# Chapter 9
# Preclinical Chemosensitization by PARP Inhibitors

**David R. Shalinsky, Cherrie K. Donawho, Gerrit Los and Joann P. Palma**

**Abstract** Preclinical research has provided a strong rationale for employing PARP inhibitors (PARPi) as chemosensitisers in combination with cytotoxic agents, radiosensitisers in combination with radiation, as well as monotherapy to induce synthetic lethality in human malignancies. The primary aim of this chapter is to describe the rationale and preclinical pharmacology of potent PARPi (such as veliparib, rucaparib and olaparib) as chemosensitisers studied in cytotoxic combination regimens. Additional aims are to review: (1) emerging strategies for employing PARPi as monotherapy to induce synthetic lethality; and (2) potentially novel mechanisms by which PARPi may enhance antitumour therapy.

Pharmacodynamic (PD) biomarkers have been integral to the successful early development of PARPi, allowing the development team to demonstrate -on-mechanism (target inhibition), guide Phase 2 dose selection (PK/PD relationship), and generate preliminary antitumour activity data to help guide "Go, No Go" decisions for promoting compounds into advanced development. The measurement of PARP activity in blood by a fit-for-purpose analytically-validated assay is the PD biomarker that has been initially developed and tested the most in Phase 1/2 clinical trials.

The ultimate clinical need is to identify patient-selection markers to enrich for patients who will respond to therapy, e.g., the overarching goal of personalized medicine. The lack of patient-selection biomarkers is a major deficit that has contributed to the lack of clinical registration of PARPi in cytotoxic combination regimens. Nevertheless, PARPi combination therapy offers an attractive prospect to potentially broaden clinical benefit, but predicting the best combination for achieving

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N. J. Curtin, R. A. Sharma (eds.), *PARP Inhibitors for Cancer Therapy,*
Cancer Drug Discovery and Development 83, DOI 10.1007/978-3-319-14151-0_9

clinical benefit remains a challenge. As monotherapy, preliminary clinical results with the PARPi, olaparib, suggest that BRCA1/BRCA2-deficiency may indeed serve as a useful patient-enrichment tool to select the right patients who will respond to PARPi therapy. Additional synthetic lethal strategies are also being explored with PARPi. Development of a clinically-validated patient-selection marker (companion diagnostic) will depend on the results of Phase 3 clinical oncology trials.

In conclusion, this chapter reviews the rationale and experimental data generated for PARPi studies in preclinical models with a primary focus on chemosensitisation. These results provide a strong rationale for employing PARP inhibitors in antitumour therapies.

**Keywords** PARP inhibitor · Chemosensitizer · Radiosensitizer · PAR · pADPr · PARP-trapping · CO-338 · AG014699 · Rucaparib · ABT-888 · Veliparib · AZD-2281 · Olaparib · MK-4827 · Niraparib · BMN 673

## 9.1 Introduction

### *9.1.1 Rationale for Employing PARP Inhibitors as Chemosensitisers in Cancer Therapy*

Cancer therapies employ chemotherapy and radiotherapy to induce DNA-damage and inhibit the growth of human malignancies. The rationale for employing poly(ADP-ribose) polymerase (PARP) inhibitors (PARPi) in chemotherapy and radiotherapy is to prevent PARP-mediated DNA repair [1]. This chapter will focus on the preclinical pharmacology of PARPi as chemosensitisers in combination regimens with DNA-damaging agents (e.g., alkylators, platinum agents) with the exception of topoisomerase inhibitors (discussed in a separate chapter)[1]. We will also summarize the pharmacology of PARPi as reported in the literature when tested side-by-side with DNA- damaging agents, the use of PARPi combined with radiotherapy, which has been comprehensively reviewed elsewhere in this book volume[2], and the rationale for employing PARPi in synthetic lethal strategies as an important route for gaining regulatory approval to use PARPi in clinical oncology. Synthetic lethal strategies have been reviewed in depth elsewhere in this book volume[3].

[1] Combination regimens with topoisomerase inhibitors have been reviewed in Chap. 10 (Murai and Pommier)

[2] Combination regimens with radiotherapy have been reviewed in Chap. 11 (Fouillade)

[3] Synthetic lethal concepts have been reviewed in Chaps. 13–17 (Bryant and Shall; Leszczynska; Okita and Shibata; Stanley and Yang, Cornell, Johnson and Shapiro; respectively)

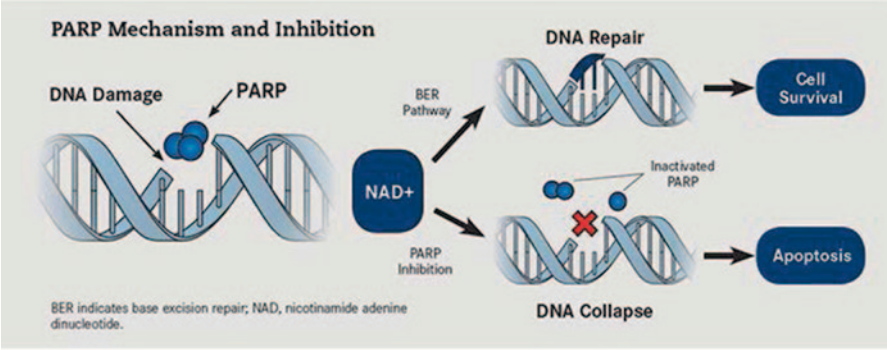


**Fig. 9.1** PARP mechanism of DNA repair. (Reprinted with permission [18])

### *9.1.2 History and Mechanistic Basis for the Development of PARP Inhibitors as Chemosensitisers in Cancer Therapy*

Pioneering basic and molecular research from the 1960's onwards identified PARP-1 as a key enzyme activated by DNA strand breaks [2–4] leading to the central hypothesis that PARP-1 is involved in DNA repair providing a rationale for using PARPi to enhance the cytotoxicity of alkylating agents in cancer therapy [5]. The initial synthesis of low potency and broad specificity PARPi, such as 3-aminobenzimide (3-AB) enabled mechanistic studies [6]. Substantial chemistry and biological studies ensued [5, 7–10, 6] and led to the design and development of many agents to inhibit the PARP family of enzymes [11–15]. PARP-1, a key PARP in this multifunctional family of enzymes, is thought to play a key role in base excision repair (BER) [16], by facilitating repair of single-strand DNA breaks (SSBs) (Fig. 9.1) [17, 18]. PARP also binds to more lethal radiation- or chemotherapy-induced double-strand breaks (DSBs) apparently protecting the lesion and signaling repair, ibid [19, 1]. Comprehensive reviews of the modes of action of PARP on DNA repair and detailed mechanisms and structure-activity-relationships (SAR) by which PARPi modulate repair have been reported elsewhere [20–22, 6, 23]. Subsequent studies on PARP knock-out mice exhibiting radiosensitisation further supported the role of PARP in facilitating DNA repair [24].

Various combination therapies with PARPi have been and are being explored: EGFR [25, 26], cyclophosphamide [27, 28], bortezomib [29], 5-FU [30, 31], irinotecan [32, 33] and recently, targeted therapies such as phosphoinositide 3-kinase (PI3K) inhibitors [34] owing to its role in sensing DNA strand breaks [35–37].

