Synthetic Lethality and Cancer Therapy: Lessons Learned from the Development of PARP Inhibitors

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Abstract

The genetic concept of synthetic lethality, in which the combination or synthesis of mutations in multiple genes results in cell death, provides a framework to design novel therapeutic approaches to cancer. Already there are promising indications, from clinical trials exploiting this concept by using poly(ADP-ribose) polymerase (PARP) inhibitors in patients with germline *BRCA1* or *BRCA2* gene mutations, that this approach could be beneficial. We discuss the biological rationale for BRCA-PARP synthetic lethality, how the synthetic lethal approach is being assessed in the clinic, and how mechanisms of resistance are starting to be dissected. Applying the synthetic lethal concept to target non-*BRCA*-mutant cancers also has clear potential, and we discuss how some of the principles learned in developing PARP inhibitors might also drive the development of additional genetic approaches.

GENETIC CONCEPTS IN CANCER THERAPY

Purposefully or not, many of the existing treatments for cancer exploit weaknesses or dependencies present in tumor cells that are absent, or at least less pronounced, in normal cells. For example, one relatively pervasive characteristic of cancers is genomic instability. In some cases, tumor cell defects in the repair of damaged DNA contribute to this phenotype. Defects that drive genomic instability also impart vulnerabilities that may make tumor cells sensitive to particular DNA-damaging chemotherapies (reviewed in Reference 1). Building on this concept of tumor cell vulnerabilities, there have been efforts to improve the therapeutic window of cancer treatments by identifying targets that are far more tumor cell selective, in the hope that the antitumor effect is enhanced, whereas the deleterious side effects caused by normal cell toxicity are minimized (2).

Perhaps the most pervasive genetic concept has been that of "oncogene addiction" (3). Oncogene addiction (**Figure 1*a***) occurs when a tumor cell is totally dependent on the activity of a mutated gene. A clear example is the oncogene addiction of chronic myelogenous leukemia (CML) cells to constitutive ABL (Abelson murine leukemia viral oncogene homolog 1) kinase activity caused by the *BCR-ABL* fusion gene. This fusion gene is formed as a result of a chromosomal translocation, and the addiction to it has been exploited in the treatment of CML with drugs, such as imatinib, that inhibit ABL kinase activity (4). Similarly, breast and other tumors with amplification of the *ERBB2/HER2* gene become addicted to the elevated activity of the protein product of this gene (HER2) and are sensitive to drugs such as the antibody trastuzumab, which targets HER2 (5).

Somewhat more complex is the concept of "synthetic lethality," a term coined in the middle of the twentieth century by geneticists working on the fruit fly (6) (**Figure 1*b***). Synthetic lethality describes the situation where a defect in one gene or protein is compatible with cell viability but results in cell death when combined (synthesized) with another gene or protein defect.

As an extension of these concepts, Fraser and colleagues coined the term "induced essentiality" (7) (**Figure 1*c***). This phrase explains why many synthetic lethal and gene addiction effects might exist. As cells lose tumor suppressor gene function or gain new oncogene activity, these events, as well as driving the tumorigenic phenotype, probably have deleterious effects. In an attempt to minimize these deleterious effects, molecular networks within a cell facilitate compensatory alterations that allow the cell to survive, a form of "functional buffering" (8). The cell in its new, tumorigenic state now becomes dependent on or addicted to these induced effects, and they become essential to its survival. For example, many oncogenes enhance proliferative rate; in doing so they often induce a state of "replicative stress," a broad term that describes the slowing or stalling of DNA replication forks (9). In some cases, chronic stalling of replication forks leads to DNA damage, which would ultimately be deleterious to the cell. To minimize the impact of this effect and to maintain the fitness of the cell, the activation of oncogenes is often associated with compensatory molecular changes, processes mediated in part by the ATM (ataxia telangiectasia mutated) and ATR (ataxia telangiectasia and Rad3-related) protein kinases (9). These fundamental processes, as described below, have important implications for the development of new therapeutic approaches for the treatment of cancer.

SYNTHETIC LETHAL APPROACHES TO CANCER TREATMENT

The idea of exploiting synthetic lethality to target cancer was first highlighted by Hartwell and colleagues (10) and subsequently championed by Kaelin (11). The synthetic lethal principle provided a conceptual basis for targeting tumors with a known tumor suppressor defect: If genes and proteins could be identified that were synthetically lethal with specific tumor suppressor gene

