Asperen *et al.* 2005). Although most familial *BRCA1* or *BRCA2* mutations are inherited as heterozygous mutations, rare, biallelic germ-line mutations do occur in patients with Fanconi anaemia (Howlett *et al.* 2002, Domchek *et al.* 2013, Meyer *et al.* 2014, Sawyer *et al.* 2015). Furthermore, in addition to germ-line mutations in these genes, somatic *BRCA1* and *BRCA2* mutations are also found in breast, prostate, ovarian and pancreatic cancers, as is somatic hypermethylation of the *BRCA1* gene promoter. An analysis of tumours from individuals with *BRCA1* or *BRCA2* mutations indicates that the wild-type allele is generally lost (Futreal *et al.* 1994,

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0228

This paper forms part of a thematic review section on 20 Years of BRCA 1 and 2. The Guest Editors for this section were Kokichi Sugano and William Foulkes

Collins *et al*. 1995, Gudmundsson *et al*. 1995), suggesting that loss of heterozygosity at the *BRCA1* and *BRCA2* loci appears to be an important event for tumourigenesis.

Both *BRCA1* and *BRCA2* are relatively large genes, which display limited sequence homology to each other. *BRCA1* comprises 24 exons that translate to a 1863 amino acid protein with a RING domain with E3 ubiquitin ligase activity, a coiled-coil domain in the largely unstructured central region important for binding with another tumour suppressor protein, PALB2, and BRCT (BRCA1 carboxy terminal) repeats important for interaction with phosphorylated proteins (Wu *et al*. 1996, Brzovic *et al*. 2001, Xia *et al*. 2006, Sy *et al*. 2009, Zhang *et al*. 2009*b*). *BRCA2* comprises 27 exons that translate to a 3418 amino acid protein that includes amino-terminal BRC repeats, which mediate binding of BRCA2 to PALB2 and the DNA recombinase RAD51, a central DNA binding domain, and nuclear localisation and RAD51 control domains at the carboxy-terminus (Sharan *et al*. 1997, Wong *et al*. 1997, Yang *et al*. 2002).

Small insertions/deletions (in-dels) or nonsense mutations leading to truncations are the most common *BRCA* gene mutation types observed in cancer patients. At least 1790 distinct mutations, polymorphisms and variants have been identified in *BRCA1* to date and over 2000 of them have been identified in *BRCA2* according to the Breast Cancer information Core (BIC) and ClinGen. Mutations are distributed across the entire coding sequence for both genes with over 50% of observed mutations being unique to particular individuals. In addition to known pathogenic mutations, there are a large number of missense, in-frame deletion and silent mutations known as 'variants of unknown significance', which have unclear pathogenic potential, making clinical interpretation of genetic testing difficult in cancer patients harbouring these variants.

Breast tumours in *BRCA1* mutation carriers tend to have a basal-like transcriptional signature and more often than not exhibit a 'triple-negative' phenotype, lacking expression of the oestrogen and progesterone receptors and lacking amplification of the *ERBB2* (*HER2*) oncogene (Foulkes *et al*. 2003). This triple-negative phenotype precludes the use of targeted oestrogen receptor-based or ERBB2-specific therapies, and in general, BRCA1 mutant breast cancers are treated with traditional genotoxic chemotherapy agents. In contrast, breast tumours in *BRCA2* mutation carriers tend to better reflect the hormone receptor and ERBB2 status of breast cancers in the non-BRCA mutant population (Jonsson *et al*. 2010, Waddell *et al*. 2010).

BRCA1 and BRCA2 functions in DNA repair and replication fork protection

Tumourigenesis occurs in the absence of BRCA1 and BRCA2 as both proteins play important roles in the repair of DNA double-strand breaks (DSBs) (Moynahan & Jasin 2010) and the stability of replication forks. DSBs, the most toxic type of DNA lesions, can be catastrophic for the cell if left unrepaired as they compromise the double helix structure of DNA. The two main methods of DSB repair are the error-prone non-homologous end-joining (NHEJ) pathway and the error-free homologous recombination (HR) pathway. NHEJ, used predominantly in the G_1 phase of the cell cycle, can result in loss of genetic information proximal to the DSB site. In contrast, HR, active during S and G_2 phases, uses homologous sequence from a sister chromatid for error-free repair of DSBs. In HR, after the initial detection of the DSB, the broken DNA ends are enzymatically resected to generate 3′ single-stranded DNA (ssDNA). The ssDNA is coated by the replication protein A (RPA) complex, which is then replaced by the RAD51 recombinase. The binding of multiple RAD51 molecules onto ssDNA enables strand invasion, in which ssDNA from the damaged DNA site invades the double helix of intact DNA, a process that facilitates the identification of a homologous DNA sequence that is used as a template for DNA repair across the break site. Efficient resolution of the resulting intermediates completes the process, and the genetic integrity of broken DNA is restored.

BRCA1 and BRCA2 play key roles in HR. BRCA1 is required for CtIP-mediated resection of DSBs to generate single-stranded DNA (ssDNA), which is coated by the RPA complex (Yu & Baer 2000, Chen *et al*. 2008). BRCA1-mediated resection is a key step in committing to repair by HR as opposed to the error-prone NHEJ pathway (Kass & Jasin 2010). CDK-phosphorylated CtIP protein binds BRCA1 BRCT repeats, is localised to the DSB and mediates resection through the MRN (MRE11-RAD50-NBS1) complex (Wong *et al*. 1998, Yu *et al*. 1998, Sartori *et al*. 2007, Chen *et al*. 2008). In addition to CtIP localisation to DSBs, BRCA1 also counteracts 53BP1 function, and in doing so impairs NHEJ (Bouwman *et al*. 2010, Bunting *et al*. 2010). Additionally, both BRCA1 and BRCA2, bridged by PALB2, are required for the recruitment of the DNA recombinase RAD51 to damaged DNA, where it forms a nucleoprotein complex (or 'filament') with ssDNA that mediates strand invasion (Xia *et al*. 2006, Sy *et al*. 2009, Zhang *et al*. 2009*a*,*b*, Tischkowitz & Xia 2010). BRCA2 is not only required for localisation of RAD51 to RPA-coated DNA

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0228

Published by Bioscientifica Ltd.

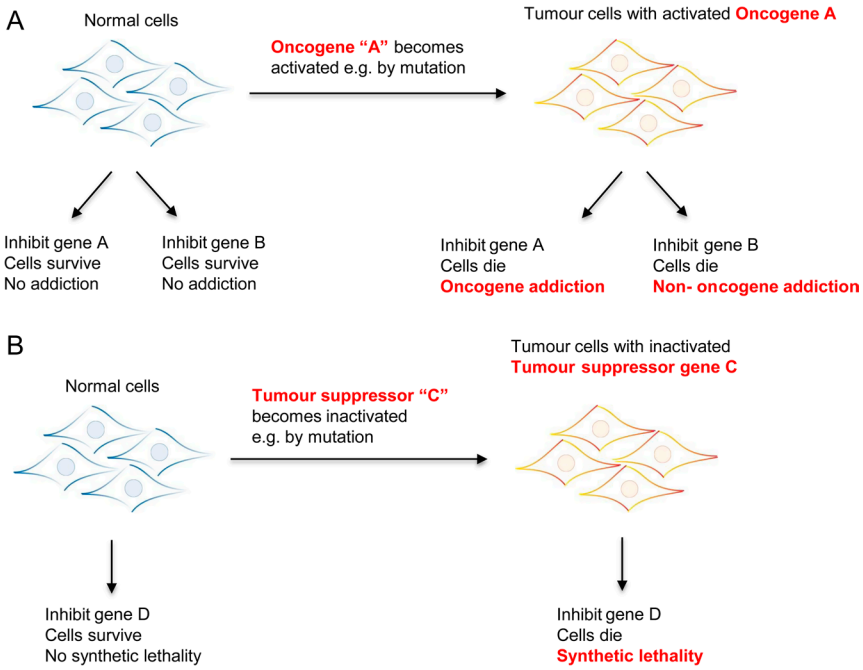

Figure 1
Oncogene addiction and synthetic lethality. Oncogene and tumour suppressor gene mutations drive the oncogenic process. In addition to driving the oncogenic process, alterations in oncogenes and tumour suppressor also impart a distinct set of genetic dependencies on tumour cells that are not present in normal cells, which are known as oncogene addictions, non-oncogene addictions and synthetic lethal effects. (A) Oncogene addiction is the situation where a tumour cell becomes totally dependent on the activity of a mutated gene (Gene A is shown as an example). An analogous scenario, known as non-oncogene addiction, exists when tumour cells with an alteration in an oncogene A become addicted to the activity of a non-oncogene, B. (B) Synthetic lethality is a scenario in which loss of either gene A or gene B function is tolerated but simultaneous loss of both genes is not. In normal cells, inhibition of either A or B does not result in cell death. In tumour cells in which gene B is rendered dysfunctional (for example by mutation), inhibition of gene A results in cell death.

but also for stabilising the RAD51 nucleofilament that is formed by blocking RAD51-mediated ATP hydrolysis.

When the progression of replication forks is halted (replication fork stalling), which can be caused by a variety of factors including damaged DNA lesions being encountered by the replication fork or the relative absence of the requisite nucleotides, preventing the disintegration or collapse of the fork structure is key to the continued fitness of cells. One of the molecular events that challenges fork stability in this setting is the activity of the nuclease MRE11, which if not tightly controlled degrades the newly synthesised (nascent) DNA at the replication fork, potentially forcing fork collapse. In addition to their roles in DSB repair, BRCA1 and BRCA2 prevent the degradation of nascent DNA at stalled replication forks (Schlacher *et al*. 2011, Schlacher *et al*. 2012, Pathania *et al*. 2014). For example, Schlacher and coworkers found using single-molecule DNA fibre analysis that once replication forks are stalled with hydroxyurea (HU), tracts of nascent DNA produced before fork stalling are degraded in the absence of BRCA2 by MRE11. This protection of nascent DNA at replication forks appears to be mediated by a conserved C-terminal region in BRCA2 that stabilises RAD51 nucleoprotein filaments but is not required for RAD51 loading or homologous recombination *per se* (Schlacher *et al*. 2011). Using Brca1-deficient embryonic stem (ES) cells, Schlacher and coworkers later found that Brca1 also prevents fork degradation by MRE11 (Schlacher *et al*. 2012).

The loss of DNA repair and fork stability functions of BRCA1 and BRCA2 is the likely cause of the genomic instability seen in *BRCA1* or *BRCA2* mutant tumours. Cells deficient in either protein have been shown to have reduced efficiency of HR (Moynahan *et al*. 2001*a*,*b*). BRCA1/2-deficient cells also exhibit spontaneous and DNA damage-induced genetic instability, which subsequently contributes to tumourigenesis. Additionally, BRCA1/2-deficient cells are sensitive to DNA-damaging agents, especially those that form crosslinks on DNA such as cisplatin (Narod 2010). This particular phenotype has been exploited in the clinic to treat BRCA-deficient tumours. In fact, cisplatin and its derivative, carboplatin, have been shown to be particularly effective in the treatment of *BRCA1*- and *BRCA2*-associated cancers, particularly in ovarian cancers (Boyd *et al*. 2000, Cass *et al*. 2003, Chetrit *et al*. 2008, Tan *et al*. 2008, Vencken *et al*. 2011). However, chemoresistance to platinum compounds is a very significant clinical problem and has a negative impact on patient survival. Therefore, identification of additional drugs that can effectively treat HR-deficient cancers by exploiting synthetic lethal gene interactions is essential (Fig. 1).