Whilst the mechanism by which PARP(s) engage DNA and its repair is much more complex than may have been originally appreciated [19] there is a substantial body of research supporting the hypothesis that catalytic PARP inhibition would abrogate repair to serve as sensitizers of chemotherapy [38, 11, 39] and radiotherapy [38, 40]. In addition, the very nature of PARP-1 binding to damaged DNA [20] and more recent preclinical data indicate that PARP inhibitors can exert or modulate cytotoxicity by forming PARP-DNA complexes (PARP-trapping) to inhibit DNA repair [41, 42].

### 9.1.3 Synthetic Lethality: BRCA1/BRCA2

In the early 2000s, a therapeutic paradigm known as *synthetic lethality* [43, 44] arose with PARPi and *BRCA*-deficiency serving as the poster child for this paradigm [18]. Synthetic lethality is the concept that two defects, which alone are benign, can be lethal when combined (Fig. 9.2). If SSBs are left unrepaired, they have the potential to develop into lethal DSBs, which would lead to cell death. Suppressing PARP activity prevents SSB repair via the BER pathway, but other DNA repair pathways such as homologous recombination (HRR) and non-homologous end joining (NHEJ) may take over to repair the DSBs. Therefore, if PARP inhibitors are used against tumours in which there is already a DNA repair defect, e.g. the inability to repair DSBs, the combination of endogenous defect and pharmacologic inhibition by PARPi drives synthetic lethality.

### 9.1.4 Emerging Synthetic Lethal Concepts

*A) HRR-Deficient Tumours* Once it was established that *BRCA1* and *BRCA2* are central to the repair of DSBs by HRR, it was determined that deficiency of several genes or related proteins (such as RAD51, ATR/ATM, CHK1/2) involved in HRR were associated with hypersensitivity to PARPi [45]. These results have stimulated a therapeutic strategy to potentially employ PARPi against a wide range of tumours with HRR deficiency caused by mechanisms distinct from *BRCA*-deficiency [45].

Research has identified additional endogenous genes or proteins (e.g., *CDK1, PTEN, PI3K*) that may combine with PARPi to induce synthetic lethality. For example, combining a PI3K inhibitor and the PARPi, AZD-2281, enhances antitumour activity in spontaneous *BRCA1*-deficient mammary tumours arising in MMTV-Cre*Brca1f/fTrp53*$^{+/-}$ mice [46]. Further, AZD-2281 acts in a synthetic lethal manner in cancer cells with the mutant tumour suppressor gene, phosphatase and tensin homolog (*PTEN*). PTEN deficiency causes a HRR defect that can be exploited preclinically in combination with PARP inhibition [47].

*B) BRCA- or HRR-Proficient Tumours* PI3K inhibition can also impair *BRCA1/BRCA2* expression and sensitize *BRCA*-proficient triple negative breast cancer cells to PARPi [36]. The strategy of inducing synthetic lethality in *BRCA*-proficient tumours is further supported by the observation that depletion or inhibition of CDK1 compromises the ability of *BRCA*-wildtype cancer cells to repair DNA damage by HRR [48]. It has also been reported that HER2 overexpression sensitises tumour cells through inhibition of the NF-kB signaling driven by HER2 independently of HRR-defects [49]. These results suggest that PARPi may have clinical utility for improving cancer therapy in genetic backgrounds including but not limited to *BRCA* mutations and that novel drug combinations may have utility in HRR-proficient malignancies, or even independently of HRR, rendering tumours sensitive to broader combination therapies [48, 36, 46, 37].

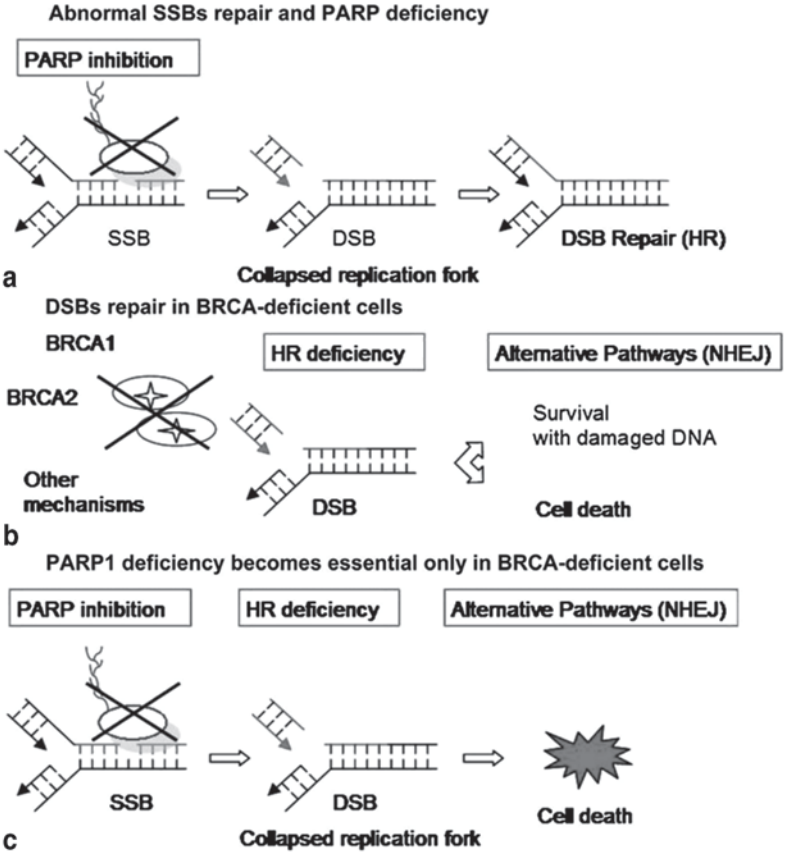


**Fig. 9.2** Abnormal single-strand breaks (*SSBs*) repair and poly(adenosine diphosphate [ADP]-ribose) polymerase (*PARP*) deficiency. **a** Abnormal single-strand breaks (*SSBs*) repair and poly(adenosine diphosphate [ADP]-ribose) polymerase (*PARP*) deficiency. PARP inhibition causes an increase in persistent SSBs, which, when encountered by replication fork, cause fork collapse and the formation of double-strand breaks (*DSBs*) that are repaired by homologous recombination repair (*HRR*). (HR in the published figure = HRR). **b** DSBs repair in BRCA-deficient cells. Cells deficient in proteins of the HRR pathway (such as BRCA1 and 2) are unable to repair the DSBs that accumulate. **c** PARP-1 deficiency becomes essential only in BRCA-deficient cells. In the case of excessive SSBs, resultant collapsed replication forks and accumulation of DSBs and HRR-deficient cells exposed to PARP inhibitors are unable to maintain the integrity of their genome and become non-viable. (Reprinted with permission [43])

### *9.1.5 Complexity Underlying PARPi Mechanisms of Action*

In as much as PARP and phospho(ADP-ribose) molecules (pADPr/PAR) are ubiquitously expressed in cells, and depend upon cellular $NAD^+$ (and ATP) levels to function, DNA repair may be inhibited by NAMPT inhibition [50] or by extensive cellular use of $NAD^+$ consumed when cells act to repair DNA damage induced

by chemotherapy. This can lead to apoptosis or cell death through disregulation of AMP:mTORC1 signaling [3] or may abrogate other DNA fidelity mechanisms that exist in cells unrelated to HRR-DNA repair [2]. Further, other PARPs can also impact DNA repair (e.g., PARP-3 in NHEJ [6]). The interplay of PARP family members on repair processes merits deeper investigation. Thus, the mechanisms by which PARP and its inhibition can be modulated to improve cancer therapy are complex, multifactorial and challenging to understand and exploit *(ibid*).