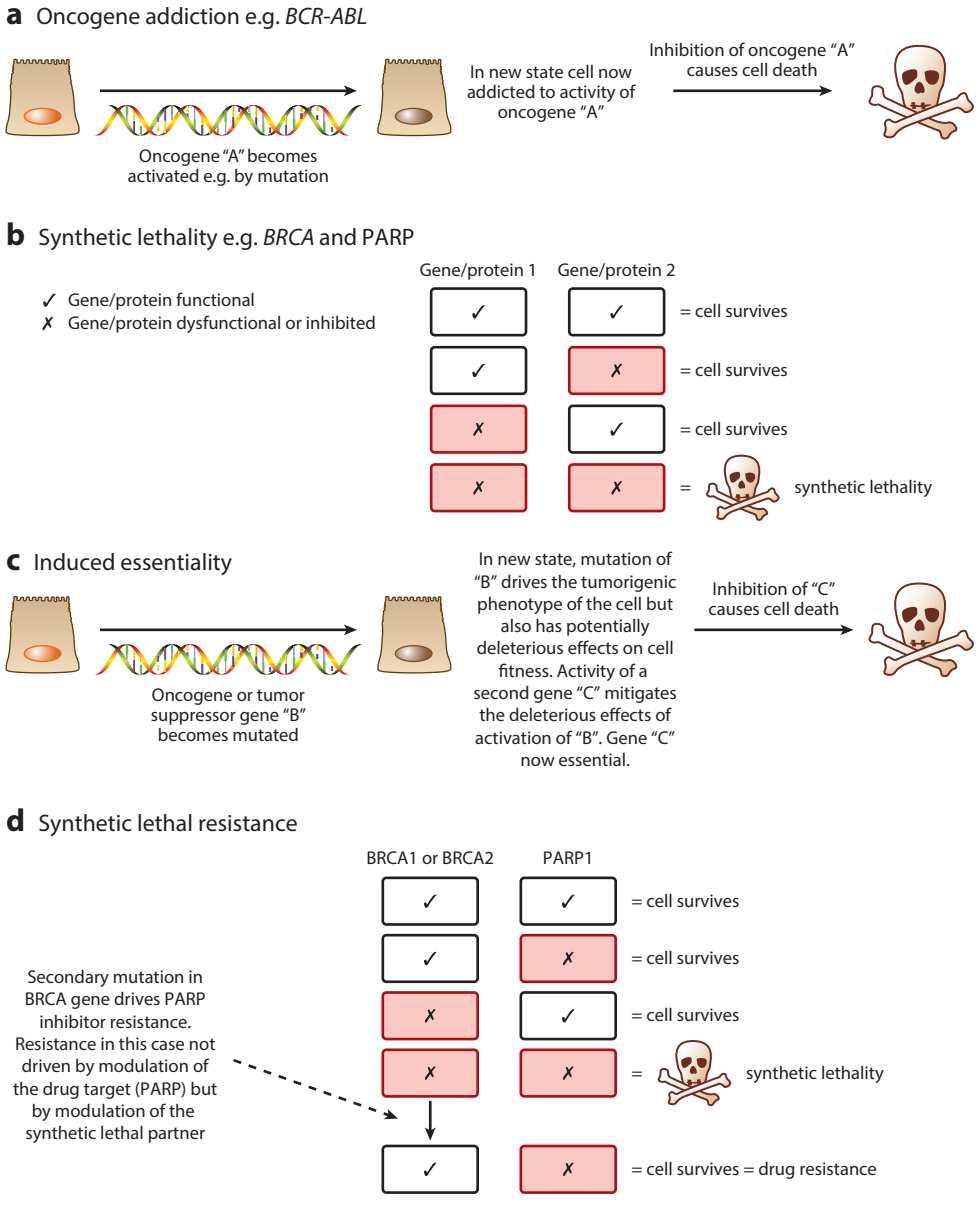

Figure 1

Genetic concepts in cancer therapy. (*a*) Oncogene addiction (3) is the situation where a tumor cell becomes totally dependent on the activity of a mutated gene. (*b*) Synthetic lethality (6) occurs when a combination (synthesis) of gene/protein defects results in cell death. (*c*) Induced essentiality (7) extends the concept of synthetic lethality. When applied to cancer, the term describes the scenario where an alteration in an oncogene or tumor suppressor induces dependency on a second gene, which if targeted can result in death of the tumor cell. (*d*) Synthetic lethal resistance (82) is a form of drug resistance that is mediated not by modulation of the drug target but by an alteration in the drug target.

defects, then in principle, targets could be identified that would likely elicit tumor-cell-specific death without deleterious effects on normal cells, which do not harbor tumor suppressor gene loss.

Perhaps the clearest example thus far of the synthetic lethal principle, when applied to cancer treatment, has come from studies that seek approaches to treat cancers resulting from tumor-specific loss of either *BRCA1* or *BRCA2* tumor suppressor gene function. *BRCA1* and *BRCA2* were originally identified as familial (i.e., inherited) breast and ovary cancer predisposition genes, and mutations also occur in sporadic forms of the disease (12, 13). In many cases, the disease-causing

DSB: double-strand break

HR: homologous recombination

TNBC: triple-negative breast cancer

BRCA mutations truncate the open reading frame of the gene, encoding dysfunctional forms of either protein. The normal forms of *BRCA1* and *BRCA2* encode proteins that play key roles in the repair of DNA double-strand breaks (DSBs) by homologous recombination (HR), a process also mediated by the DNA recombinase RAD51. Tumor cells with defective BRCA1 or BRCA2 lack the ability to localize RAD51 to damaged DNA and are unable to perform HR efficiently (14). The use of alternative, error-prone DNA repair mechanisms leads to an accumulation of genetic aberrations, which likely foster tumorigenesis (15). Early efforts to selectively target *BRCA*-mutant tumors focused on exploiting the role of HR in the repair of DNA crosslinks caused by platinum salts, which generate DNA lesions that are in part repaired by HR (16). On this basis, a proof-of-concept phase III clinical trial assessing the efficacy of carboplatin in women with triple-negative breast cancer (TNBC), including those with mutations in either *BRCA1* or *BRCA2*, was initiated (17).