Therapeutic exploitation of *BRCA* gene defects with PARP inhibitors

The intricate dissection of *BRCA1* and *BRCA2* function, and in particular the discovery that these tumour suppressor proteins were required for effective HR,

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0228

Published by Bioscientifica Ltd.

made a significant impact on the discovery and mechanistic understanding of therapeutic approaches that target *BRCA1* or *BRCA2* (*BRCA1/2)* gene mutant cancers. To date, most agents proposed to selectively inhibit *BRCA1/2* mutant tumour cells likely do so by causing the stalling and collapse of DNA replication forks. Specifically, these agents cause replication fork damage that requires HR for repair (Fig. 2). In the absence of *BRCA1* or *BRCA2* gene function, and therefore functional HR, tumour cells most likely attempt to repair replication forks via non-HR forms of DNA repair. These alternative repair strategies cause large-scale chromosomal abnormalities, which ultimately impair the fitness of cells and induce cell death. Early evidence of this phenomenon was suggested by work illustrating the sensitivity of *BRCA* gene-defective cells to platinum salts (Fedier *et al*. 2003, Bartz *et al*. 2006, Evers *et al*. 2008) or topoisomerase inhibitors such as camptothecin (Rahden-Staron *et al*. 2003). Platinum salts most likely stall replication forks by causing intra-strand and inter-strand crosslinks in DNA through covalent interaction with nucleophilic N-7 sites on purine residues (Sikov 2015). These 'lesions' within the DNA structure prevent normal unwinding of the DNA double helix before replication (Fig. 2, Sikov 2015). DNA topoisomerase enzymes bind DNA and unwind its helical structure (Champoux 2001), a prerequisite for multiple processes such as DNA replication, transcription, recombination and chromatin remodelling (Champoux 2001). Topoisomerase inhibitors (also known as topo-poisons) such as camptothecin fix or 'trap' topoisomerase on DNA (O'Connell *et al*. 2010, Lord & Ashworth 2012). Presumably, this trapped form of topoisomerase provides a bulky structure, which prevents the progression of the replication fork (Fig. 2, O'Connell *et al*. 2010).

At least in *in vitro* tissue culture models, platinum salts and topoisomerase inhibitors selectively target *BRCA1/2* gene mutant tumour cells, compared with cells with 'wild-type' function, but still have relatively profound inhibitory effects on wild-type cells (Evers *et al*. 2008). Conversely, work from two teams in 2005 suggested that small molecule inhibitors of the DNA repair enzyme – poly (ADP-ribose) polymerase (PARP) – caused profound cell inhibitory effects in *BRCA1* (Farmer *et al*. 2005) or *BRCA2* mutant (Bryant *et al*. 2005, Farmer *et al*. 2005)

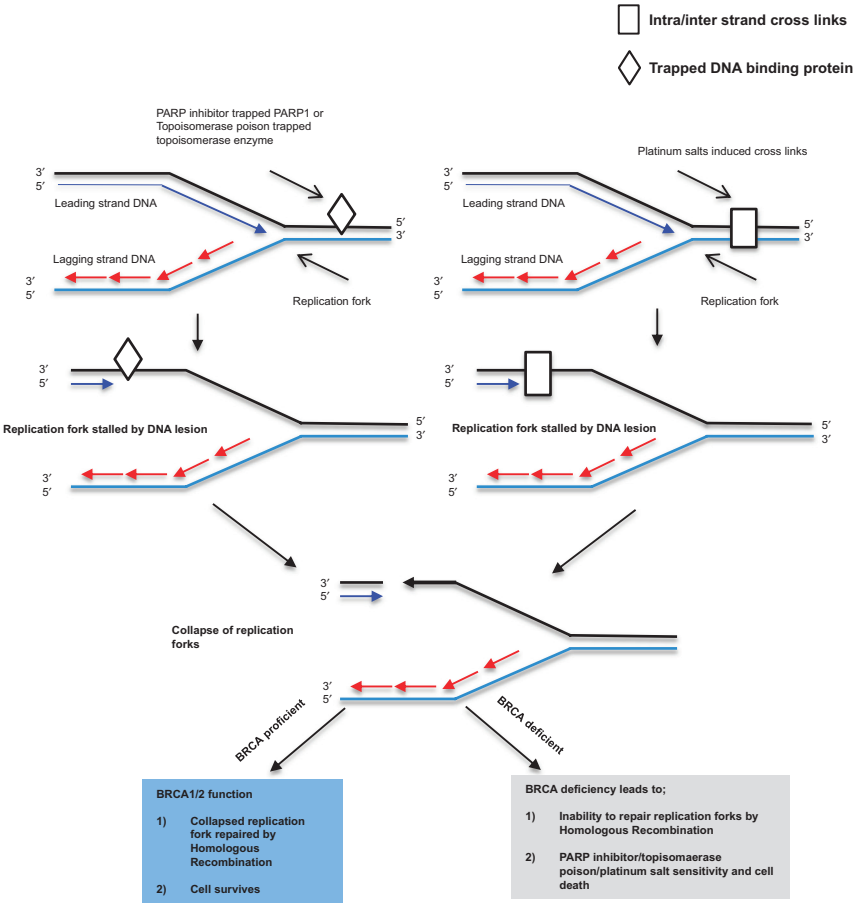

Figure 2
DNA lesions causing replication fork collapse. A working model of the DNA damage response (DDR) to replication fork stalling agents is shown. Drug-like PARP inhibitors trap PARP on DNA. Likewise, topoisomerase poisons trap topoisomerase enzymes on DNA. Platinum salts cause DNA crosslinks. Each of these events stalls the progression of replication forks in S phase. Stalled forks often collapse, forming double-strand DNA breaks. DSBs in this setting are often cytotoxic if not repaired. The normal DNA repair process, homologous recombination, is controlled by BRCA1 and BRCA2. BRCA1 function is required for the processing of DNA ends before repair, a process known as end resection. Once end resection is complete, BRCA2 localises the key DNA recombinase enzyme, RAD51, to DNA at the site of DNA damage. The binding of RAD51 to DNA allows damaged DNA to invade an intact DNA double helix with homologous DNA sequence to that at the site of DNA damage (often in the sister chromatid), which is used as a template on which new DNA could be synthesised as part of the DNA repair process. In the absence of functional BRCA1 and BRCA2, cells either fail to effectively repair DNA (which can lead to apoptosis) or to use orthogonal DNA repair processes such as non-homologous end joining, which increase the frequency of complex DNA rearrangements, events that ultimately impair the fitness of cells.

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0228

Published by Bioscientifica Ltd.

tumour cells but had minimal effects in wild-type cells with functional HR. PARP1 is an enzyme that uses β-NAD+ as a co-factor to synthesise poly(ADP-ribose) chains (PAR) on target proteins and has a known role in the repair of single-strand DNA breaks (breaks in one strand of the DNA double helix) (Hottiger *et al*. 2010). At the time, it was thought that the inhibition of PARP activity might cause an accumulation of DNA damage that requires HR for its repair (Bryant *et al*. 2005, Farmer *et al*. 2005). Subsequently, this hypothesis has been refined by data suggesting that the key cytotoxic DNA lesion in PARP inhibitor-exposed tumour cells is PARP 'trapped' on DNA (Fig. 2, Murai *et al*. 2012, 2014), a mechanism reminiscent of that used to explain the BRCA selectivity of topoisomerase inhibitors. PARP binds damaged DNA and then initiates a series of PARylation events. One of these events is autoPARylation (PARylation of PARP itself), which causes the release of PARP once its role in the initial phase of DNA repair is complete (Murai *et al*. 2012). It seems possible that some catalytic inhibitors of PARP impair autoPARylation, thus trapping PARP on the double helix, where it is able to stall and collapse replication forks (Fig. 2).

A number of years after the pre-clinical observation of PARP inhibitor/*BRCA* gene synthetic lethality, clinical trials, including those which studied breast cancer patients, confirmed the potential of PARP inhibitors as treatments for *BRCA* gene mutant cancers. Although these trials have recently been reviewed in detail elsewhere (Balmana *et al*. 2011, Livraghi & Garber 2015, Lord *et al*. 2015), the key trials can be summarised as follows:

Early phase 1 trials show sustained anti-tumour responses in germ-line *BRCA* mutant cancers

Although the first-in-human PARP inhibitor clinical trial assessed the safety of rucaparib (Pfizer), olaparib (aka AZD2281, Lynparza, KuDOS/AZ) was the first PARP inhibitor to be formally assessed in *BRCA1/2* gene mutant patients. In a phase 1 clinical trial of olaparib (Fong *et al*. 2009), 60 patients were treated with 400 mg twice daily olaparib, 19 of whom had germ-line mutations in either *BRCA1* or *BRCA2*. In this subset of *BRCA1/2* gene mutant patients, 63% exhibited a clinical benefit from olaparib treatment, as defined by radiological and/or tumour marker responses or disease stabilisation for a period greater than 4 months (Fong *et al*. 2009). Even though dose-limiting myelosuppression and central nervous system side effects were seen in some patients, many of the sustained anti-tumour responses were not associated with the deleterious side-effect profile normally associated with classical chemotherapy (Fong *et al*. 2009). Based on these promising results, the same phase I trial was subsequently expanded to include a total of 50 germ-line *BRCA1/2* mutant carriers with ovarian, primary peritoneal or fallopian tube carcinoma. Here, an overall response rate (ORR) of 40% and a disease control rate (DCR) of 46% were observed (Fong *et al*. 2010). In a retrospective analysis of this study, a significant correlation between a good response to prior platinum salt treatment and subsequent therapeutic response to olaparib was seen (Fong *et al*. 2010). One explanation for this correlation is that both platinum salts and PARP inhibitors stall replication forks and require HR for the repair of the subsequent DNA lesions caused (Fig. 2).

Key phase 2 clinical trials in germ-line or somatic *BRCA* mutated cancers

The promising results from this phase I clinical study prompted two phase 2 clinical trials investigating single-agent olaparib in patients with *BRCA* gene mutant chemotherapy-resistant breast (Tutt *et al*. 2010) or ovarian cancers (Audeh *et al*. 2010). These trials, which used either a 400 mg or 100 mg twice-daily treatment regimen, established an ORR of 33% in ovarian cancer patients in the 400 mg twice-daily treatment schedule and 13% of the 100 mg twice-daily group, with a median progression-free survival (PFS) of 5.8 months and 1.9 months, respectively (Audeh *et al*. 2010). Similar response rates were observed in the breast cancer cohort, where a response rate (RR) of 22% was seen in the 100 mg twice-daily cohort (PFS 3.8 months), whereas a RR of 41% was observed in the cohort receiving a higher dose of olaparib (PFS 5.7 months) suggesting that the higher dose was essential to achieve a maximal clinical response.