### *9.1.6 Promise of Translating Preclinical PARPi Pharmacology into Clinical Benefit*

Despite a plethora of promising PARPi preclinical data (single agent and in combination), many agents having entered into clinical oncology trials (reviewed by Plummer and Drew, Chap. 20, Irshad, Chap. 21 and Clift, Coupe and Middleton Chap. 22 in this book volume), none had been approved prior to 2014, which had been quite disappointing[4,] [51, 18]. However, renewed efforts to exploit synthetic lethality have arisen due to seminal preclinical research [43, 44] that promoted clinical testing of PARPi in *BRCA*-deficient patients expected to benefit most from this approach [52–54]. Promising clinical findings were highlighted at the 2013 ASCO meeting [18] and the recent US[5] and EU[6] approvals of olaparib in BRCA-deficient ovarian cancer have provided evidence to clinically-validate this approach. Thus there are intense, renewed efforts underway and hope that exploitation of synthetic lethality will lead the way to the broad registration of PARPi for use in cancer therapy.

### *9.1.7 Commonly-Studied PARP Inhibitors*

Table 9.1 lists preclinical properties of common PARP inhibitors. The primary focus is on preclinical research related to ABT-888 (veliparib) [27, 13], CO-338 (rucaparib) [55][7] and AZD-2281 (olaparib) [14][8], agents which have been studied most extensively and reported in the literature. Other PARPi under development (e.g., BMN-673 [19], MK-427 [56]) have also been described or cited as appropriate.

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[4] Ruth Plummer, personal communication

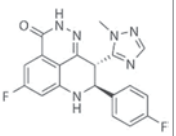
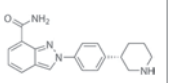
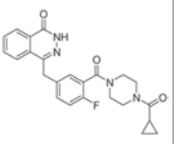
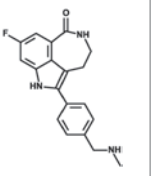
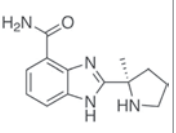
[5] FDA approval of olaparib 19 Dec 2014

[6] EU approval of olaparib 09 Jan 2015

[7] Formerly known as AG14699, AG-14699 or AG014699 as named by Agouron Pharmaceuticals, Inc. AG014699 was renamed PF-01367338 after Pfizer Inc. purchased Agouron (2001). PF-01367338 was renamed CO-338 after Clovis Oncology outlicensed AG014699 from Pfizer Inc. (2011). For simplicity, PARPi inhibitors will generally be referred to by the compound numbers numbers or generic names as designated by their respective organizations.

[8] Formerly known as KU59436, KU 59436 or KU0059436 as named by KuDos Pharmaceuticals Limited. KU59436 was renamed AZD-2281 after Astrazeneca purchased KuDos Pharmaceuticals Limited (2006).

**Table 9.1** Basic pharmacokinetic and pharmacology attributes of commonly-studied PARP inhibitors

| Agent | Molecular weight (g/mol) | Structure | Oral BA[a] | Terminal $t_{½}$ (h)[b] | $K_i$ and/or $IC_{50}$ for PARP-1 (nM)[c] | $K_i$ and/or $IC_{50}$ for PARP-2 (nM)[c] | Reference(s) |
|---|---|---|---|---|---|---|---|
| BMN 673 | 380.35 |  | Yes | NP[f] | $K_i$: 0.57 to 1.2<br>$IC_{50}$: 2.5 | $K_i$: 0.85<br>$IC_{50}$: NP | [19] |
| Niraparib (MK-4827) | 320.39 |  | Yes | – | $K_i$: unclear<br>$IC_{50}$: 3.8 vs. PARP-1; 4.0 in cell-based assay | $K_i$: unclear<br>$IC_{50}$: 2.1 vs. PARP-2 | [56] |
| Olaparib (AZD-2281)[d] | 435.08 |  | Yes | 0.9 | $K_i$: 5<br>$IC_{50}$: 53.8 | $K_i$: 1<br>$IC_{50}$: 12.1 | [14] |
| Rucaparib (CO-338)[e] | 421.36 |  | Yes | NP[f] | $K_i$: 1.4<br>$IC_{50}$: NP | $K_i$ = 0.2<br>$IC_{50}$: NP | [55] |
| Veliparib (ABT-888) | 244.29 |  | Yes (56–92%) | 1.2–2.7 | $K_i$: 5.2 nM<br>$IC_{50}$: 5.5 nM | $K_i$: 4.7 nM<br>$IC_{50}$: 4.9 nM | [13, 27] |

[a]Bioavailability
[b]In preclinical models
[c]$K_i$ values reported (by respective company) in enzyme assays and $IC5_{50}$ values in cellular functional assays have not been produced under standard conditions, enzyme constructs/cell lines, or analytical methods. Results should be interpreted with caution
[d]formerly known as KU59436
[e]formerly known as AG014699 and PF-01367338
[f]Not published

## 9.2 Chemosensitisation by PARPi in Combination Chemotherapy Regimens

### *9.2.1 ABT-888 (Veliparib) Studies*

#### 9.2.1.1 Preclinical Studies

As reviewed above, PARP senses DNA SSB through the BER pathway and promotes HRR DSB repair. Veliparib is a potent PARP 1/2 inhibitor with excellent oral (PO) bioavailability that readily crosses the blood-brain barrier. It has been shown to enhance the activity of multiple DNA damaging agents and is currently being investigated in a number of Phase 3 clinical studies in multiple indications (http://clinicaltrials.gov/). Early *in vitro* work relying on short term proliferation assays were found not to be predictive of *in vivo* preclinical efficacy. Achievable pharmacokinetics (PK) determined by *in vivo* metabolism of the drug, tissue residency time, target expression as well as target engagement (on and off rates) may account for some differences in tissue culture and a complete biological system in an *in vivo* setting.

Subsequently, preclinical work with veliparib has been extensively investigated and reported in preclinical tumour models [27, 57, 58, 12, 59, 60, 1, 61, 54]. Veliparib is orally (PO) bioavailable and has favourable pharmacokinetics for *in vivo* testing (Table 9.1). Veliparib significantly enhanced the antitumour activity of DNA damaging agents in a variety of preclinical tumour models, including melanoma, breast, prostate, colon, and glioma. Significant inhibition of PARP activity (tumour pADPr/PAR levels) at doses similar similar to those associated with antitumour effects was also observed. This observation provided an opportunity to use PARP activity as a potential biomarker to assess veliparib exposure and activity, e.g., to develop a pharmacokinetic/pharmacodynamic (PK/PD) relationship for inhibition of PARP activity and tumour growth, in preclinical tumour models.

#### 9.2.1.2 PK/PD Studies of Veliparib in Tumour Models

To develop a PK/PD biomarker, different dosing regimens were used with veliparib in combination with TMZ in mice bearing B16F10 tumours (Fig. 9.3). Comparable

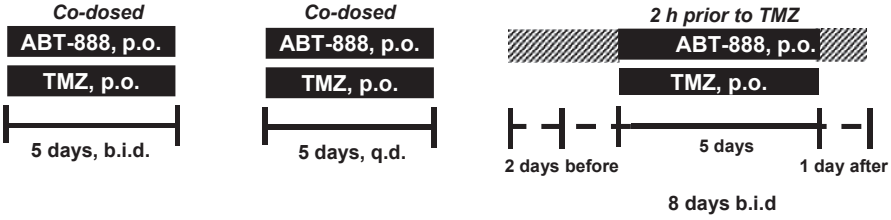



**Fig. 9.3** Schematic diagram of dosing schedules used in veliparib/TMZ studies. (Reprinted with permission [58])

veliparib exposures were demonstrated after PO, IV or administration by osmotic minipump, enabling broad studies of veliparib in combination regimens in xenograft, orthotopic and syngeneic tumour models as well as high dose monotherapy, single agent activity [27, 61– 63] (Figs. 9.4, 9.5 and 9.6; Table 9.2).