In 2005, two groups demonstrated the potential of PARP1 inhibition as a targeted, synthetic lethal approach to treating *BRCA*-mutant tumors (18, 19). PARP1 is an enzyme also involved in DNA repair, in particular, DNA single-strand break repair. Upon the formation of a single-strand break, PARP1 binds DNA and enzymatically synthesizes PARP chains onto a series of protein substrates (PARylation). By posttranslationally modifying substrate proteins, PARP1 is thought to recruit effectors of DNA repair, such as XRCC1, to the site of DNA damage and to orchestrate their activity (20). As part of this DNA repair process, PARP1 eventually PARylates itself (autoPARylation), an event that mediates its release from damaged DNA (20). Drug-like small-molecule inhibitors of PARP1, which also inhibit the similar enzyme PARP2, are profoundly selective for *BRCA*-deficient tumor cells (18, 19). These small-molecule PARP inhibitors are, in general, mimics of the PARP1 cofactor β-NAD^+ and bind the catalytic domain of the protein (21). In some cases, *BRCA*-mutant tumor cells are more than 1,000 times more sensitive to PARP inhibitors than wildtype cells (18). Although PARP inhibitors had previously been proposed as chemosensitizers and had entered clinical assessment in 2003 as a combination therapy with temozolomide (22), the level of *BRCA* selectivity achieved with PARP inhibitors alone in preclinical studies provided the impetus for PARP inhibitors to be tested in clinical trials as single agents.

INITIAL CLINICAL TRIALS WITH PARP INHIBITORS

In 2005, a phase I clinical trial began that established preliminary clinical evidence that a synthetic lethal strategy using a single-agent PARP inhibitor could have efficacy in patients with *BRCA*-mutant tumors. In an initial accelerated dose-escalation phase, the maximum tolerated dose of the oral PARP inhibitor olaparib (AZD2281, AstraZeneca/KuDOS) was established at 400 mg b.i.d. (23). This trial included 19 *BRCA1/2*-mutant patients with breast, ovarian, or prostate cancer; in these patients, an objective response rate (ORR) of 47% was observed, in addition to a disease control rate (DCR) of 63%, suggesting that the preclinical observation of synthetic lethality could also operate clinically. Importantly, the toxicities observed at the maximum tolerated dose in this trial (myelosuppression and central nervous system side effects) were milder than those elicited with standard-of-care chemotherapies and were resolved upon cessation of treatment (23). A subsequent expansion of this phase I trial, using 200 mg b.i.d. olaparib, in patients with ovarian, fallopian tube, or peritoneal cancer with germline *BRCA1/2* mutations ($n = 50$) demonstrated an ORR of 40% and DCR of 46% (24), further supporting the rationale for using single-agent PARP inhibitors in *BRCA1/2*-mutant patients. A retrospective analysis established that the most favorable clinical responses to olaparib occurred in patients who had demonstrated prior platinum sensitivity; those with platinum-refractory disease showed an olaparib response rate (RR) of 23%,

whereas the platinum-sensitive patients showed an olaparib RR of 69% (24). The relationship between prior platinum response and clinical PARP inhibitor sensitivity is most likely explained by the ability of both PARP inhibitors and platinum salts to stall replication forks and cause DNA damage that is repaired by BRCA1- and BRCA2-mediated HR.

On the basis of the promising phase I data, two phase II trials evaluated single-agent olaparib therapy, either at 400 mg b.i.d. or 100 mg b.i.d., in patients with chemotherapy-refractory breast (25) and ovarian cancer (26). The lower dose was predicted from a phase I biomarker assessment to inhibit PARP1 to the same extent as the higher dose (23). However, a RR of 13% was seen in the 100 mg b.i.d. ovarian cancer cohort, whereas a RR of 33% was observed in the 400 mg b.i.d. ovarian cancer cohort, suggesting that the higher dose was required for maximal clinical response (26). Similarly, the RR in the 400 mg b.i.d. breast cancer cohort was 41% [median progression-free survival (PFS) of 5.7 months] but only 22% in the 100 mg b.i.d. breast cancer cohort (PFS 3.8 months) (25). Importantly, olaparib responses were observed in *BRCA1/2*-mutant breast cancer patients with either estrogen receptor (ER)–positive or -negative tumors, suggesting the *BRCA*-mutation genotype of the tumors might be a better determinant of PARP inhibitor response than tumor subtype.

CLINICAL TRIALS IN TRIPLE-NEGATIVE BREAST CANCER AND HIGH-GRADE SEROUS OVARIAN CANCER

On the basis of the promising proof-of-concept phase II trials in breast (25) and ovarian cancer (26), two additional phase II studies were initiated. First, Gelmon et al. (27) assessed single-agent olaparib in sporadic advanced ovarian cancer and TNBC. TNBC is defined as breast tumors that do not express the estrogen or progesterone receptors and do not feature the *ERBB2* amplification event. The selection of these two patient subtypes was largely predicated on a subset of sporadically occurring TNBC and also high-grade serous ovarian cancer (HGSOC) sharing a number of molecular, histopathological, and clinical similarities with *BRCA*-mutant familial tumors [a concept termed BRCAness (28)], including a relatively high frequency of somatically occurring *BRCA* mutations and an enhanced response to DNA-damaging chemotherapy. In the Gelmon study, patients were classified according to whether they possessed *BRCA* mutations or not, and they received 400 mg olaparib b.i.d. (27). A number of sustained antitumor responses were observed in the ovarian cancer cohort, even in the absence of *BRCA* mutations; again the olaparib responses correlated with prior platinum sensitivity (27). In the breast cancer cohort, although tumor-reduction effects were noted in the *BRCA*-mutant patients and the frequency of disease stabilization was higher in the *BRCA*-mutant group than in the nonmutant patients (63% versus 13%), no sustained responses were achieved [as defined by RECIST (Response Evaluation Criteria In Solid Tumors) criteria] in either the *BRCA*-mutant patients ($n = 10$) or the nonmutant group ($n = 16$) (27).