As discussed later, many sporadic ovarian serous and non-familial triple-negative breast cancers display many of the molecular and histopathological features found in germ-line *BRCA1/2* gene mutant tumours, which are often driven by somatic mutations in *BRCA1*, *BRCA2* and other HR-modifying genes, a concept termed 'BRCAness' (Turner *et al*. 2004). On this basis, olaparib was also assessed as a monotherapy in sporadic cancers thought to display the BRCAness phenotype, namely high-grade serous ovarian cancer (HGSOvCa) and triple-negative breast cancers (described earlier). In patients with HGSOvCa both *BRCA1/2* gene mutant and non-mutant patients demonstrated a number of sustained therapeutic responses to olaparib, a number of which were also associated with prior platinum sensitivity

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0228

Published by Bioscientifica Ltd.

(Gelmon *et al*. 2011). In triple-negative breast cancer patients, those with *BRCA1/2* gene mutations exhibited a higher frequency of disease stabilisation in response to olaparib treatment than those without *BRCA1/2* gene mutations (63% vs 13%), but unlike in the ovarian cancer cohort, no sustained responses were achieved in either the *BRCA1/2*-mutant or non-mutant patients (Gelmon *et al*. 2011). Alongside these studies, olaparib was assessed as a maintenance therapy (i.e. a therapy used to reduce disease recurrence after chemotherapy) in patients with HGSOvCa who had previously received carboplatin, a platinum salt chemotherapy ((Ledermann *et al*. 2012) NCT00753545, Study 19). In this study, 136 patients received olaparib after chemotherapy, with 129 receiving a placebo instead. A preliminary analysis of this trial suggested that when used as a maintenance monotherapy, olaparib significantly improved PFS, and time to first and second subsequent therapy or death compared with the use of a placebo in the maintenance setting, with *BRCA1/2* mutant patients (be it germ-line or somatic) in the trial deriving the greatest benefit from olaparib. However, an effect on OS in either *BRCA1/2* gene mutant or non-*BRCA1/2* gene mutant patients was not seen (Ledermann *et al*. 2014). Nevertheless, the improvements in PFS were sufficient to warrant an approval by the FDA and EMA for olaparib as a maintenance monotherapy in HGSOv cancer characterised by *BRCA1/2* gene mutation, making this PARP inhibitor not only the first synthetic lethal treatment for cancer to be approved but also the first treatment for an inherited cancer (Kim *et al*. 2015). A retrospective analysis of data from study 19, conducted after 77% of the patients had died, has now shown an overall survival benefit from olaparib maintenance monotherapy. In the *BRCA* mutant patients, this OS benefit was not only most pronounced (median OS 34.9 months for olaparib vs 30.2 months for placebo, hazard ratio (HR) of 0.62) but was also seen in the entire dataset, which included both *BRCA1/2* mutant and non-*BRCA1/2* mutant patients (OS 29.8 months (olaparib) vs 27.8 (placebo), HR 0.73) (Ledermann *et al*. 2016).

Additional clinical trials observed responses to olaparib in patients with *BRCA1/2* mutations in a spectrum of other *BRCA1/BRCA2*-associated cancers including pancreatic and prostate cancers (Kaufman *et al*. 2015). Notable among these studies has been the TOPARP phase II clinical trial assessing the efficacy of olaparib in men with metastatic, castration-resistant prostate cancer (Mateo *et al*. 2015). Of the 49 patients who underwent prior treatment (docetaxel, the androgen synthesis inhibitor abiraterone or the androgen receptor inhibitor enzalutamide) and received oral olaparib at 400 mg twice daily, 14 showed a response to olaparib; seven of these patients harboured *BRCA2* defects and four exhibited tumour-specific *ATM* defects, raising the possibility that other genes involved in HR, such as ATM, might also be good predictive biomarkers of olaparib response (Mateo *et al*. 2015). On the basis of this study, olaparib has now been given breakthrough status in prostate cancer, and an expansion of the TO-PARP trial to a larger number of patients with HR gene defects is now underway.

Although not all PARP inhibitor trials have delivered such positive results (Lord *et al*. 2015), the clinical responses in the phase 2 trials described previously, alongside the favourable side-effect profile of PARP inhibitors, such as olaparib, talazoparib, rucaparib, niraparib and veliparib, have provided the impetus for initiating a series of phase III trials, including those in breast cancers. It is expected that within a few years, the data from these trials will provide some of the definitive information that could support or refute the case for using PARP inhibitors in cancers other than HGSOv cancer.

Additional *BRCA*-directed therapy

As described previously, in addition to PARP inhibitors, a number of conventional chemotherapies routinely used in the management of cancer might also provide an approach to target *BRCA1/2* mutant tumours. These include not only platinum agents, topoisomerase I inhibitors (topotecan and camptothecin) and topoisomerase II inhibitors (doxorubicin and etoposide) described previously but also nucleoside analogous such as gemcitabine, which prevents DNA synthesis when incorporated into DNA by preventing chain elongation during DNA replication (Gandhi *et al*. 1996, Lord & Ashworth 2016). The common mechanism of action of these agents is that they can stall the normal progression of replication forks and likely require *BRCA* and HR function for the repair of the DNA lesions they cause. These agents have been assessed both pre-clinically (Fedier *et al*. 2003, Rahden-Staron *et al*. 2003, Bartz *et al*. 2006) and clinically and have shown selectivity in *BRCA1/2*-defective backgrounds (Kilburn & Group 2008, Silver *et al*. 2010).

One of characteristics of *BRCA1/2* mutant tumours is an elevated mutational load, compared with non-*BRCA1/2* mutant tumours, a likely effect of defective HR. Clinical responses to immune checkpoint inhibitors such as anti-PD-1 and anti-PD-L1 antibodies have previously been associated with hypermutated cancers, including lung carcinomas and melanomas. It seems possible that similar approaches could be used to target *BRCA* mutant tumours.

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0228

Published by Bioscientifica Ltd.

To investigate this, Strickland and coworkers recently predicted neoantigen load in *BRCA1/2*-mutated HGSOv tumours and found that this was elevated compared with tumours without HR gene defects, as were the presence of CD3+ and CD8+ tumour-infiltrating lymphocytes and PD-1 and PD-L1 expression in tumour-associated immune cells (Strickland *et al*. 2016). Such an analysis therefore supports the clinical assessment of combinations of PARP inhibitors with PD-L1 inhibitors in breast and ovarian cancers (e.g. NCT02484404 (Lee *et al*. 2016)), as do data from a pre-clinical study illustrating the efficacy of an anti-CTLA4 antibody in combination with the PARP inhibitor veliparib in a mouse model carrying a *Brca1* mutant tumour (Higuchi *et al*. 2015).

Drug resistance in a *BRCA* mutant setting

Although PARP inhibitors have shown to be useful for the treatment of BRCA1/2-associated cancers, PARP inhibitor resistance is likely to be a major obstacle to the overall effectiveness of treatment (Fong *et al*. 2009, Tutt *et al*. 2010). PARP inhibitor resistance, in *BRCA1* or *BRCA2* mutant cancers, can occur due to the reversal of synthetic lethality by several mechanisms including restoration of DSB repair by HR, loss of PARP1 expression, loss of 53BP1 expression and upregulation of PARP inhibitor efflux from cells (Fig. 3).

Restoration of BRCA1/2 function as a mechanism of PARP inhibitor resistance

We and others hypothesized that restoration of HR may be able to reverse chemosensitivity to DNA-damaging drugs in BRCA1/2-deficient cells based on the observation that spontaneously occurring secondary genetic alterations could compensate for the initial disease-causing mutations in some patients with Fanconi anaemia (FA), including reversal of DNA damaging agent sensitivity in cells (Hirschhorn 2003, Ikeda *et al*. 2003, Wiegant *et al*. 2006). We hypothesized that acquired secondary intragenic *BRCA1* or *BRCA2* mutations may reverse the effect of the initial disease-causing *BRCA1/2* mutations in tumours and result in resistance to PARP inhibitors and DNA crosslinking drugs such as cisplatin and carboplatin (Edwards *et al*. 2008, Sakai *et al*. 2008, 2009, Swisher *et al*. 2008).

Evidence for secondary *BRCA1/2* mutations as a method of reversing PARP inhibitor-related synthetic lethality was first demonstrated in several *in vitro* and *in vivo* drug-selected *BRCA2* mutated cell lines (Edwards *et al*. 2008, Sakai *et al*. 2008, 2009). PARP inhibitor- or cisplatin-selected clones of the pancreatic cancer cell line CAPAN-1 (*BRCA2.6147delT*) and ovarian cancer cell line PEO1 (*BRCA2.5193C>G*) acquired secondary *BRCA2* mutations that restore the open reading frame and express functional BRCA2 protein (Edwards *et al*. 2008, Sakai *et al*. 2008, 2009). PARP inhibitor-resistant clones had internal insertions or deletions in the *BRCA2* gene that eliminated the truncating effect of the parental c.6147delT mutation in CAPAN-1 cells and changed the nonsense mutation in PEO1 cells to a missense mutation. PEO4 ovarian cancer cells derived from the same patient as PEO1 cells, after the onset of clinical resistance, were resistant to both PARP inhibitor and cisplatin as a result of a secondary *BRCA2* mutation that converts the parental nonsense mutation, p.Y1655X, to a silent mutation p.Y1655Y. The same silent mutation was also found in the drug-resistant tumour sample from the same patient.

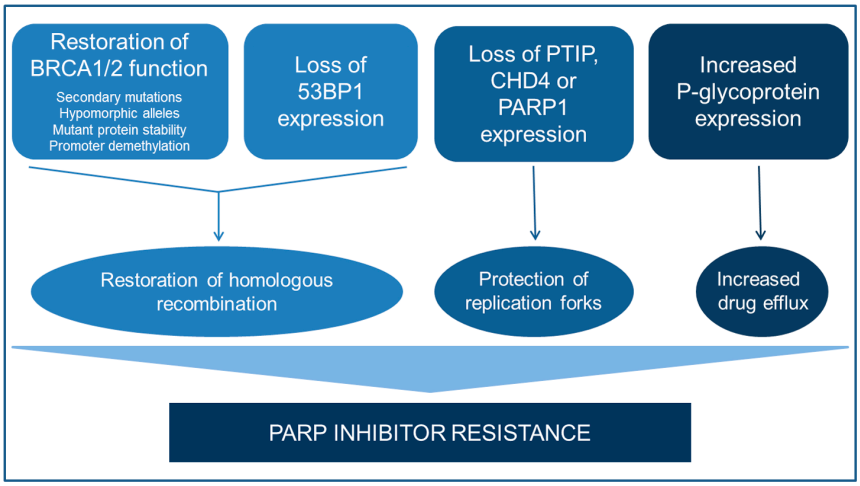

Figure 3
Mechanisms of PARP inhibitor resistance in BRCA1/2-associated cancers. Loss of PARP inhibitor resistance in BRCA1/2 mutated cancers can occur via (1) restoration of BRCA1/2 function and HR by secondary intragenic BRCA1/2 mutations, expression of hypomorphic BRCA1 alleles, stabilisation of mutant BRCA proteins and demethylation of the *BRCA1* promoter; (2) restoration of HR as a result of relief from 53BP1-mediated block on end-resection (only in *BRCA1* mutant tumour cells); (3) protection of replication forks, from MRE11-mediated degradation, due to loss of PTIP, CHD4 or PARP1 expression; and (4) increased efflux of PARP inhibitor from cancer cells as a result of increased P-glycoprotein expression.