Determining the optimal efficacious dose of PARPi in models is a challenge because these agents are not cytotoxic and often lack single agent activity (at low doses) in preclinical models. Preclinically, one must determine whether drug actually reaches the tumour after dosing and whether exposure is associated with on-target activity or inhibition of PARP activity. Thus, a key preclinical objective was to identify PK attributes associated with a pharmacodynamic (PD) biomarker of PARP inhibition (e.g., PK/PD relationship for veliparib dose responsive inhibition of PARP activity and tumour growth) to guide the setting of an appropriate dose for veliparib in early clinical trials (Fig. 9.4, dose responsive data not shown [58]). This approach was taken initially by the research team at Abbott Laboratories with the goal to inform the optimum biological dose for use in clinical trials.[9]

Veliparib did not inhibit tumour growth in the B16F10 syngeneic model as monotherapy (low dose) but markedly potentiated the activity of temozolomide (TMZ) [58]. TMZ has been widely studied based on the potentiating or enhanced activities reported for the combination of TMZ and other PARPi in preclinical models representing those with poor treatment responses, e.g., melanomas and gliomas [5]. The *in vivo* efficacy of veliparib and TMZ was also investigated in the syngeneic orthotopic 9L rat glioma model, an aggressive model with similarities to the clinical disease in which all control rats succumb to the disease within 3 weeks of tumour implantation [27]. Demonstrable concentrations of veliparib were measured in orthotopic tumours after PO and osmotic minipump dosing, demonstrating that veliparib crosses the blood-brain barrier. Veliparib dose-dependently potentiated the efficacy of TMZ, reflected by decreased tumour volume and prolonged survival, whereas TMZ alone produced minimal efficacy (Fig. 9.5) [27]. These studies suggest a broad potential for using veliparib as a chemopotentiator with TMZ that was further confirmed by subsequent studies [61]. The mechanism of potentiation of TMZ cytotoxicity by veliparib was explored *in* vitro [57]. It was shown that cells treated with TMZ need to be exposed to veliparib for at least 17–24 h to achieve maximal cytotoxicity and that cytotoxicity correlated with γH2AX levels, indicative of the level of DSBs. In synchronized cells, DNA damage induced by TMZ in the presence of veliparib during the S phase generated high levels of DNA DSBs, presumably due to conversion of DNA SSBs resulting from the cleavage of the methylated nucleotides to DSBs through DNA replication, leading to higher levels of cytotoxicity.

Veliparib has shown significant ability to potentiate multiple DNA damaging agents in a spectrum of tumours from multiple histological types [62]. In studies using cytotoxic combinations with veliparib (cisplatin, cyclophosphamide, and TMZ), a marked increase in efficacy over cytotoxic monotherapies was observed, especially in the MX-1 breast cancer model. It was shown by genotyping that the MX-1

[9] Abbott Laboratories divested its pharmaceutical division forming AbbVie Inc. on January 1, 2012. ABT-888 is now part of the AbbVie oncology drug portfolio.

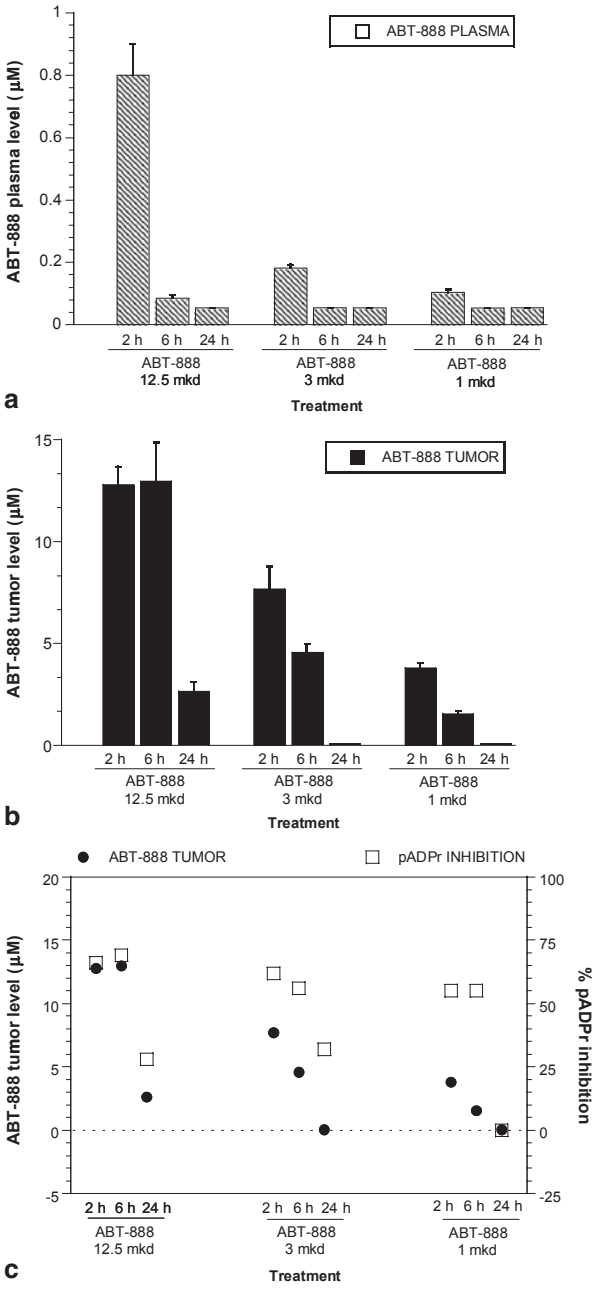



**Fig. 9.4** Drug level and correlation of the reduction in pADPr polymer by veliparib *in vivo*. The level of veliparib [12.5, 3 and 1 mg/kg/d (mkd)] in **a** plasma and **b** tumour were analyzed after a single dose at 2, 6, and 24 h. **c** Corresponding pADPr levels were also analyzed by ELISA. Data represent 3–5 mice per treatment group; *bars*, SE. (Reprinted with permission [58])