In a separate phase II trial, commonly referred to as "study 19," Ledermann et al. (29) assessed the utility of olaparib as maintenance therapy after an initial response to platinum in HGSOC. A retrospective analysis of PFS and overall survival (OS) in this study suggested that patients with germline or somatically occurring *BRCA1* or *BRCA2* mutations exhibited the greatest PFS benefit from olaparib maintenance treatment; the median PFS for patients with either germline or somatically occurring *BRCA* mutations was 11.2 months versus 4.3 months for those who received placebo ($p < 0.0001$, hazard ratio 0.19). Although the survival analysis from this study is yet to be finalized, an interim analysis suggests a trend toward an OS benefit from olaparib treatment in patients with either germline or somatically occurring *BRCA* mutations (34.9 months versus 31.9 months, $p = 0.2$, hazard ratio 0.74) (30).

While the Gelmon (27) and Ledermann (29) studies were under way, a phase II clinical trial in TNBC using iniparib (BiPAR/Sanofi-Aventis) suggested that PARP inhibitors might have potential in this hard-to-treat disease. Iniparib was originally proposed to be a noncompetitive inhibitor of PARP1, mediating its activity by disrupting the interaction between PARP1 and DNA (31). When used in combination with gemcitabine and carboplatin, iniparib achieved a substantially better RR in patients with TNBC compared to gemcitabine and carboplatin without iniparib (32). However, the phase III trial that was based on these promising phase II results failed to deliver the predefined improvements in either PFS or OS, causing significant confusion in the field (33, 34). Many believe that iniparib is not a bona fide PARP inhibitor; this drug has very low PARP inhibition in vitro (35), and its mechanism of action in vivo is unclear (34). The consensus is now that there is little reason to regard the relative failure of iniparib in the phase III trial as a PARP-inhibitor class effect (33, 34).

PHASE III CLINICAL TRIALS

A number of additional PARP inhibitors have shown at least some synthetic lethal activity in *BRCA*-mutant patients. These include BMN 673 (Biomarin) (36), niraparib (Merck/Tesaro) (37), rucaparib (38, 39), and veliparib (Abbvie) (40). In addition to activity in the *BRCA*-mutant cohort, responses to niraparib have also been identified in non-*BRCA*-mutation-linked cancers, including sporadic castration-resistant prostate cancer and non–small cell lung cancer (37).

Although phase II trials in *BRCA* mutation carriers provided supporting evidence that PARP inhibitors could indeed elicit synthetic lethality in the clinic, for some time the clinical development of these agents was delayed (33). In part, this might have been because of the lack of OS benefit in the olaparib maintenance study by Ledermann et al. (29), the absence of sustained responses in the Gelmon study (27), and the confusion caused by the promising phase II but disappointing phase III results with iniparib (32, 33). Nevertheless, the enthusiasm for moving a synthetic lethal strategy toward registration appears to be reignited, and other phase III trials have now been registered (**Table 1**). Most of these trials have returned to the original synthetic lethal principle and either mandate a pathogenic, loss-of-function *BRCA* mutation as an entry criterion or include an assessment of *BRCA* mutation status within the trial analysis. In general, the designs of these trials fall into three categories: (*a*) use of single-agent PARP inhibitor as a maintenance therapy following platinum treatment in ovarian cancer (compared to a placebo-treated cohort); (*b*) use of single-agent PARP inhibitor compared to placebo in the post-chemotherapy adjuvant setting or to standard-of-care chemotherapy in the advanced disease setting; or (*c*) combination therapy trials involving chemotherapy in both advanced disease and neoadjuvant therapy settings.

DRUG RESISTANCE TO A SYNTHETIC LETHAL TREATMENT

While the clinical development of PARP inhibitors progressed, a number of preclinical studies identified candidate mechanisms of drug resistance. Many of the pathogenic *BRCA1* and *BRCA2* mutations are frameshift or nonsense mutations that encode truncated, dysfunctional proteins that lack the C-terminal domains of either BRCA1 or BRCA2. By exposing large populations of *BRCA2*-mutant tumor cells to either olaparib (41) or a platinum salt (42), two independent groups identified the emergence of drug-resistant clones with additional, secondary mutations in *BRCA2* (41, 42). These secondary mutant alleles either restored the wildtype BRCA2 protein coding sequence or encoded a novel form of BRCA2 that, although not identical to the wildtype protein, did encode a critical C-terminal region of BRCA2 not found in the truncated protein. In each case, the secondary mutations restored BRCA2 function, HR capability, and PARP inhibitor

Table 1 Phase III trials (as of April 1, 2014) assessing PARP inhibitors in cancer as a synthetic lethal approach