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0228

Published by Bioscientifica Ltd.

The restored BRCA2 proteins in CAPAN-1, PEO1 and PEO4 cells are functional as evidenced by the restoration of ionizing radiation-induced RAD51 foci formation, reduced DNA damage-induced chromosomal aberrations and cross-resistance to cisplatin. Non-BRCA2-restored clones of CAPAN-1 or PEO1 had neither secondary BRCA2 mutations nor restoration of damage-induced RAD51 foci formation. Importantly, depletion of BRCA2 by siRNA reversed the drug resistance in BRCA2-restored clones, and ectopic expression of the mutant BRCA2 proteins found in resistant clones led to drug resistance in BRCA2-deficient backgrounds.

Although most pre-clinical studies identified mutations in BRCA2, and not BRCA1, that were associated with therapy resistance, several clinical studies have demonstrated that this is an effect that likely operates for both of the tumour suppressor genes. Norquist and coworkers evaluated PARP inhibitor response in cisplatin/carboplatin-resistant ovarian tumours from patients with *BRCA1* or *BRCA2* mutations (Norquist *et al*. 2011). Of the three non-BRCA1/2-restored tumours, two showed complete response to PARP inhibitor and one showed a partial response. As expected, two of the three BRCA1/2-restored tumours with secondary *BRCA1/2* mutations did not show response as the disease progressed presumably due to restored HR, whereas the third showed partial response (Norquist *et al*. 2011).

In another study, Barber and coworkers found evidence of secondary *BRCA2* mutations in two PARP inhibitor-resistant tumours that were not present in matched treatment-naïve tumour samples from the same patients (Barber *et al*. 2013). A breast tumour from a male carrying the *BRCA2c.9106C>T* nonsense mutation had acquired a secondary mutation that changed the nonsense (p.Q2960X) to a missense (p.Q2960E) mutation. The second observation was a high-grade serous ovarian carcinoma from a patient carrying the *BRCA2c.4705_4708delGAAA* mutation, who was previously treated for breast cancer. In this case, the BRCA2 open reading frame was restored as a result of a larger deletion, *BRCA2c.4697_4709delAAA TACTGAAAG*, which encompassed the germ-line *BRCA2* deletion mutation. Though not formally tested, both secondary *BRCA2* mutations likely restore at least partially functional BRCA2 protein that cancels PARP inhibitor-associated synthetic lethality (Barber *et al*. 2013).

In a recent study, whole-genome sequencing and analysis of high-grade serous ovarian carcinomas revealed five individuals with platinum-resistant disease who had secondary *BRCA1/2* mutations out of a total of ten patients analysed, who had germ-line *BRCA1/2* mutations (Patch *et al*. 2015). One of the two patients, whose tumour was also cross-resistant to PARP inhibitor, had at least 12 distinct secondary deletion mutations in *BRCA2* identified from multiple metastatic sites. The second patient had two distinct secondary *BRCA1* mutations that changed the germ-line nonsense mutation to missense mutations in platinum-resistant cells.

A recent study by Jonkers and coworkers has also provided strong evidence for BRCA1 restoration as an important mechanism for PARP inhibitor and cisplatin resistance in BRCA1-deficient, triple-negative breast cancer (Ter Brugge *et al*. 2016). The analysis of patient-derived xenograft (PDX) models of triple-negative breast cancer included those derived from BRCA1-deficient tumours with *BRCA1* promoter hypermethylation and a frameshift mutation leading to a premature stop (*BRCA1.c2210delC*). In line with previous observations in *BRCA1*-mutated tumours, *BRCA1 c2210delC* therapy-resistant tumours had intragenic deletions that restore the *BRCA1* open reading frame to reinstate BRCA1 protein expression and IR-induced RAD51 foci formation. Interestingly, demethylation of the *BRCA1* promoter was shown to be the major mechanism of resistance in therapy-resistant tumours derived from BRCA1 promoter hypermethylated tumours. *BRCA1* gene fusions with other chromosome 17 genes also allowed the bypass of *BRCA1* promoter hypermethylation to allow BRCA1 expression in a few drug-resistant tumours. Analysis of post-treatment tumours from individuals with *BRCA1* promoter hypermethylation in pre-treatment samples showed a significant decrease in BRCA1 promoter methylation, which correlated with a similar increase in *BRCA1* mRNA. Taken together, data from these studies provide strong evidence of BRCA1 restoration by multiple mechanisms, including *BRCA1* promoter demethylation, as an important driver of PARP inhibitor and cisplatin resistance in BRCA1-deficient breast cancer.

Though clinical examples of secondary *BRCA1/2* mutations in PARP inhibitor-resistant breast and other cancers remain few, more extensive data are available for secondary *BRCA1/2* mutations in platinum-resistant cancers (Swisher *et al*. 2008, Dhillon *et al*. 2011, Norquist *et al*. 2011). Data from cell line models and the limited clinical samples suggest that BRCA1/2-restored, platinum-resistant tumours have a high likelihood of being cross-resistant to PARP inhibitor. Additionally, secondary *BRCA1/2* mutations resulting in PARP inhibitor and cisplatin resistance are likely driven by the convergence of at least three different factors: increased mutation rate

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0228

Published by Bioscientifica Ltd.

due to exposure to genotoxic agents, the lack of error-free DNA repair and a selective advantage for BRCA1/2-restored cells when patients are treated with PARP inhibitors or platinum salts.

Several examples of BRCA1/2 restoration by means other than secondary mutation also exist. For example, Johnson and coworkers showed that stabilisation of a normally undetectable mutant BRCA1 protein can lead to PARP inhibitor resistance in rucaparib-selected clones derived from the MDA-MB-436 breast cancer cell line, harbouring a *BRCA1.5396+1C>A* splice donor site mutation (Johnson *et al.* 2013). Expression of an HSP90-stabilised, carboxy terminus-truncated BRCA1 protein results in restoration of damage-induced RAD51 foci formation and decreased PARP inhibitor-induced chromosomal aberrations. Reduced 53BP1 expression was also observed in rucaparib-resistant cells, which may allow increased BRCA1-independent CtIP-mediated resection, though 53BP1 loss alone was not sufficient to render cells resistant to the PARP inhibitor. The combination of mutant protein stabilisation and reduced 53BP1 expression was also observed in a clinical cisplatin-resistant ovarian cancer sample though the response to PARP inhibitor is unknown.

In another study, Wang and coworkers demonstrated that breast cancer cell lines with mutations in the central large exon 11 of *BRCA1* expressing the Δ11q splice variant show partial PARP inhibitor resistance and strong ionizing radiation-induced BRCA1 and RAD51 foci formation (Wang *et al.* 2016*a*). Depletion of the Δ11q splice variant reduced foci formation and sensitized cells to PARP inhibitor and cisplatin. Moreover, 5-year overall survival in individuals with the exon 11 mutations was similar to those with wild-type *BRCA1* and worse than those carrying mutations outside of exon 11.

Several groups have provided evidence for hypomorphic activity of two additional *BRCA1* mutations that contribute to PARP inhibitor and cisplatin resistance (Drost *et al.* 2011, Drost *et al.* 2016, Powell 2016, Wang *et al.* 2016*b*). The *BRCA1.C61G* mutation in the RING domain abolishes BRCA1 ubiquitin ligase activity, while still promoting tumourigenesis. Importantly, mouse *Brca1.C61G* cells are not sensitive to PARP inhibition suggesting the mutant protein retains at least partial function. Drost and coworkers also showed recently that a BRCA1 variant missing the RING domain ('RING-less' BRCA1) can be detected in cells from a mouse model carrying the *Brca1.185STOP* mutation and in the human breast cancer cell line SUM1315MO2 with the *BRCA1.185delAG* mutation (Drost *et al.* 2016). Importantly, expression of 'RING-less' BRCA1 renders cells partially resistant to PARP inhibitor and cisplatin, suggesting that its intact carboxy terminus provides partial function. Moreover, Wang and coworkers showed that PARP inhibitor and cisplatin-resistant clones of the SUM1315MO2 cell line had increased expression of the 'RING-less' BRCA1 variant that results from translation at an alternative start site (Wang *et al.* 2016*b*). Ectopic overexpression of this BRCA1 variant resulted in partial resistance to PARP inhibitor and cisplatin *in vitro* and *in vivo*. Interestingly, Drost and coworkers did not observe increased expression of the 'RING-less' BRCA1 consistently in cisplatin-resistant clones. The existence of partial function mutants warrants a better understanding of how specific mutations impact response to PARP inhibitor, cisplatin and other therapies and the clinical management of BRCA-deficient breast and other types of cancers.

Loss of 53BP1 expression

BRCA1 and 53BP1 play important roles in choice of DSB repair by HR or NHEJ: BRCA1 promotes HR, whereas 53BP1 tips the balance in favour of NHEJ. Several groups have shown that loss of the 53bp1 in a *Brca1-null* or *Brca1Δexon11* mice rescues embryonic lethality observed in Brca1-deficient mice (Cao *et al.* 2009, Bouwman *et al.* 2010, Bunting *et al.* 2010). Brca1/53bp1-deficient cells and mice also have restored growth, decreased chromosomal aberrations, increased RAD51 foci formation and at least partially restored HR relative to Brca1-deficient mice. Importantly, the loss of 53bp1 in Brca1-deficient mice renders them resistant to PARP inhibitor. Additionally, a subset of olaparib-resistant Brca1/P glycoprotein-deficient murine tumours had lost 53bp1 expression, whereas several others had heterogeneous expression (Jaspers *et al.* 2013). Bouwman *et al* also found reduced 53BP1 expression in clinical *BRCA1/2*-associated and triple-negative breast cancers (Bouwman *et al.* 2010). Lower 53BP1 expression was correlated with lower metastasis-free survival, presumably due to reduced response to therapy. Together these data suggested that 53BP1 loss in a BRCA1-deficient background is a mechanism of PARP inhibitor resistance in mice and humans. The prevalence of 53BP1 loss in patients with BRCA1-associated and triple-negative breast cancer remains to be determined.

Replication fork protection

Studies investigating the mechanisms that mediate replication fork stability in the absence of *BRCA1* or

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0228

Published by Bioscientifica Ltd.