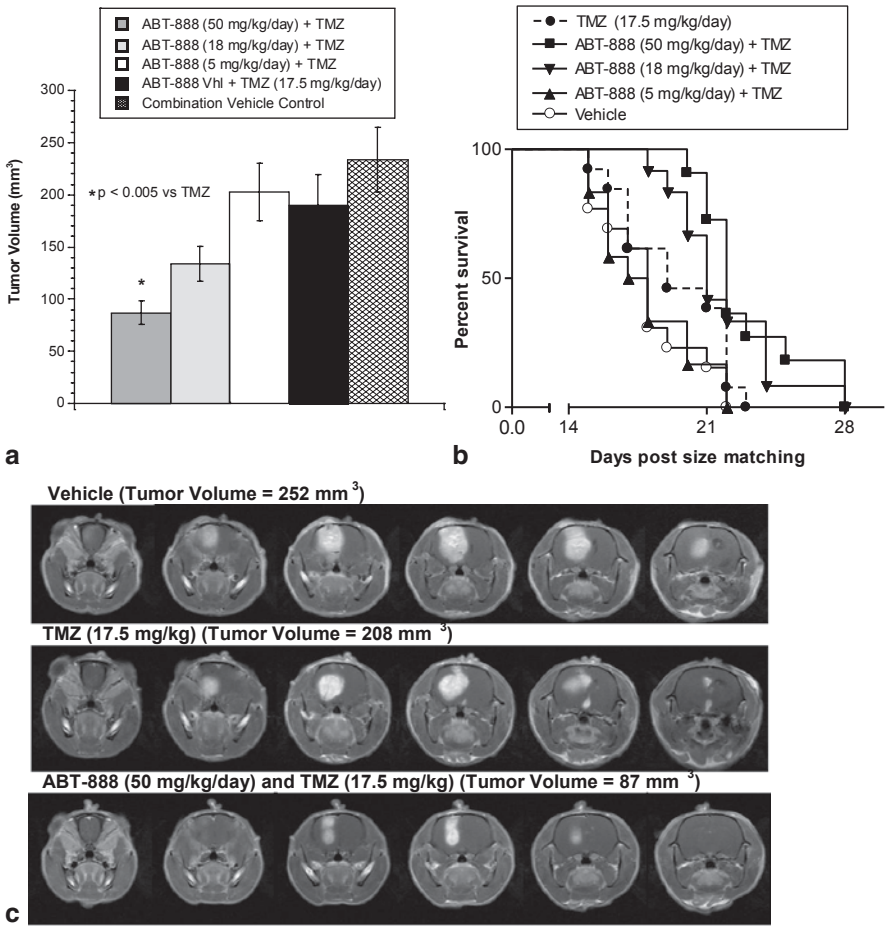



**Fig. 9.5** *In vivo* efficacy of veliparib in combination with temozolomide in a syngeneic orthotopic 9L rat glioma model. **a** glioma tumour volumes at day 14 using contrast-enhanced magnetic resonance imaging. Treatment of veliparib began on day 3 following tumour cell inoculation and continued for 13 d. TMZ was administered from day 4 to 8. Columns, mean of 11–12 rats per treatment group; *bars*, SE. veliparib as a single agent at 50 mg/kg/d was not efficacious in this model (data not shown). **b** Kaplan-Meier analysis of orthotopic 9L rat glioma model (same experimental set of animals in A). Survival end point was based when animals showed signs of irreversible illness (e.g., impaired movement and greater than 20% weight loss). Median survival times for the vehicle, TMZ, and 5, 18, and 50 veliparib mg/kg/d TMZ combination groups were 18, 19, 17.5, 21, and 22 d, respectively. **c** representative contrast-enhanced magnetic resonance images of orthotopic glioma (bright intensity areas) with the tumour volumes representative of the average for the treatment groups of vehicle, TMZ alone, and veliparib and TMZ. Row, transverse slices from a single rat taken on day 14. (Reprinted with permission [27])

breast cancer cell line was HRR-defective (*BRCA1*-deleted and *BRCA2*-mutated) and very sensitive to PARP inhibition. Studies with both the MX-1 breast line and the Capan-1 (*BRCA2* deficient) pancreatic cancer cell line showed that veliparib (25 mg/kg/day, PO, BID x5) demonstrated enhancement of TMZ (50 mg/kg/day, PO, QD x5) activity, with regressions compared to TMZ alone (81–97% TGI, TGI, tumour growth inhibition). Continuous dosing of veliparib at 4x-8x higher doses (100 and 200 mg/kg/day PO, BID x21) demonstrated a significant single agent, dose-responsive activity (27–74% TGI). Further, the combination treatment of veliparib (100 and 200 mg/kg/day, PO, BID x21) with carboplatin, carbotaxol, radiation, gemcar, cyclophosphamide, topotecan and TMZ also demonstrated a measurable advantage over either veliparib or cytotoxic agent alone (Fig. 9.6 and Table 9.2). Both high dose single agent veliparib and the cytotoxic combination therapy were well-tolerated and there were no observable safety concerns in animals. This indicated the feasibility of using the high dose, single agent monotherapy and cytotoxic combination regimens in the clinic. Furthermore, analysis of tumours for the reduction of pADPr after veliparib treatment demonstrated a correlative and significant reduction by western blot indicating the ability of veliparib to inhibit PARP activity *in* vivo (Fig. 9.4c). Altogether, these studies show enhanced activity and tolerability of veliparib in combination with various cytotoxic agents (Table 9.2).

Chemosensitisation was observed similar to that published for other PARPi across experimental tumour models such as melanoma, glioma, colorectal, breast and hematopoietic malignancies [11, 64, 54]. The most suitable combination therapy may likely be determined by factors such as, therapeutic index (potential overlapping clinical toxicity profiles), combination PK (influence of one agent combined with another), DNA repair mechanism and underlying resistance factors, level of DNA damage, dosing regimen, biomarkers etc. While PARPi combination therapy offers an attractive prospect to potentially broaden clinical benefit, to date, predicting the best therapeutic combination beyond methylating agents and radiotherapy remains a challenge.

#### 9.2.1.3 Biomarker Studies

As mentioned, a strong PD signal demonstrating inhibition of PARP activity is needed to guide early clinical development. To enable this, a facile ELISA was developed to detect the formation of pADPr (or PAR) in cells [60, 65, 58]. This assay uses clinically accessible material, is biologically relevant to the mechanism of action, and reflects activity in preclinical tumours that correlates with PK parameters (PK/PD relationship). As indicated prior, veliparib did not affect the growth of B16F10 tumours; TMZ was moderately active as monotherapy [58]. In combination, TMZ co-administered with veliparib demonstrated robust efficacy in the various dosing regimens tested in the B16F10 model consistent with the hypothesized mechanism of PARP inhibition of DNA damage induced by TMZ (*ibid.*), and therefore was a relevant model to evaluate PK/PD relationships. Tumour and blood concentrations of veliparib were evaluated in mice after dosing and PARP activity