Clinical Trials.gov[a] reference	Trial name (if available)	PARP inhibitor used	Are *BRCA*-mutant patients assessed as part of trial?	Histology	Trial type
NCT01924533	NOVA	Niraparib	Yes—*BRCA* mutation is an entry requirement	Ovarian	PARP inhibitor used as single-agent maintenance therapy following chemotherapy
NCT01844986	SOLO1	Olaparib	Yes—*BRCA* mutation is an entry requirement	Ovarian	PARP inhibitor used as single-agent maintenance therapy following chemotherapy
NCT01874353	SOLO2	Olaparib	Yes—*BRCA* mutation is an entry requirement	Ovarian	PARP inhibitor used as single-agent maintenance therapy following chemotherapy
NCT02032823	Olympia	Olaparib	Yes—*BRCA* mutation is an entry requirement but high-risk HER2-negative patients without *BRCA* mutation are also included	Breast	PARP inhibitor used as single-agent adjuvant therapy following adjuvant or neoadjuvant chemotherapy
NCT01968213	ARIEL3	Rucaparib	Not in entry criteria but assessed prospectively	Ovarian/gynae	PARP inhibitor used as single-agent maintenance therapy following chemotherapy
NCT01905592	BRAVO	Niraparib	Yes—*BRCA* mutation is an entry requirement	Breast	PARP inhibitor used as single-agent maintenance therapy following chemotherapy
NCT02032277	Brightness	Veliparib	No	Triple-negative breast cancer	PARP inhibitor used in combination with a platinum chemotherapy combination
NCT01924533	Gold	Olaparib	No	Gastric	PARP inhibitor used in combination with paclitaxel
NCT01945775	EMBRACA	BMN 673	Yes—*BRCA* mutation is an entry requirement	Breast	PARP inhibitor used as a single agent and compared to physician's choice of treatment
NCT02000622	OlympiAD	Olaparib	Yes—*BRCA* mutation is an entry requirement	Breast	PARP inhibitor used as a single agent and compared to physician's choice of licensed standard of care

[a]**http://www.clinicaltrials.gov**.

resistance (41, 42). Similar secondary mutation events were subsequently observed in both *BRCA2*- and *BRCA1*-mutant ovarian cancer patients who had become resistant to carboplatin (41–43). In a study by Norquist et al., ovarian cancer patients with secondary *BRCA* mutations were treated with a PARP inhibitor after platinum treatment and were also found to be resistant to the second-line therapy (43). In a recent study (44), two patients, a man with familial *BRCA2*-mutant breast cancer (**Figure 2*b***) and a woman with familial *BRCA2*-mutant breast and ovarian cancer, both showed an