BRCA2 have led to a number of additional mechanisms of drug resistance to be proposed. As discussed earlier, replication forks in *BRCA1/2* mutant cells are liable to degradation via MRE11 (Schlacher *et al*. 2011, 2012, Pathania *et al*. 2014). Chaudhuri and coworkers recently found that in *Brca2* mutant cells, loss of PTIP improved cell viability, protected HU-stalled replication forks from MRE11-mediated degradation and decreased genetic instability (Chaudhuri *et al*. 2016). These effects were not caused by restoration of HR, but are best explained by PTIP's role in localising MRE11 to replication forks. In the absence of PTIP, replication fork degradation via MRE11 was reduced, which in turn led to a reduction in replication fork degradation. Chaudhuri and coworkers also found that BRCA1/2-deficient cells with co-occurring PTIP defects also showed a reduced number of chromosomal abnormalities when exposed to either cisplatin or a PARP inhibitor, suggesting that these processes could influence BRCA1/2 mutant tumour cell response to therapy (Chaudhuri *et al*. 2016). In a series of *Brca2*-deficient, PARP inhibitor-resistant mouse tumours, RAD51 foci formation was not restored, but replication fork degradation after HU challenge was reduced, suggesting that HR restoration was not the cause of drug resistance in this case, but that restoration of fork stability could be (Chaudhuri *et al*. 2016). Taken together, these data provide a case for assessing biomarkers of replication fork stability in clinical trials involving *BRCA1/2* mutant cancer patients.

PARP inhibitor resistance due to increased efflux

Increased efflux of PARP inhibitor from cancer cells also contributes to PARP inhibitor resistance in the *BRCA1/2* mutation context. Rottenberg and coworkers showed that mammary tumours in *Brca1/p53* double-mutant mice that are initially very responsive to olaparib eventually become resistant to the drug (Rottenberg *et al*. 2008). PARP inhibitor resistance in these tumours is mediated by increased expression of P-glycoprotein (Pgp) transporter genes *Abcb1a* and *Abcb1b* and can be reversed by inhibiting Pgp activity with tariquidar. Knockout of the Pgp *Mdr 1a/b* gene in a *Brca1* mutant background improved the response of mammary tumours to PARP inhibitor, though they eventually became resistant due to other mechanisms (Jaspers *et al*. 2013). Additionally, multidrug resistance, to drugs including olaparib, observed in a *Brca2*-mutated mouse model of mammary mesenchymal carcinosarcomas was, in part, due to increased Pgp expression (Jaspers *et al*. 2015). Though increased efflux via Pgp transporter upregulation leads to PARP inhibitor resistance in *Brca1* and *Brca2* mutant mouse models of breast cancer, it is yet to be reported in the clinic.

BRCAness

In addition to patients with germ-line *BRCA1* or *BRCA2* gene mutations (*gBRCA*), it seems very likely that significant numbers of cancer patients without *gBRCA* mutations have tumours that resemble, at the molecular and histological level, *gBRCA* mutant tumours, a phenomenon termed BRCAness (reviewed recently in (Lord & Ashworth 2016)). In some cases, these shared molecular features might also drive the same defect in HR that could lead to sensitivity to BRCA synthetic lethal treatments such as PARP inhibitors.

BRCAness might be driven by several different mechanisms. With the onset of large-scale tumour sequencing, it is clear that in addition to germ-line *BRCA* gene mutations, a significant proportion of non-familial cancers have somatic alterations in *BRCA1*, *BRCA2* or the growing number of genes associated with HR. For example, triple-negative breast cancers, HGSOvCa, metastatic, castration-resistant prostate cancer and pancreatic ductal adenocarcinomas exhibit somatic alterations in *BRCA1, BRCA2* or BRCAness genes such as *ATM, ATR, BAP1, CDK12, CHK1, CHK2*, the Fanconi anaemia proteins (*FANCA, C, D2, E, F), PALB2*, *NBN, WRN*, the *RAD51* homologs *RAD51B, C* and *D, MRE11A, BLM* and *BRIP1* (reviewed in (Lord & Ashworth 2016)). Many of these genes have been shown in pre-clinical models to cause PARP inhibitor sensitivity when dysfunctional (McCabe *et al*. 2006, Blazek *et al*. 2011, Bajrami *et al*. 2014, Joshi *et al*. 2014), extending the causative link between HR dysfunction and sensitivity to these drugs.

There is also growing evidence for BRCAness in tumours that have a particular spectrum or pattern of mutations. One of the key observations made from the genomic profiling of tumours is the classification of tumours according to the type of mutations they possess, a *mutational scar*, rather than the specific genes that are mutated. In some instances, these mutational scars reflect the natural history of a tumour, and particularly the types of DNA damage and repair that have moulded the genome over successive cell cycles. For example, *BRCA1* and *BRCA2* mutant tumours exhibit a mutational scar that appears to be caused by the elevated use of NHEJ, a DNA repair process that predominates in the absence of HR. For example, recent work from Nik-Zainal and coworkers, based on data from the whole-genome sequences of

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0228

Published by Bioscientifica Ltd.

560 breast tumours, confirmed the presence of three distinct genomic rearrangement signatures associated with the loss of HR in tumours, each characterised by tandem DNA duplications or deletions. One of these signatures appears to be associated with loss of BRCA1 function, the second being associated with defective BRCA1 or BRCA2, with the aetiology of the third signature remaining largely unknown (Nik-Zainal *et al*. 2016). The discovery of these genomic signatures in part reflects observations made in genetically engineered mouse cell lines with either *Brca1* or *Brca2* mutations, where the use of non-conservative forms of DNA repair such as NHEJ results in an elevated frequency of DNA deletions flanked by short, tandem DNA repeats at the break points of the deletion (Moynahan *et al*. 2001*b*, Tutt *et al*. 2001, Xia *et al*. 2001). Mutational scars similar to those seen in *gBRCA* mutant tumours are also seen in non-*gBRCA* mutant tumours, and even in those without a detectable germ-line or somatic alteration in an HR gene, suggesting that similar DNA repair defects might be operating in these tumours. Importantly, there is now a growing body of evidence, which suggests that the presence of such BRCAness mutational scars also correlates with clinical responses to HR-targeting agents such as platinum salts and PARP inhibitors (Birkbak *et al*. 2013), correlations which are driving the development of clinically applicable BRCAness mutational scar assays. Most of these assays use genome-wide DNA copy number profiling to estimate the extent of chromosomal rearrangements characteristic of an HR defect (Birkbak *et al*. 2013).

Extending the utility of the synthetic lethal paradigm

It seems reasonable to question whether synthetic lethality as a concept might have a wider applicability in the search for optimised treatments for breast cancer. The progress in the molecular profiling of breast tumours means that there is now a working list of driver gene defects in the disease that in principle could be targeted with a synthetic lethal approach. For example, many of the tumour suppressor gene defects that recurrently occur in breast cancer, such as *TP53, PTEN* and *RB1*, might be amenable to synthetic lethal approaches; already a number of candidate synthetic lethal targets for these genes have been identified (Edgar *et al*. 2005, Gordon & Du 2011, Reaper *et al*. 2011, Mendes-Pereira *et al*. 2012, Emerling *et al*. 2013, Mereniuk *et al*. 2013, Morandell *et al*. 2013, Origanti *et al*. 2013). Many of the efforts to identify synthetic lethal interactions that are relevant to breast cancer have been driven by advances in functional genomic approaches such as RNA interference screening and more recently CRISPR-based screens (Gilbert *et al*. 2014, Wang *et al*. 2015, Morgens *et al*. 2016). The synthetic lethal approach might also be applied to target relatively common oncogene amplification events in breast cancer such as *MYC* amplification, which is present in over 22% of all breast tumours (Cerami *et al*. 2012, Gao *et al*. 2013, Ciriello *et al*. 2015). *MYC* encodes a transcription factor, which might be challenging to directly target with drug-like small molecules, and so using synthetic lethal strategies to targeting *MYC* amplification seems a reasonable approach. Already synthetic lethal interactions between *MYC* and the DR5 death receptor pathway (Wang *et al*. 2004) or inhibition of the splicesome in MYC-dependent breast tumours have been identified (Hsu *et al*. 2015). This latter observation might be explained by an increased dependency in MYC-amplified tumours on pre-mRNA processing (Hsu *et al*. 2015).

Although there are opportunities to more widely exploit the synthetic lethal concept in breast cancer, there are also clear challenges. For a synthetic lethal effect to be clinically actionable and to have significant utility, there are certain qualities the synthetic lethal relationship must exhibit, many of which are common to all ideal therapeutic approaches, synthetic lethal or not. Firstly, the therapeutic window between tumour and normal cell inhibition/toxicity achieved with the synthetic lethal target must be profound. Second, ideal synthetic lethal effects must be highly penetrant – i.e. the presence of the predictive biomarker (e.g. a mutation in a breast cancer driver gene) must be highly predictive of sensitivity to inhibition of the synthetic lethal target. If this is not the case, then a novel synthetic lethal treatment might only work in a minority of patients or a minority of tumour cell clones within an individual. Third, ideal synthetic lethal interactions must be relatively resilient to additional molecular changes that might reverse the synthetic lethal effect. This is critical if clinical synthetic lethal effects are to be effective in breast tumours, whose inherent molecular heterogeneity and ability to evolve and survive in the face of negative selective pressure is well documented (Alizadeh *et al*. 2015, Brooks *et al*. 2015). Despite advances in the ability to identify synthetic lethal effects in breast tumour cells, somewhat less attention is often given to whether these effects also fulfil these ideal criteria.

One concept that might gain further scrutiny in the future is the idea of exploiting combinations of different synthetic lethal effects in the same tumour, each of

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0228

Published by Bioscientifica Ltd.

which focuses on a different breast cancer driver gene or phenotype. For example, in triple-negative breast cancers with germ-line or somatic *BRCA* gene mutations, *TP53* mutations also co-occur. It seems reasonable to suggest that a drug combination strategy that involves a PARP inhibitor (to synthetically lethal target the *BRCA* gene defect), used alongside a *TP53* synthetic lethal therapy, might be more effective than PARP inhibitor monotherapy, which might be limited by the emergence of secondary mutant *BRCA1/2* alleles. This idea of targeting multiple co-occurring driver mutations in the same tumour might be most effective when mutations that occur early on in the disease process, and so are more likely present in most subclones in a tumour, are selected.

Conclusions and future prospects

The cloning of *BRCA1* and *BRCA2* stimulated a large body of work, from many investigators, that ultimately resulted in the first clinically approved treatment for a genetically defined cancer syndrome. Although this work provides a very compelling narrative that illustrates the influence pre-clinical and clinical research can have, several important questions still remain. Some of these pertain directly to the use of PARP inhibitors, whereas others are also relevant to the treatment of cancer in general. For example, although olaparib has been approved for use as a maintenance therapy after platinum treatment in HGSOv cancer, a role for first-line PARP inhibitor treatment in *BRCA1* or *BRCA2* mutant patients, or those with BRCAness, remains to be established. There is also very little understanding about what might constitute the optimal drug combination strategies involving PARP inhibitors or how patients with PARP inhibitor resistance might best be treated. It seems reasonable to suggest that some of the answers to these questions will come from clinical studies but also will be informed by pre-clinical research and a continued focus on the molecular biology of the BRCA1 and BRCA2 genes. Beyond the BRCA/PARP inhibitor paradigm, the wider clinical applicability of the synthetic lethal concept is still not established, although it is hoped the continued pre-clinical research activity in this area will ultimately lead to further clinical trials drug approvals that deliver more effective treatments of cancer patients.