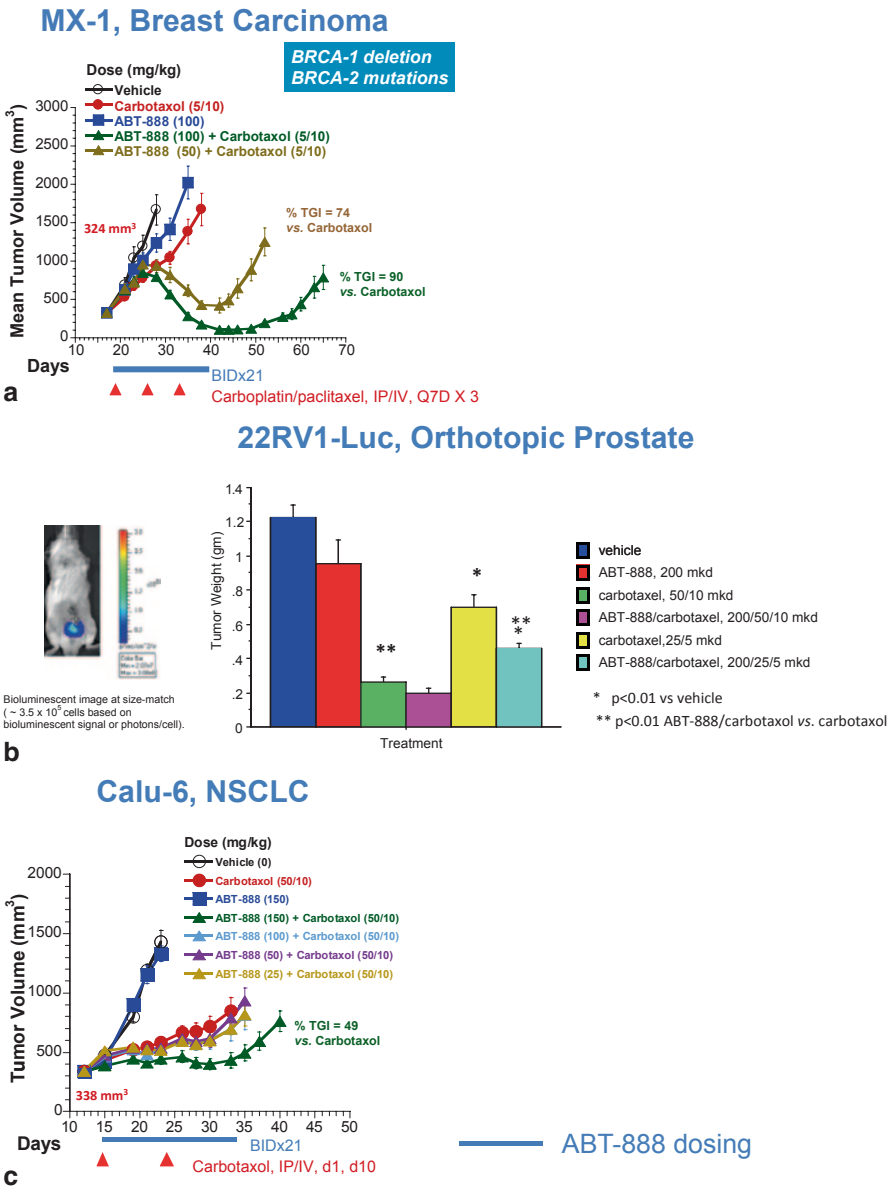



**Fig. 9.6** Veliparib combination efficacy with carbotaxol in preclinical models [62]. Tumour growth inhibition following treatment with veliparib, 200 mkd, carbotaxol 50/10 mkd or 25/5 mkd, veliparib/carbotaxol 200/50/10 mkd or 200/25/5 mkd. Treatment started 4 days post inoculation of tumour cells. The dose, route and regimen are specified in the legend. Each group represents the mean of ten tumours, except where noted. Error *bars* depict the standard error of the mean. The 22Rv1-Luc cell line was a generous gift from Dr. Michael Henry (Univ. of Iowa) [63]

**Table 9.2** AbbVie data summarizing veliparib preclinical studies, monotherapy and potentiation of chemotherapies as well as radiation [27, 61, 62]

| Combination Therapies with ABT-888[a] | % Tumor growth inhibition<br>+≥50%<br>+/− 30–49%<br>−≤29% |
|---|---|
| Cyclophosphamide | + |
| Carbotaxol | +/− |
| Gemcitabine/carboplatin | +/− |
| Radiation | + |
| Topotecan | +/− |
| Temozolomide | + |
| *Combination therapy* | *Xenograft model* |
| Gemcitabine/carbotaxol<br>Cyclophosphamide<br>Carbotaxol | MX-1[b]<br>*BRCA-1* del, *BRCA-2* mut |
| Topotecan<br>Temozolomide | Capan-1[b]<br>*BRCA-2* del, 6174delT |
| Carbotaxol | Calu-6 |
| Carbotaxol | 22Rv1 |

[a]Combination therapies with veliparib demonstrated significant efficacy compared to monotherapy cytotoxic agent in multiple xenograft models. Potent combination therapy efficacy was observed particularly in the *BRCA*-deficient models

[b]*BRCA*-deficient models that demonstrated significant monotherapy efficacy

was concomitantly measured. Veliparib plasma concentrations increased in a dose-dependent manner (1–12.5 mg/kg) after PO dosing with a maximum concentration ($C_{max}$) observed after 2 h (Fig. 9.4). High tumour:plasma ratios of veliparib were observed with more rapid clearance from plasma than tumour. Further, pADPr levels decreased in a dose- and time-dependent manner after veliparib dosing whereas TMZ did not produce a consistent trend in pADPr levels [58]. Based on these preclinical experiments, the authors postulated that marked decreases (≥70%) in pADPr may be required for tumour growth inhibition. Different combination therapies may require different thresholds for pADPr inhibition (or increased DNA damage due to inactivation of PARP) for efficacy, *i.e.*, lower doses for PARPi for cytotoxic combination therapy compared to higher doses of PARPi for monotherapy.

In summary, these studies demonstrated a strong PK/PD relationship for veliparib associated with tumour growth inhibition. Further, these studies facilitated development of an ELISA biomarker assay for PARP activity for use as a PK/PD marker of veliparib in clinical trials [60, 65, 58]. A phase 0 study was conducted to assess pharmacokinetics and PARP activity as a pharmacodynamic marker in tumour and peripheral blood mononuclear cells (PBMCs) in blood. The study demonstrated good oral bioavailability of veliparib and tolerability in patients. In addition, significant inhibition of PARP activity (reduction in pADPr) was observed in tumour biopsies and PBMCs at the 25 mg and 50 mg dose levels. These results showed the

feasibility of assessing a relevant PD marker in surrogate tissue (PBMCs) to generate mechanistic proof-of-concept data in Phase 0 as an early guide to dose selection in Phase 1 clinical trials. These results supported investigation of veliparib as monotherapy and in cytotoxic combination regimens in the clinic. Currently these are being assessed in the further clinical development of veliparib.

### *9.2.2 CO-338 (Rucaparib) Studies*

Rucaparib was identified in a collaboration between the Northern Institute of Cancer Research ((NICR), University of Newcastle upon Tyne, UK), Cancer Research UK (CRUK) and Agouron Pharmaceuticals, Inc. (La Jolla, CA USA) using x-ray crystallography structure-based design [66, 23]. The collaboration led to the development of tricyclic indoles and benzimidazoles with very potent inhibition of PARP-1 as determined by SAR studies [21]. The selection process yielding AG-1447 and its phosphate salt pro-drug, AG014699 (rucaparib) as a clinical candidate has been published [67]. However, a fuller understanding of the pharmacology of rucaparib is attained by reviewing the preclinical research published for a key precursor, AG14361, AG1477 and its phosphate salt pro-drug, AG014699 (rucaparib).

### *9.2.3 AG14361*[10]

AG14361 was a key precursor to the development of rucaparib (AG014699/CO-338). Lead optimization studies identified AG14361 as a promising tool (with >1000-fold potency for PARP-1 vs. benzamides) to probe the pharmacology of PARP inhibition in preclinical cancer models [66]. The $K_i$ of AG14361 was <5 nM in purified full-length human PARP-1; The $IC_{50}$ values were 29 nM in permeabilized SW620 cells and 14 nM in intact cells, respectively (*ibid*). The metabolic stability, pharmacokinetics and distribution of AG14361 in mice and inhibition of PARP activity in subcutaneous tumours *in vivo* were favourable (Fig. 9.7), enabling advancement from *in vitro* to *in vivo* studies that are required to support early drug development.