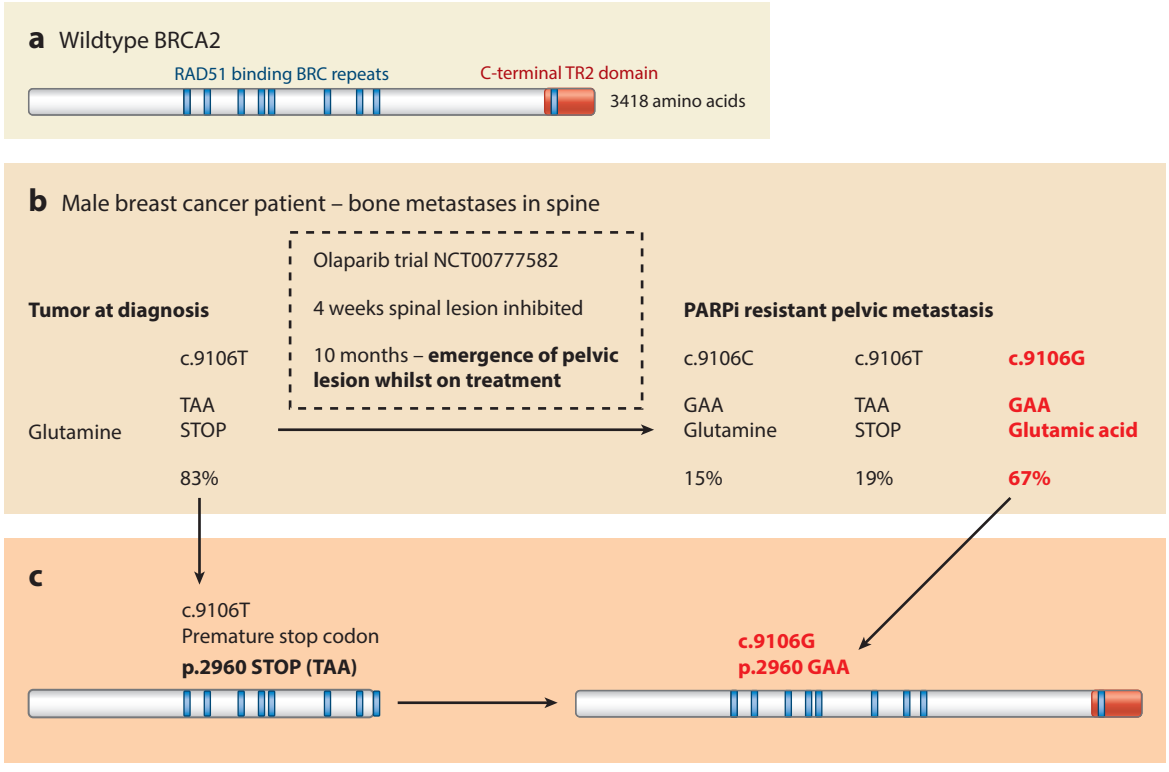

Figure 2

Synthetic lethal resistance to PARP inhibitors caused by secondary mutation in *BRCA2*. (*a*) Schematic of the protein domain structure of wildtype BRCA2. This 3418–amino acid protein contains RAD51 binding domains (shown in *blue*), known as BRC repeats, and a C-terminal region, TR2, essential for homologous recombination. (*b*) Frequency of different tumor *BRCA2* alleles in a male breast cancer patient with PARP inhibitor resistance (44). This patient was originally diagnosed with breast cancer and multiple bone metastases and received surgery, radiotherapy, and chemotherapy. As this patient possessed a germline heterozygous *BRCA2* c.9106C > T mutation, predicted to encode a truncated BRCA2 protein (p.Q2960X), he was subsequently enrolled in a phase I study of olaparib and received 400 mg b.i.d.. Within four weeks on olaparib therapy, the patient's symptoms improved, and a decline in carcinoma antigen levels and a reduction in a metastatic lesion in the thoracic spine were noted. After 10 months on olaparib treatment, tumor regrowth was noted in the right ischium and hemipelvis. Deep sequencing of DNA from a biopsy indicated the presence of a secondary mutant *BRCA2* allele. From the archival diagnostic tissue, the vast majority of DNA reads obtained by deep sequencing encompassed the *BRCA2* c.9106T mutation, with a minority being derived from the *BRCA2* wildtype allele, presumably originating from stromal contamination. In the olaparib-resistant biopsy, the wildtype and *BRCA2* c.9106T alleles were still detected, but an additional *BRCA2* allele was identified with a *BRCA2* c.9106G mutation predicted to encode a glutamic acid residue in place of the premature stop codon encoded by *BRCA2* c.9106T. (*c*) Schematic of the protein domain structures of BRCA2 proteins encoded by the *BRCA2* c.9106T and *BRCA2* c.9106G mutant alleles. The *BRCA2* c.9106T allele (the predominant form in the treatment-naïve biopsy) is predicted to encode a dysfunctional form of BRCA2 that is truncated at amino acid 2960 and lacks the N-terminal TR2 domain. In contrast, the *BRCA2* c.9106G allele (the predominant form in the olaparib-resistant biopsy) encodes a full-length protein including the TR2 domain. This *BRCA2* c.9106G/p.2960 form is predicted to be functional and is likely to explain the olaparib resistance.

initial response to olaparib followed by the emergence of lesions that exhibited profound PARP inhibitor resistance. In each case, the predominant *BRCA2* allele in the treatment-naïve tumor biopsies encoded a truncated BRCA2 protein, whereas in the PARP inhibitor–resistant tumor lesions, *BRCA2* alleles with secondary mutations that restored the open reading frame of the gene were now predominant (44) (**Figure 2**). The gradual emergence of this form of resistance during treatment suggests a possible Darwinian selective pressure effect (45) in which a possibly preexisting but rare secondary mutant clone has a selective advantage once the selective pressure of PARP inhibitor treatment is applied, with the secondary mutant clone eventually dominating the tumor population. It also suggests that efforts to target these secondary mutant clones, or at least strategies to delay their emergence, are warranted. Importantly, secondary *BRCA* mutations that have the potential to mediate PARP inhibitor resistance also emerge in response to platinum chemotherapy, an observation that has implications for the sequential use of these two drug classes; confirming the presence of secondary *BRCA* mutations, either as the predominant allele or as rare alleles within the tumor, prior to either therapy seems sensible. Conceptually, the identification of resistance via secondary *BRCA* mutation confirmed the synthetic lethality between BRCA and PARP and might be regarded as synthetic lethal resistance (**Figure 1*d***), where drug resistance is driven not by an alteration in the drug target, PARP1, but by a change in the synthetic lethal partner, BRCA1 or BRCA2 (1, 46).

In addition to this form of synthetic lethal resistance, a number of alternative mechanisms of PARP inhibitor resistance have been identified in preclinical models. These include pharmacological resistance caused by elevated p-glycoprotein (PgP) expression (47). PgP efflux pumps, also known as multidrug-resistance proteins or MDRs, mediate small-molecule cellular efflux, a defense against the effects of foreign compounds. As a number of small-molecule PARP inhibitors are substrates of PgP, enhanced PgP activity reduces the intracellular concentration of PARP inhibitors and restricts their antitumor activity (47). In addition, the HR defect caused by loss of BRCA1 function can be restored by loss of 53BP1, a process that also drives PARP inhibitor resistance (48); the antagonistic activities of BRCA1 and 53BP1 determine whether HR, driven by BRCA1, is used to repair a DNA DSB, or whether nonhomologous end joining (NHEJ), driven by 53BP1, is utilized (49). In *BRCA1*-null tumor cells, 53BP1 activity inactivates HR such that PARP inhibitor–induced DNA lesions are repaired by NHEJ, a process that is often cytotoxic. If 53BP1 function is abrogated along with BRCA1 loss, the suppression of HR is relieved and PARP inhibitor lesions are effectively repaired without causing cytotoxicity (48, 50). Hence, the result of simultaneous BRCA1 and 53BP1 loss of function is PARP inhibitor resistance (48, 50). Recent reports suggest that this 53BP1-dependent mechanism of resistance might be accompanied in some cases by heat shock protein–mediated stabilization of mutant BRCA1 protein (51). Altered 53BP1 expression has been reported inTNBC, suggesting that this process might operate in clinical disease as well as in experimental models (52).