Declaration of interest

C J L is a named inventor on patents describing the use of PARP inhibitors and stands to gain from their use as part of the ICR Rewards to Inventors Scheme.

Funding

This work was funded as follows: K K D is supported by the Thomsen Family Breast Cancer Research Fellowship; T T is the recipient of support from the Howard Hughes Medical Institute, the Fanconi Anemia Research Fund and a V Foundation Grant in Translational Clinical Research; C J L is funded by Cancer Research UK, Breast Cancer Now and the Wellcome Trust.

Acknowledgements

The authors thank their funders (described above) for supporting their work in this area. C J L also acknowledges NHS funding to the NIHR Royal Marsden Hospital Biomedical Research Centre.

References

Alizadeh AA, Aranda V, Bardelli A, Blanpain C, Bock C, Borowski C, Caldas C, Califano A, Doherty M, Elsner M, *et al.* 2015 Toward understanding and exploiting tumor heterogeneity. *Nature Medicine* **21** 846–853. (doi:10.1038/nm.3915)

Antoniou A, Pharoah PD, Narod S, Risch HA, Eyfjord JE, Hopper JL, Loman N, Olsson H, Johannsson O, Borg A, *et al.* 2003 Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: a combined analysis of 22 studies. *American Journal of Human Genetics* **72** 5. (doi:10.1086/375033)

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)

Bajrami I, Frankum JR, Konde A, Miller RE, Rehman FL, Brough R, Campbell J, Sims D, Rafiq R, Hooper S, *et al.* 2014 Genome-wide profiling of genetic synthetic lethality identifies CDK12 as a novel determinant of PARP1/2 inhibitor sensitivity. *Cancer Research* **74** 287–297. (doi:10.1158/0008-5472.CAN-13-2541)

Balmana J, Domchek SM, Tutt A & Garber JE 2011 Stumbling blocks on the path to personalized medicine in breast cancer: the case of PARP inhibitors for BRCA1/2-associated cancers. *Cancer Discovery* **1** 29–34. (doi:10.1158/2159-8274.CD-11-0048)

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)

Bartz SR, Zhang Z, Burchard J, Imakura M, Martin M, Palmieri A, Needham R, Guo J, Gordon M, Chung N, *et al.* 2006 Small interfering RNA screens reveal enhanced cisplatin cytotoxicity in tumor cells having both BRCA network and TP53 disruptions. *Molecular and Cell Biology* **26** 9377–9386. (doi:10.1128/MCB.01229-06)

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)

Blazek D, Kohoutek J, Bartholomeeusen K, Johansen E, Hulinkova P, Luo Z, Cimermancic P, Ule J & Peterlin BM 2011 The Cyclin K/Cdk12 complex maintains genomic stability via regulation of expression of DNA damage response genes. *Genes & Development* **25** 2158–2172. (doi:10.1101/gad.16962311)

Bouwman P, Aly A, Escandell JM, Pieterse M, Bartkova J, van der Gulden H, Hiddingh S, Thanasoula M, Kulkarni A, Yang Q, *et al.*

Published by Bioscientifica Ltd.

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)

Boyd J, Sonoda Y, Federici MG, Bogomolniy F, Rhei E, Maresco DL, Saigo PE, Almadrones LA, Barakat RR, Brown CL, *et al.* 2000 Clinicopathologic features of BRCA-linked and sporadic ovarian cancer. *JAMA* **283** 2260–2265. (doi:10.1001/jama.283.17.2260)

Breast Cancer Linkage Consortium 1999 Cancer risks in BRCA2 mutation carriers. *Journal of National Cancer Institute* **91** 1310–1316. (doi:10.1093/jnci/91.15.1310)

Brooks MD, Burness ML & Wicha MS 2015 Therapeutic implications of cellular heterogeneity and plasticity in breast cancer. *Cell Stem Cell* **17** 260–271. (doi:10.1016/j.stem.2015.08.014)

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)

Brzovic PS, Rajagopal P, Hoyt DW, King MC & Klevit RE 2001 Structure of a BRCA1-BARD1 heterodimeric RING-RING complex. *Nature Structural Biology* **8** 833–837. (doi:10.1038/nsb1001-833)

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)

Cao L, Xu X, Bunting SF, Liu J, Wang RH, Cao LL, Wu JJ, Peng TN, Chen J, Nussenzweig A, *et al.* 2009 A selective requirement for 53BP1 in the biological response to genomic instability induced by Brca1 deficiency. *Molecular Cell* **35** 534–541. (doi:10.1016/j.molcel.2009.06.037)

Cass I, Baldwin RL, Varkey T, Moslehi R, Narod SA & Karlan BY 2003 Improved survival in women with BRCA-associated ovarian carcinoma. *Cancer* **97** 2187–2195. (doi:10.1002/cncr.11310)

Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, *et al.* 2012 The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discovery* **2** 401–404. (doi:10.1158/2159-8290.CD-12-0095)

Champoux JJ 2001 DNA topoisomerases: structure, function, and mechanism. *Annual Review of Biochemistry* **70** 369–413. (doi:10.1146/annurev.biochem.70.1.369)

Chaudhuri AR, Callen E, Ding X, Gogola E, Duarte AA, Lee JE, Wong N, Lafarga V, Calvo JA, Panzarino NJ, *et al.* 2016 Replication fork stability confers chemoresistance in BRCA-deficient cells. *Nature* **535** 382–387. (doi:10.1038/nature18325)

Chen L, Nievera CJ, Lee AY & Wu X 2008 Cell cycle-dependent complex formation of BRCA1.CtIP.MRN is important for DNA double-strand break repair. *Journal of Biological Chemistry* **283** 7713–7720. (doi:10.1074/jbc.M710245200)

Chetrit A, Hirsh-Yechezkel G, Ben-David Y, Lubin F, Friedman E & Sadetzki S 2008 Effect of BRCA1/2 mutations on long-term survival of patients with invasive ovarian cancer: the national Israeli study of ovarian cancer. *Journal of Clinical Oncology* **26** 20–25. (doi:10.1200/JCO.2007.11.6905)

Ciriello G, Gatza ML, Beck AH, Wilkerson MD, Rhie SK, Pastore A, Zhang H, McLellan M, Yau C, Kandoth C, *et al.* 2015 Comprehensive molecular portraits of invasive lobular breast cancer. *Cell* **163** 506–519. (doi:10.1016/j.cell.2015.09.033)

Collins N, McManus R, Wooster R, Mangion J, Seal S, Lakhani SR, Ormiston W, Daly PA, Ford D, Easton DF, *et al.* 1995 Consistent loss of the wild type allele in breast cancers from a family linked to the BRCA2 gene on chromosome 13q12-13. *Oncogene* **10** 1673–1675.

Dhillon KK, Swisher EM & Taniguchi T 2011 Secondary mutations of BRCA1/2 and drug resistance. *Cancer Science* **102** 663–669. (doi:10.1111/j.1349-7006.2010.01840.x)

Domchek SM, Tang J, Stopfer J, Lilli DR, Hamel N, Tischkowitz M, Monteiro AN, Messick TE, Powers J, Yonker A, *et al.* 2013 Biallelic deleterious BRCA1 mutations in a woman with early-onset ovarian cancer. *Cancer Discovery* **3** 399–405. (doi:10.1158/2159-8290.CD-12-0421)

Drost R, Bouwman P, Rottenberg S, Boon U, Schut E, Klarenbeek S, Klijn C, van der Heijden I, van der Gulden H, Wientjens E, *et al.* 2011 BRCA1 RING function is essential for tumor suppression but dispensable for therapy resistance. *Cancer Cell* **20** 797–809. (doi:10.1016/j.ccr.2011.11.014)

Drost R, Dhillon KK, van der Gulden H, van der Heijden I, Brandsma I, Cruz C, Chondronasiou D, Castroviejo-Bermejo M, Boon U, Schut E, *et al.* 2016 BRCA1185delAG tumors may acquire therapy resistance through expression of RING-less BRCA1. *Journal of Clinical Investigation* **126** 2903–2918. (doi:10.1172/JCI70196)

Edgar KA, Belvin M, Parks AL, Whittaker K, Mahoney MB, Nicoll M, Park CC, Winter CG, Chen F, Lickteig K, *et al.* 2005 Synthetic lethality of retinoblastoma mutant cells in the Drosophila eye by mutation of a novel peptidyl prolyl isomerase gene. *Genetics* **170** 161–171. (doi:10.1534/genetics.104.036343)

Edwards SM, Kote-Jarai Z, Meitz J, Hamoudi R, Hope Q, Osin P, Jackson R, Southgate C, Singh R, Falconer A, *et al.* 2003 Two percent of men with early-onset prostate cancer harbor germline mutations in the BRCA2 gene. *American Journal of Human Genetics* **72** 1–12. (doi:10.1086/345310)

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)

Emerling BM, Hurov JB, Poulogiannis G, Tsukazawa KS, Choo-Wing R, Wulf GM, Bell EL, Shim HS, Lamia KA, Rameh LE, *et al.* 2013 Depletion of a putatively druggable class of phosphatidylinositol kinases inhibits growth of p53-null tumors. *Cell* **155** 844–857. (doi:10.1016/j.cell.2013.09.057)

Evers B, Drost R, Schut E, de Bruin M, van der Burg E, Derksen PW, Holstege H, Liu X, van Drunen E, Beverloo HB, *et al.* 2008 Selective inhibition of BRCA2-deficient mammary tumor cell growth by AZD2281 and cisplatin. *Clinical Cancer Research* **14** 3916–3925. (doi:10.1158/1078-0432.CCR-07-4953)

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)

Fedier A, Steiner RA, Schwarz VA, Lenherr L, Haller U & Fink D 2003 The effect of loss of Brca1 on the sensitivity to anticancer agents in p53-deficient cells. *International Journal of Oncology* **22** 1169–1173. (doi:10.3892/ijo.22.5.1169)

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)

Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P, Bishop DT, Weber B, Lenoir G, Chang-Claude J, *et al.* 1998 Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The Breast Cancer Linkage Consortium. *American Journal of Human Genetics* **62** 676–689. (doi:10.1086/301749)

Foulkes WD, Stefansson IM, Chappuis PO, Begin LR, Goffin JR, Wong N, Trudel M & Akslen LA 2003 Germline BRCA1 mutations and a basal

Published by Bioscientifica Ltd.

epithelial phenotype in breast cancer. *Journal of National Cancer Institute* **95** 1482–1485. (doi:10.1093/jnci/djg050)

Futreal PA, Liu Q, Shattuck-Eidens D, Cochran C, Harshman K, Tavtigian S, Bennett LM, Haugen-Strano A, Swensen J, Miki Y, *et al.* 1994 BRCA1 mutations in primary breast and ovarian carcinomas. *Science* **266** 120–122. (doi:10.1126/science.7939630)

Gandhi V, Legha J, Chen F, Hertel LW & Plunkett W 1996 Excision of 2′,2′-difluorodeoxycytidine (gemcitabine) monophosphate residues from DNA. *Cancer Research* **56** 4453–4459.

Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, *et al.* 2013 Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Science Signaling* **6** pl1. (doi:10.1126/scisignal.2004088)

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)

Gilbert LA, Horlbeck MA, Adamson B, Villalta JE, Chen Y, Whitehead EH, Guimaraes C, Panning B, Ploegh HL, Bassik MC, *et al.* 2014 Genome-scale CRISPR-mediated control of gene repression and activation. *Cell* **159** 647–661. (doi:10.1016/j.cell.2014.09.029)

Gordon GM & Du W 2011 Targeting Rb inactivation in cancers by synthetic lethality. *American Journal of Cancer Research* **1** 773–786.

Gudmundsson J, Johannesdottir G, Bergthorsson JT, Arason A, Ingvarsson S, Egilsson V & Barkardottir RB 1995 Different tumor types from BRCA2 carriers show wild-type chromosome deletions on 13q12-q13. *Cancer Research* **55** 4830–4832.

Higuchi T, Flies DB, Marjon NA, Mantia-Smaldone G, Ronner L, Gimotty PA & Adams SF 2015 CTLA-4 Blockade synergizes therapeutically with parp inhibition in BRCA1-deficient ovarian cancer. *Cancer Immunology Research* **3** 1257–1268. (doi:10.1158/2326-6066.CIR-15-0044)

Hirschhorn R 2003 In vivo reversion to normal of inherited mutations in humans. *Journal of Medical Genetics* **40** 721–728. (doi:10.1136/jmg.40.10.721)

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)

Howlett NG, Taniguchi T, Olson S, Cox B, Waisfisz Q, De Die-Smulders C, Persky N, Grompe M, Joenje H, Pals G, *et al.* 2002 Biallelic inactivation of BRCA2 in Fanconi anemia. *Science* **297** 606–609. (doi:10.1126/science.1073834)

Hsu TY, Simon LM, Neill NJ, Marcotte R, Sayad A, Bland CS, Echeverria GV, Sun T, Kurley SJ, Tyagi S, *et al.* 2015 The spliceosome is a therapeutic vulnerability in MYC-driven cancer. *Nature* **525** 384–388. (doi:10.1038/nature14985)

Ikeda H, Matsushita M, Waisfisz Q, Kinoshita A, Oostra AB, Nieuwint AW, De Winter JP, Hoatlin ME, Kawai Y, Sasaki MS, *et al.* 2003 Genetic reversion in an acute myelogenous leukemia cell line from a Fanconi anemia patient with biallelic mutations in BRCA2. *Cancer Research* **63** 2688–2694.

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)

Jaspers JE, Sol W, Kersbergen A, Schlicker A, Guyader C, Xu G, Wessels L, Borst P, Jonkers J & Rottenberg S 2015 BRCA2-deficient sarcomatoid mammary tumors exhibit multidrug resistance. *Cancer Research* **75** 732–741. (doi:10.1158/0008-5472.CAN-14-0839)

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)

Jonsson G, Staaf J, Vallon-Christersson J, Ringner M, Holm K, Hegardt C, Gunnarsson H, Fagerholm R, Strand C, Agnarsson BA, *et al.* 2010 Genomic subtypes of breast cancer identified by array comparative genomic hybridization display distinct molecular and clinical characteristics. *Breast Cancer Research* **12** R42. (doi:10.1186/bcr2596)

Joshi PM, Sutor SL, Huntoon CJ & Karnitz LM 2014 Ovarian cancer-associated mutations disable catalytic activity of CDK12, a kinase that promotes homologous recombination repair and resistance to cisplatin and poly(ADP-ribose) polymerase inhibitors. *Journal of Biological Chemistry* **289** 9247–9253. (doi:10.1074/jbc.M114.551143)

Kass EM & Jasin M 2010 Collaboration and competition between DNA double-strand break repair pathways. *FEBS Letters* **584** 3703–3708. (doi:10.1016/j.febslet.2010.07.057)

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)

Kilburn LS & Group TNTTM 2008 'Triple negative' breast cancer: a new area for phase III breast cancer clinical trials. *Clinical Oncology* **20** 35–39. (doi:10.1016/j.clon.2007.09.010)

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)

King MC 2014 "The race" to clone BRCA1. *Science* **343** 1462–1465. (doi:10.1126/science.1251900)

King MC, Marks JH & Mandell JB 2003 Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. *Science* **302** 643–646. (doi:10.1126/science.1088759)

Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, Scott C, Meier W, Shapira-Frommer R, Safra T, *et al.* 2012 Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. *New England Journal of Medicine* **366** 1382–1392. (doi:10.1056/NEJMoa1105535)

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)

Ledermann JA, Harter P, Gourley C, Friedlander M, Vergote I, Rustin GJS, Scott CL, Meier W, Shapira-Frommer R, Safra T, *et al.* 2016 (OS) in patients (pts) with platinum-sensitive relap(OS) in patients (pts) with platinum-sensitive relapsed serous ovarian cancer (PSR SOC) receiving olaparib maintenance monotherapy: an interim analysis. *Journal of Clinical Oncology* **34** (supplement; abstr 5501).

Lee J ZA, Lipkowitz S, Annunziata CM, Ho TW, Chiou VL, Minasian LM, Houston ND, Ekwede I & Kohn EC 2016 Phase I study of the PD-L1 inhibitor, durvalumab (MEDI4736; D) in combination with a PARP inhibitor, olaparib (O) or a VEGFR inhibitor, cediranib (C) in women's cancers (NCT02484404). *Journal of Clinical Oncology* **34** (suppl; abstr 3015).

Livraghi L & Garber JE 2015 PARP inhibitors in the management of breast cancer: current data and future prospects. *BMC Medicine* **13** 188. (doi:10.1186/s12916-015-0425-1)

Lord CJ & Ashworth A 2012 The DNA damage response and cancer therapy. *Nature* **481** 287–294. (doi:10.1038/nature10760)

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

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0228

Published by Bioscientifica Ltd.

Lord CJ, Tutt AN & Ashworth A 2015 Synthetic lethality and cancer therapy: lessons learned from the development of PARP inhibitors. *Annual Review of Medicine* **66** 455–470. (doi:10.1146/annurev-med-050913-022545)

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)

McCabe N, Turner NC, Lord CJ, Kluzek K, Bialkowska A, Swift S, Giavara S, O'Connor MJ, Tutt AN, Zdzienicka MZ, *et al.* 2006 Deficiency in the repair of DNA damage by homologous recombination and sensitivity to poly(ADP-ribose) polymerase inhibition. *Cancer Research* **66** 8109–8115. (doi:10.1158/0008-5472.CAN-06-0140)

Mendes-Pereira AM, Lord CJ & Ashworth A 2012 NLK is a novel therapeutic target for PTEN deficient tumour cells. *PLoS ONE* **7** e47249. (doi:10.1371/journal.pone.0047249)

Mereniuk TR, El Gendy MA, Mendes-Pereira AM, Lord CJ, Ghosh S, Foley E, Ashworth A & Weinfeld M 2013 Synthetic lethal targeting of PTEN-deficient cancer cells using selective disruption of polynucleotide kinase/phosphatase. *Molecular Cancer Therapy* **12** 2135–2144. (doi:10.1158/1535-7163.MCT-12-1093)

Meyer S, Tischkowitz M, Chandler K, Gillespie A, Birch JM & Evans DG 2014 Fanconi anaemia, BRCA2 mutations and childhood cancer: a developmental perspective from clinical and epidemiological observations with implications for genetic counselling. *Journal of Medical Genetics* **51** 71–75. (doi:10.1136/jmedgenet-2013-101642)

Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, *et al.* 1994 A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* **266** 66–71. (doi:10.1126/science.7545954)

Morandell S, Reinhardt HC, Cannell IG, Kim JS, Ruf DM, Mitra T, Couvillon AD, Jacks T & Yaffe MB 2013 A reversible gene-targeting strategy identifies synthetic lethal interactions between MK2 and p53 in the DNA damage response in vivo. *Cell Reports* **5** 868–877. (doi:10.1016/j.celrep.2013.10.025)

Morgens DW, Deans RM, Li A & Bassik MC 2016 Systematic comparison of CRISPR/Cas9 and RNAi screens for essential genes. *Nature Biotechnology* **34** 634–636. (doi:10.1038/nbt.3567)

Moynahan ME & Jasin M 2010 Mitotic homologous recombination maintains genomic stability and suppresses tumorigenesis. *Nature Reviews Molecular Cell Biology* **11** 196–207. (doi:10.1038/nrm2851)

Moynahan ME, Cui TY & Jasin M 2001*a* Homology-directed DNA repair, mitomycin-c resistance, and chromosome stability is restored with correction of a Brca1 mutation. *Cancer Research* **61** 4842–4850.

Moynahan ME, Pierce AJ & Jasin M 2001*b* BRCA2 is required for homology-directed repair of chromosomal breaks. *Molecular Cell* **7** 263–272. (doi:10.1016/S1097-2765(01)00174-5)

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)

Murai J, Huang SY, Renaud A, Zhang Y, Ji J, Takeda S, Morris J, Teicher B, Doroshow JH & Pommier Y 2014 Stereospecific PARP trapping by BMN 673 and comparison with olaparib and rucaparib. *Molecular Cancer Therapy* **13** 433–443. (doi:10.1158/1535-7163.MCT-13-0803)

Narod SA 2010 BRCA mutations in the management of breast cancer: the state of the art. *Natuer Reviews Clinical Oncology* **7** 702–707. (doi:10.1038/nrclinonc.2010.166)

Nik-Zainal S, Davies H, Staaf J, Ramakrishna M, Glodzik D, Zou X, Martincorena I, Alexandrov LB, Martin S, Wedge DC, *et al.* 2016 Landscape of somatic mutations in 560 breast cancer whole-genome sequences. *Nature* **534** 47–54. (doi:10.1038/nature17676)

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'Connell BC, Adamson B, Lydeard JR, Sowa ME, Ciccia A, Bredemeyer AL, Schlabach M, Gygi SP, Elledge SJ & Harper JW 2010 A genome-wide camptothecin sensitivity screen identifies a mammalian MMS22L-NFKBIL2 complex required for genomic stability. *Molecular Cell* **40** 645–657. (doi:10.1016/j.molcel.2010.10.022)

Origanti S, Cai SR, Munir AZ, White LS & Piwnica-Worms H 2013 Synthetic lethality of Chk1 inhibition combined with p53 and/or p21 loss during a DNA damage response in normal and tumor cells. *Oncogene* **32** 577–588. (doi:10.1038/onc.2012.84)

Ozcelik H, Schmocker B, Di Nicola N, Shi XH, Langer B, Moore M, Taylor BR, Narod SA, Darlington G, Andrulis IL, *et al.* 1997 Germline BRCA2 6174delT mutations in Ashkenazi Jewish pancreatic cancer patients. *Nature Genetics* **16** 17–18. (doi:10.1038/ng0597-17)

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)

Pathania S, Bade S, Le Guillou M, Burke K, Reed R, Bowman-Colin C, Su Y, Ting DT, Polyak K, Richardson AL, *et al.* 2014 BRCA1 haploinsufficiency for replication stress suppression in primary cells. *Nature Communications* **5** 5496. (doi:10.1038/ncomms6496)

Powell SN 2016 BRCA1 loses the ring but lords over resistance. *Journal of Clinical Investigation* **126** 2802–2804. (doi:10.1172/JCI89209)

Rahden-Staron I, Szumilo M, Grosicka E, Kraakman van der Zwet M & Zdzienicka MZ 2003 Defective Brca2 influences topoisomerase I activity in mammalian cells. *Acta Biochimica Polonica* **50** 139–144.