Dose tolerance studies in mice were then investigated (data not shown) prior to studying the effects of AG14361 and TMZ on xenograft tumour growth (Fig. 9.8) (*ibid.*). At 5 and 15 mg/kg, AG14361 did not affect tumour growth. In contrast, TMZ as monotherapy modestly delayed the growth of LoVo xenografts (Fig. 9.8 panel A) and 68 and 136 mg/kg TMZ dose-dependently delayed the growth of SW620 xenografts (Fig. 9.8 panel B). In combination, AG14361 and TMZ had markedly enhanced anticancer activity over that produced by TMZ monotherapy alone with

[10] Also referred to as AG014361. AG14361 has been used in text as designated by Agouron Pharmaceuticals, Inc.

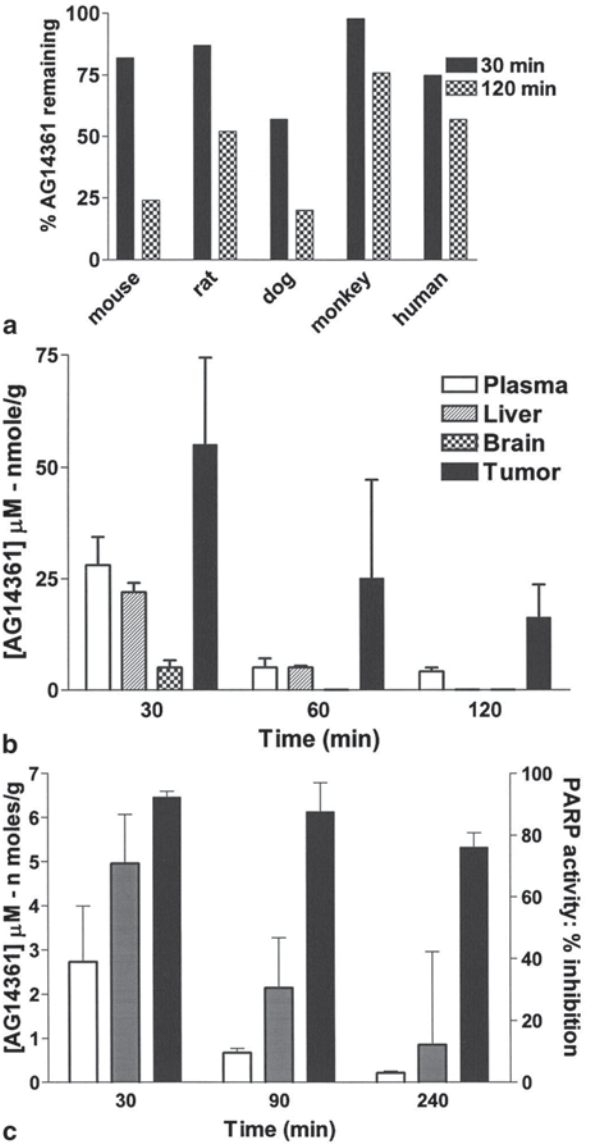



**Fig. 9.7** AG14361 metabolic stability, tissue distribution and tumour poly(ADP ribose) polymerase 1 (PARP-1) inhibition. **a** Metabolic stability of AG14361 (initial concentration = 5 μM) after 30 and 60 min incubations with hepatic microsomes from the indicated mammalian species. Data are mean of duplicate experiments. **b** Plasma pharmacokinetic and distribution of AG14361 at indicated times in several organs and LoVo xenograft tumours after IP administration of 50 mg/kg AG14361. Data are mean from three tumour-bearing mice. **c** Concentrations of AG14361 in plasma (open bars) and SW620 xenograft tumours (*shaded bars*) and inhibition of PARP-1 activity in SW620 xenograft tumour homogenates (*solid bars*) after IP administration of 10 mg/kg AG14361. Data are the mean from three tumour-bearing mice. The *right* y-axis shows inhibition of PARP-1 activity; the *left* y-axis shows the concentration of AG14361 in plasma and tumour tissue. **b** and **c** Error *bars* represent 95% confidence intervals. (Reprinted with permission [66])

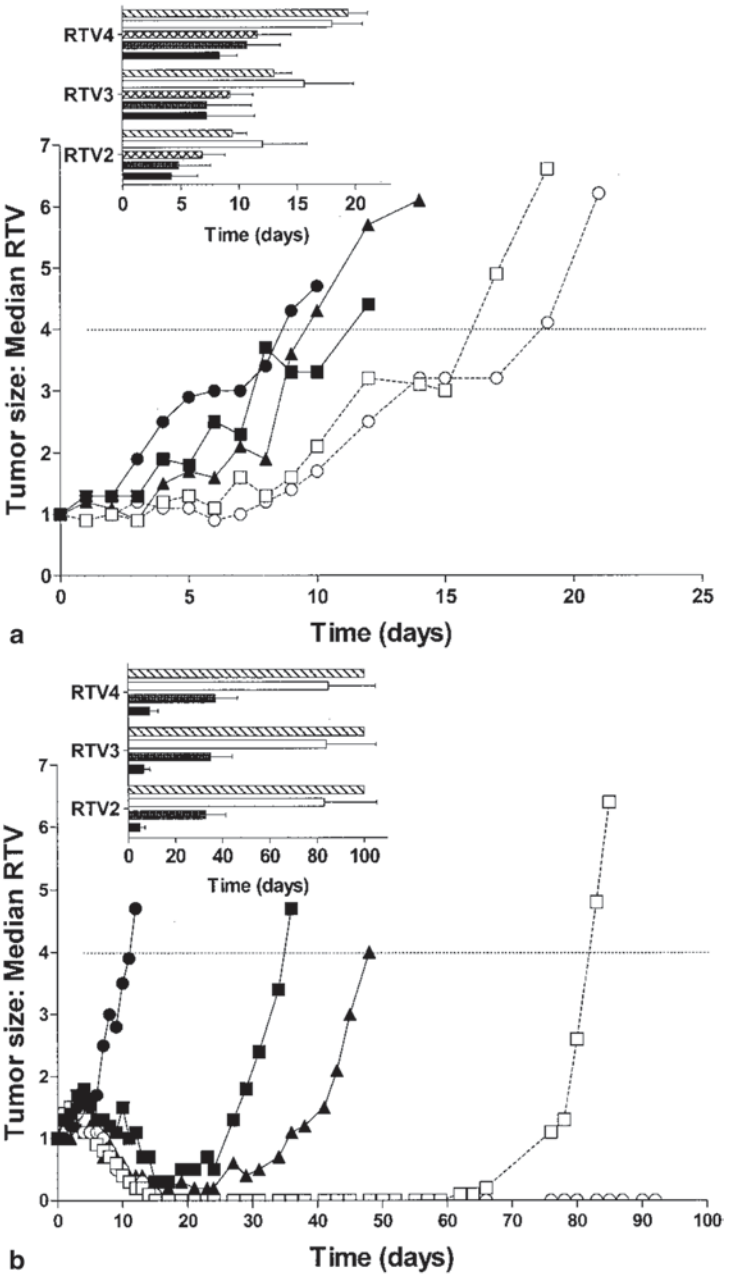



**Fig. 9.8** Enhancement of temozolomide activity by AG14361 in tumour-bearing mice. Tumour growth is presented as the median relative tumour volume (RTV, five mice per group). Insets represent time taken in days for tumours to reach RTV2, RTV3 and RTV4 (95% confidence intervals). **a** Growth of LoVo tumour xenografts after 5 daily treatments with vehicle alone (*solid circles, solid bars*), TMZ at 68 mg/kg (*solid squares*, *shaded bars*), TMZ at 138 mg/kg (*solid triangles, cross-hatched bars*), 5 mg/kg AG14361+68 mg/kg TMZ (*open squares, open bars*), or 15 mg AG14361+68 mg/kg TMZ (*open circles, hatched bars*). **b** Growth of SW620 tumour xenografts after 5 daily treatments with same vehicle and agents described in panel A. (Reprinted with permission [66])

the most prominent effect observed in SW620 xenografts (*ibid*). AG14361 has also been reported to preferentially restore sensitivity to TMZ in mismatch-repair (MMR) proficient tumour cells [68] and to potentiate the effects of radiation therapy in preclinical tumour models (data not shown) [66]. In addition, AG14361 has also been observed to potentiate topo I poison-mediated cytotoxicity presumably through a PARP-dependent base excision repair mechanism [69].