It is also possible that alterations in PARP1 itself could modulate how tumor cells and normal cells respond to PARP inhibitors. Indeed, in in vitro screens in HR functional cells, loss of PARP1 drives an enhanced level of olaparib resistance (53). It seems likely that the ability of some PARP inhibitors to trap PARP1 on DNA underlies this effect (54). For example, Murai et al. (55) recently demonstrated that a novel PARP inhibitor, BMN 673 (56), is approximately 100-fold more potent than olaparib at trapping PARP1-DNA complexes and that olaparib is, in turn, a more potent PARP1 trapper than veliparib, another clinical PARP inhibitor (54, 55). In some respects, this trapping potency reflects the relative cytotoxic profiles of these agents; in the SUM149 BRCA1-deficient breast tumor cell line model, BMN 673 has an SF_{50} value (concentration required to cause 50% cell inhibition) of 10 pM, compared to 10 nM for olaparib, rucaprib, and niraparib, and 1 μM for veliparib (56). These observations are in part consistent with the original descriptions of PARP1

function; non-PARylated PARP1 tightly binds damaged DNA, but once it is autoPARylated, it is released from DNA (20). The fact that loss of PARP1 drives resistance to PARP inhibitors in HR-competent cells might suggest that noncancerous cells with elevated PARP1 expression might be somewhat more sensitive to PARP inhibitors than are those with reduced PARP1 levels, potentially explaining some deleterious side effects.

ADDITIONAL GENETIC DETERMINANTS OF PARP INHIBITOR SENSITIVITY

One aim of preclinical studies has been to identify genetic determinants of PARP inhibitor sensitivity other than *BRCA1* and *BRCA2*. As early as 2006, additional proteins that control the cellular response to DNA lesions that stall replication forks, such as ATM, ATR, CHEK1, CHEK2, DSS1, RAD51, NBS1 and those whose deficiency causes Fanconi anemia, had been implicated as possible determinants of the tumor cell response to PARP inhibitors (57). More recently, the *PTEN* (phosphatase and tensin homolog) tumor suppressor gene has been suggested to have a role in maintaining genomic stability (58, 59) and PARP inhibitor sensitization (60–62). Although it seems that the PARP inhibitor–PTEN synthetic lethality does not operate in all tumor cell models tested (63), where it does occur, the phenotype is likely explained by a nuclear function of PTEN and potentially a role in controlling the nuclear localization of the DNA recombinase RAD51 in response to DNA damage (59, 62). The ability of a cell to localize RAD51 to the nucleus and to perform HR was recently found to depend on a posttranslational SUMOylation of PTEN at amino acid 254 (59).

Of potential clinical relevance were the observations that tumor cells with *TMPRSS2-ERG* or *EWSR1-FLI1* translocations, found in prostate cancer or Ewing's sarcoma, respectively, exhibited profound PARP inhibitor sensitivity (64–66). The sensitivity of Ewing's sarcoma cell line models with the *EWSR1-FLI1* translocation has led to clinical trials such as NCT01583543, a phase II trial assessing the ORR of olaparib in adult patients with recurrent and/or metastatic Ewing's sarcoma following failure of conventional chemotherapy, which is due for completion in 2015. We and others have also recently proposed novel predictive biomarkers of PARP inhibitor response that could be assessed in the phase II setting. These include loss-of-function mutations in the kinase-encoding gene *CDK12* found in serous ovarian cancer (67–69) and loss of tumor expression of the DNA repair protein ERCC1 in non–small cell lung cancer (70, 71). Assessing these synthetic lethal effects in phase II clinical trials, once the BRCA-PARP synthetic lethality has been confirmed in phase III registration trials, could ultimately expand the utility of PARP inhibitors to patients without *BRCA* mutations. These trials could be histology specific, such as assessing the utility of PARP inhibitors as a maintenance therapy in non–small cell lung cancer following an initial cisplatin response, a similar concept to the Ledermann study 19 described above (29). Alternatively, they could be "basket studies" including a number of cancer types (72), with patients being selected for PARP inhibitor therapy based on a genetic analysis of their tumor.

EXTENDING THE SYNTHETIC LETHAL PRINCIPLE BEYOND PARP INHIBITORS

The promise of genotype-specific cell inhibition offered by synthetic lethality has stimulated a renewed interest in identifying synthetic lethal effects that pertain to other tumor-specific genotypes. For example, *TP53* (p53) mutations represent one of the most recurrent tumor-specific genetic changes in human tumors. A number of candidate synthetic lethal approaches to targeting

p53-mutant tumor cells have been proposed, including targeting the mitogen-activated protein kinase MK2 (73), components of the PI3-kinase signaling cascade (74), the DNA damage checkpoint kinase CHK1 (75), and a key determinant of replication fork stability, ATR (ataxia telangiectasia and RAD3 related) (76–78). Likewise, mutations in the oncogene *KRAS* are relatively common in a variety of malignancies including those of the lung and colon, and oncogenic *KRAS* mutations have been found to be synthetically lethal with inhibition of the ribosomal protein S6 kinase, RPS6KA2 (79), the transcription factor GATA2 (80), or ATR (81).