Reaper PM, Griffiths MR, Long JM, Charrier JD, Maccormick S, Charlton PA, Golec JM & Pollard JR 2011 Selective killing of ATM- or p53-deficient cancer cells through inhibition of ATR. *Nature Chemical Biology* **7** 428–430. (doi:10.1038/nchembio.573)

Rottenberg S, Jaspers JE, Kersbergen A, van der Burg E, Nygren AO, Zander SA, Derksen PW, de Bruin M, Zevenhoven J, Lau A, *et al.* 2008 High sensitivity of BRCA1-deficient mammary tumors to the PARP inhibitor AZD2281 alone and in combination with platinum drugs. *PNAS* **105** 17079–17084. (doi:10.1073/pnas.0806092105)

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)

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)

Sartori AA, Lukas C, Coates J, Mistrik M, Fu S, Bartek J, Baer R, Lukas J & Jackson SP 2007 Human CtIP promotes DNA end resection. *Nature* **450** 509–514. (doi:10.1038/nature06337)

Sawyer SL, Tian L, Kahkonen M, Schwartzentruber J, Kircher M, University of Washington Centre for Mendelian Genomics, FORGE Canada Consortium, Majewski J, Dyment DA, Innes AM, *et al.* 2015 Biallelic mutations in BRCA1 cause a new Fanconi anemia subtype. *Cancer Discovery* **5** 135–142. (doi:10.1158/2159-8290.CD-14-1156)

Schlacher K, Christ N, Siaud N, Egashira A, Wu H & Jasin M 2011 Double-strand break repair-independent role for BRCA2 in blocking stalled replication fork degradation by MRE11. *Cell* **145** 529–542. (doi:10.1016/j.cell.2011.03.041)

Schlacher K, Wu H & Jasin M 2012 A distinct replication fork protection pathway connects Fanconi anemia tumor suppressors to RAD51-BRCA1/2. *Cancer Cell* **22** 106–116. (doi:10.1016/j.ccr.2012.05.015)

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-16-0228

Published by Bioscientifica Ltd.

Sharan SK, Morimatsu M, Albrecht U, Lim DS, Regel E, Dinh C, Sands A, Eichele G, Hasty P & Bradley A 1997 Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking Brca2. *Nature* **386** 804–810. (doi:10.1038/386804a0)

Sikov WM 2015 Assessing the role of platinum agents in aggressive breast cancers. *Current Oncology Reports* **17** 3. (doi:10.1007/s11912-014-0428-7)

Silver DP, Richardson AL, Eklund AC, Wang ZC, Szallasi Z, Li Q, Juul N, Leong CO, Calogrias D, Buraimoh A, *et al*. 2010 Efficacy of neoadjuvant Cisplatin in triple-negative breast cancer. *Journal of Clinical Oncology* **28** 1145–1153. (doi:10.1200/JCO.2009.22.4725)

Strickland KC, Howitt BE, Shukla SA, Rodig S, Ritterhouse LL, Liu JF, Garber JE, Chowdhury D, Wu CJ, D'Andrea AD, *et al*. 2016 Association and prognostic significance of BRCA1/2-mutation status with neoantigen load, number of tumor-infiltrating lymphocytes and expression of PD-1/PD-L1 in high grade serous ovarian cancer. *Oncotarget* **7** 13587–13598. (doi:10.18632/oncotarget.7277)

Swisher EM, Sakai W, Karlan BY, Wurz K, Urban N & Taniguchi T 2008 Secondary BRCA1 mutations in BRCA1-mutated ovarian carcinomas with platinum resistance. *Cancer Research* **68** 2581–2586. (doi:10.1158/0008-5472.CAN-08-0088)

Sy SM, Huen MS & Chen J 2009 PALB2 is an integral component of the BRCA complex required for homologous recombination repair. *PNAS* **106** 7155–7160. (doi:10.1073/pnas.0811159106)

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)

Tavtigian SV, Simard J, Rommens J, Couch F, Shattuck-Eidens D, Neuhausen S, Merajver S, Thorlacius S, Offit K, Stoppa-Lyonnet D, *et al*. 1996 The complete BRCA2 gene and mutations in chromosome 13q-linked kindreds. *Nature Genetics* **12** 333–337. (doi:10.1038/ng0396-333)

Ter Brugge P, Kristel P, van der Burg E, Boon U, de Maaker M, Lips E, Mulder L, de Ruiter J, Moutinho C, Gevensleben H, *et al*. 2016 Mechanisms of therapy resistance in patient-derived xenograft models of BRCA1-deficient breast cancer. *Journal of National Cancer Institute* **108** djw148. (doi:10.1093/jnci/djw148)

Tischkowitz M & Xia B 2010 PALB2/FANCN: recombining cancer and Fanconi anemia. *Cancer Research* **70** 7353–7359. (doi:10.1158/0008-5472.CAN-10-1012)

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, 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)

van Asperen CJ, Brohet RM, Meijers-Heijboer EJ, Hoogerbrugge N, Verhoef S, Vasen HF, Ausems MG, Menko FH, Gomez Garcia EB, Klijn JG, *et al*. 2005 Cancer risks in BRCA2 families: estimates for sites other than breast and ovary. *Journal of Medical Genetics* **42** 711–719. (doi:10.1136/jmg.2004.028829)

Vencken PM, Kriege M, Hoogwerf D, Beugelink S, van der Burg ME, Hooning MJ, Berns EM, Jager A, Collee M, Burger CW, *et al*. 2011 Chemosensitivity and outcome of BRCA1- and BRCA2-associated ovarian cancer patients after first-line chemotherapy compared with sporadic ovarian cancer patients. *Annals of Oncology* **22** 1346–1352. (doi:10.1093/annonc/mdq628)

Waddell N, Arnold J, Cocciardi S, da Silva L, Marsh A, Riley J, Johnstone CN, Orloff M, Assie G, Eng C, *et al*. 2010 Subtypes of familial breast tumours revealed by expression and copy number profiling. *Breast Cancer Research and Treatment* **123** 661–677. (doi:10.1007/s10549-009-0653-1)

Wang T, Birsoy K, Hughes NW, Krupczak KM, Post Y, Wei JJ, Lander ES & Sabatini DM 2015 Identification and characterization of essential genes in the human genome. *Science* **350** 1096–1101. (doi:10.1126/science.aac7041)

Wang Y, Engels IH, Knee DA, Nasoff M, Deveraux QL & Quon KC 2004 Synthetic lethal targeting of MYC by activation of the DR5 death receptor pathway. *Cancer Cell* **5** 501–512. (doi:10.1016/S1535-6108(04)00113-8)

Wang Y, Bernhardy AJ, Cruz C, Krais JJ, Nacson J, Nicolas E, Peri S, van der Gulden H, van der Heijden I, O'Brien SW, *et al*. 2016*a* The BRCA1-Delta11q alternative splice isoform bypasses germline mutations and promotes therapeutic resistance to PARP inhibition and cisplatin. *Cancer Research* **76** 2778–2790. (doi:10.1158/0008-5472.CAN-16-0186)

Wang Y, Krais JJ, Bernhardy AJ, Nicolas E, Cai KQ, Harrell MI, Kim HH, George E, Swisher EM, Simpkins F, *et al*. 2016*b* RING domain-deficient BRCA1 promotes PARP inhibitor and platinum resistance. *Journal of Clinical Investigation* **126** 3145–3157. (doi:10.1172/JCI87033)

Wiegant WW, Overmeer RM, Godthelp BC, van Buul PP & Zdzienicka MZ 2006 Chinese hamster cell mutant, V-C8, a model for analysis of Brca2 function. *Mutation Research* **600** 79–88. (doi:10.1016/j.mrfmmm.2006.03.001)

Wong AK, Pero R, Ormonde PA, Tavtigian SV & Bartel PL 1997 RAD51 interacts with the evolutionarily conserved BRC motifs in the human breast cancer susceptibility gene brca2. *Journal of Biological Chemical* **272** 31941–31944. (doi:10.1074/jbc.272.51.31941)

Wong AK, Ormonde PA, Pero R, Chen Y, Lian L, Salada G, Berry S, Lawrence Q, Dayananth P, Ha P, *et al*. 1998 Characterization of a carboxy-terminal BRCA1 interacting protein. *Oncogene* **17** 2279–2285. (doi:10.1038/sj.onc.1202150)

Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C & Micklem G 1995 Identification of the breast cancer susceptibility gene BRCA2. *Nature* **378** 789–792. (doi:10.1038/378789a0)

Wu LC, Wang ZW, Tsan JT, Spillman MA, Phung A, Xu XL, Yang MC, Hwang LY, Bowcock AM & Baer R 1996 Identification of a RING protein that can interact in vivo with the BRCA1 gene product. *Nature Genetics* **14** 430–440. (doi:10.1038/ng1296-430)

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)

Xia B, Sheng Q, Nakanishi K, Ohashi A, Wu J, Christ N, Liu X, Jasin M, Couch FJ & Livingston DM 2006 Control of BRCA2 cellular and clinical functions by a nuclear partner, PALB2. *Molecular Cell* **22** 719–729. (doi:10.1016/j.molcel.2006.05.022)

Yang H, Jeffrey PD, Miller J, Kinnucan E, Sun Y, Thoma NH, Zheng N, Chen PL, Lee WH & Pavletich NP 2002 BRCA2 function in DNA

Published by Bioscientifica Ltd.

binding and recombination from a BRCA2-DSS1-ssDNA structure. *Science* **297** 1837–1848. (doi:10.1126/science.297.5588.1837)

Yu X & Baer R 2000 Nuclear localization and cell cycle-specific expression of CtIP, a protein that associates with the BRCA1 tumor suppressor. *Journal of Biological Chemistry* **275** 18541–18549. (doi:10.1074/jbc.M909494199)

Yu X, Wu LC, Bowcock AM, Aronheim A & Baer R 1998 The C-terminal (BRCT) domains of BRCA1 interact in vivo with CtIP, a protein implicated in the CtBP pathway of transcriptional repression. *Journal of Biological Chemistry* **273** 25388–25392. (doi:10.1074/jbc.273.39.25388)

Zhang F, Fan Q, Ren K & Andreassen PR 2009*a* PALB2 functionally connects the breast cancer susceptibility proteins BRCA1 and BRCA2. *Molecular Cancer Research* **7** 1110–1118. (doi:10.1158/1541-7786.MCR-09-0123)

Zhang F, Ma J, Wu J, Ye L, Cai H, Xia B & Yu X 2009*b* PALB2 links BRCA1 and BRCA2 in the DNA-damage response. *Current Biology* **19** 524–529. (doi:10.1016/j.cub.2009.02.018)

Received in final form 12 August 2016
Accepted 15 August 2016
Accepted Preprint 15 August 2016

Published by Bioscientifica Ltd.