Many of the experimental routes to identify synthetic lethal effects are now becoming commonplace and include the use of genetic perturbation screens (2), classical cell and molecular biology, and animal models of cancer. From the initial identification of a synthetic lethal interaction, the route through target validation, drug discovery, and development is often tortuous. One challenge is to discriminate those synthetic lethal effects that are resilient to other genetic or epigenetic changes ("hard" synthetic lethalities) from those that are easily abrogated ("soft") (82). Taking into account the extent of both intra- and intertumoral genetic and epigenetic variation, hard synthetic lethal effects may represent somewhat more tractable targets than their soft counterparts. To exemplify the extent of target validation that must often be achieved to confidently identify hard synthetic lethality, Kumar and colleagues recently carried out the genetic profiling of 26 *KRAS*-mutant or *KRAS*-wildtype lung tumor cell lines to identify GATA2 as a *KRAS* synthetic lethal target (80, 83).

Although many of the synthetic lethal effects described above are not close to being implemented in the clinic, the repositioning of already licensed drugs or those in late-stage development to be used in a synthetically lethal approach is a potentially rapid way to implement the synthetic lethal principle. Already there are efforts to systematically profile large panels of well-annotated tumor cell lines as to their drug sensitivity and use this information, in combination with genome and exome DNA sequences of the tumor cell lines, to identify previously unidentified synthetic lethal effects. For example, the recent large-scale efforts conducted at the Wellcome Trust Sanger Center, Massachusetts General Hospital, and the Broad Center (66, 84), as well as efforts focusing on particular cancer types such as breast cancer (85), have the potential to identify a plethora of synthetic lethal effects with either licensed drugs or those in late-stage development. The public availability of these datasets allows a "crowdsourcing" approach in which any number of investigators can contribute to the search for synthetic lethal effects.

LESSONS LEARNED FROM PARP INHIBITOR SYNTHETIC LETHALITY

When considering the future application of the synthetic lethal principle, a number of lessons learned from the preclinical and clinical development of PARP inhibitors might be informative. These are discussed below.

Tools for Identifying and Validating Synthetic Lethal Effects

A diversity of tools made the identification and validation of the BRCA-PARP synthetic lethality possible. These included (*a*) isogenic cell systems, i.e., cells derived from the same progenitor that differed in *BRCA* gene status; (*b*) human tumor cell line models with *BRCA* gene defects; (*c*) gene manipulation reagents and techniques, such as RNA interference, allowing the modulation of both BRCA and PARP activity; and importantly, (*d*) potent and relatively specific small-molecule PARP inhibitors (18). The scale of selectivity for *BRCA*-mutant cells elicited with small-molecule PARP inhibitors was one clear factor driving their development, and the BRCA-PARP

experience argues for continued efforts in small-molecule discovery. Subsequent work exploited mouse models of *BRCA*-mutant cancers to validate the BRCA-PARP synthetic lethality and to also identify mechanisms of drug resistance (reviewed in Reference 86), as did in vitro cell-based analysis and the analysis of clinical biopsy material (reviewed in Reference 46). Although key reagents such as small-molecule inhibitors are not always available, the work described above does reflect the continuing need for a variety of robust experimental approaches that aid the identification and validation of synthetic lethal effects.

Early Clinical Assessment of the Central Biological Hypothesis

One key aspect of the development of PARP inhibitors was that the biological and mechanistic rationale for utilizing them against *BRCA*-mutant cancers, established in preclinical studies, was taken into account in the design of many of the early clinical trials. For example, the inclusion of 19 *BRCA1/2*-mutant patients in the phase I trial assessing olaparib as a single agent (23) and the rapid assessment of olaparib as a single agent in *BRCA*-mutant breast (25) and ovarian cancer (26) in a phase II setting provided early signs of efficacy and established proof of concept that the original preclinical observation of BRCA-PARP synthetic lethality had potential as a clinical approach. If a standard phase I trial had been used, where patients could have been enrolled regardless of the molecular make-up of their disease, it is not certain the use of PARP inhibitors as single agents would have progressed.

Early Assessment of Mechanisms of Drug Resistance

The clinical development of PARP inhibitors has provided a test bed on which to assess mechanisms of drug resistance. In the case of secondary mutations in *BRCA1* and *BRCA2*, establishing this mechanism of resistance while the clinical development of PARP inhibitors was still ongoing not only highlighted why clinical responses to PARP inhibitors in *BRCA*-mutant patients might be mixed but also further supported the hypothesis that the mutational status of *BRCA1* and *BRCA2* is critical in determining the tumor cell response to PARP inhibitors. Again, using biopsy material and clinical response data from early-phase clinical trials alongside preclinical studies as a means to understand drug resistance is not specific to PARP inhibitors; it is likely to be critical for the subsequent development of any synthetic lethal strategy.

CONCLUSIONS

Results from early-phase clinical trials suggest that PARP inhibitors show promise as a synthetic lethal treatment for *BRCA*-mutant cancers. The next challenges include the issue of drug resistance and the issue of defining non-*BRCA*-mutant patient subgroups who might also respond favorably to PARP inhibitors. Applying the synthetic lethal principle to identifying additional approaches to treating the disease also has clear potential, and it is expected that a number of the synthetic lethal effects identified in preclinical studies will in time be able to be assessed in the clinic.

DISCLOSURE STATEMENT

A.A. and C.J.L. are named coinventors on a number of patents held by AstraZeneca relating to the use of PARP inhibitors. They may benefit from this financially through the Institute of Cancer Research's "Rewards to Inventors" scheme. A.N.J.T. has received "Rewards to Inventors" payments related to patents on PARP inhibitors in *BRCA 1/2*–associated malignancy.

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