In summary, AG14361 was a breakthrough compound in the NICR:CRUK:Agouron Pharmaceuticals collaboration. AG14361 was among the first, if not the first, high-potency PARP-1 inhibitor in the reported literature with the desired specificity, pharmacology, pharmaceutical properties, and preclinical chemosensitising activities *in vivo* (*ibid.*) deemed sufficient to progress Agouron's early PARPi drug development program.

### *9.2.4 AG14447 and Rucaparib (CO-338)*

AG14447 was developed leveraging knowledge gained from initial studies with AG14361. A streamlined *in vitro* to *in vivo* testing scheme led to the identification of AG14447 as the most potent PARPi within a panel of tested agents [55]. The phosphate salt of AG14447 was then synthesized as a prodrug, AG014699, with improved solubility for *in vivo* testing. Thus, AG14447 is synonymous with AG014699 (rucaparib/CO-338) in experimental models, and was studied in the earliest preclinical trials. The design of rucaparib has been reviewed in detail [23].

While potency and specificity are important determinants, pharmaceutical agents require many additional qualities to enable their development into viable drug candidates. Agouron Pharmaceuticals, Inc. had to develop first an efficient, cost-effective screening paradigm to assess the SAR of test compounds. This was accomplished combining a cell-based functional screening assay and single dose *in vivo* combination tumour growth assay (PARPi + TMZ) to correlate Ki and physical-chemical properties to identify the best chemical leads by *in vivo* testing (*ibid.*).

In single-dose efficacy experiments in mice bearing SW620 xenografts, AG14447 produced significantly greater chemosensitization of TMZ antitumour activity than AG14361. This was observed after a single dose (1 mg/kg AG14447 vs. 10 mg/kg AG14361) (Fig. 9.9, Panel A) and after repeated dosing over 5 days. In the 5 day study, AG14447 exhibited highest potency and produced the greatest antitumour efficacy in combination with TMZ; a 0.15 mg/kg mg/kg total dose of AG14447 induced complete tumour regressions whereas a total dose of 1.5 mg AG14361 produced tumour growth delays (Fig. 9.9, Panel C). Key pharmacologic properties of AG14447 are listed (*ibid.*):

- potent affinity for PARP-1
- improved aqueous solubility over benzamides
- favourable pharmacokinetics, stability and metabolism (e.g., oral absorption and ADME[11] properties amenable for *in vivo* assessment)

[11] ADME = absorption, distribution, metabolism, excretion

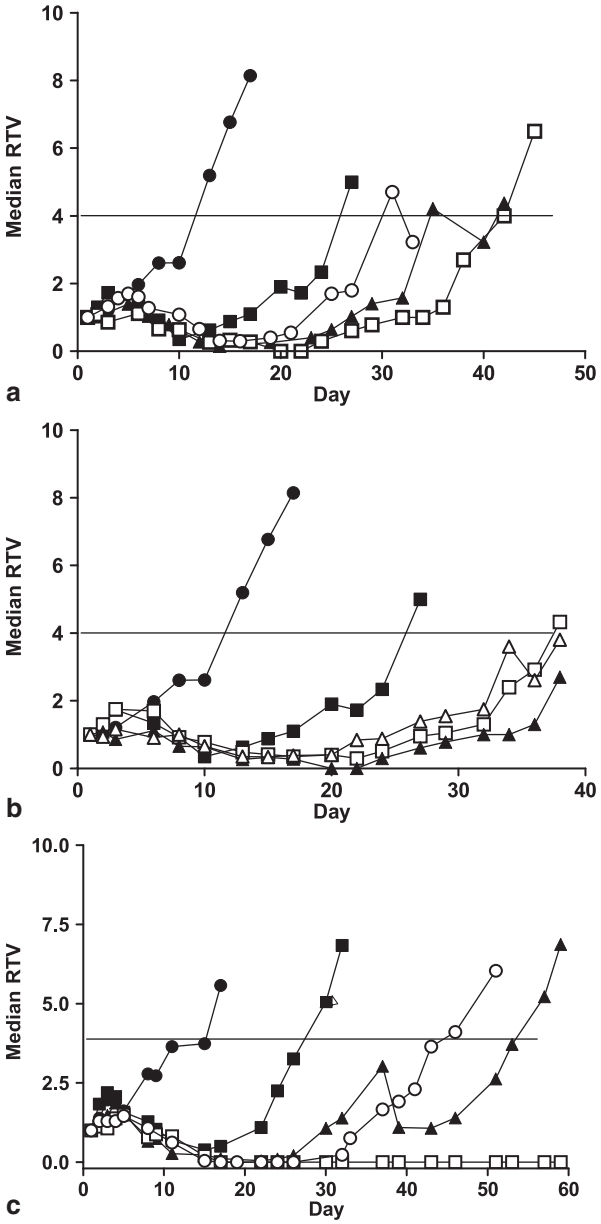



**Fig. 9.9** Comparison of in vivo chemosensitization of temozolomide activity by AG14361, AG14447 or AG144452 in tumour-bearing mice. **a** single-dose study: growth of SW620 tumour xenografts (median RTV) following a single dose of vehicle alone (●), 200 mg/kg TMZ (■), 200 mg/kg TMZ+1 mg/kg AG14361 (▲), 200 mg/kg TMZ+1 mg/kg AG14447 (Δ), or 200 mg/kg TMZ+1 mg/kg AG14452 (○). *Horizontal line*, RTV4. **b** single-dose study: growth of SW620 tumour xenografts (median RTV) following a single dose of vehicle alone (●), 200 mg/kg TMZ (■), 200 mg/kg TMZ+10 mg/kg AG14361 (□), 200 mg/kg TMZ+1 mg/kg AG14447 (▲), or 200 mg/kg TMZ+0.1 mg/kg AG14447 (Δ). *Horizontal line*, RTV4. **c** 5-d schedule: growth of SW620 tumour xenografts (median RTV) following daily treatment for 5 d with vehicle alone (●), 68 mg/kg TMZ (■), 68 mg/kg TMZ+1.5 mg/kg AG14361 (▲), 68 mg/kg TMZ+0.15 mg/kg AG14447 (□), or 200 mg/kg TMZ+5 mg/kg AG14452 (inactive analogue) (○). *Horizontal line*, RTV4. (Reprinted with permission [55])

- measurable delivery and retention into xenograft tumours after dose administration
- tolerable safety profile in animal studies
- superior potency and therapeutic index in chemosensitization studies

It was also important to demonstrate PD activity of PARP inhibition preclinically: DNA damage and PARP activity assays were used.[12] This review focuses on data related to inhibition or PARP activity. AG14447 decreased tumour PARP activity in NB-1691 xenograft tumours (Fig. 9.10) [70]. AG14447 (1 mg/kg) decreased PARP activity by ≥50% 6 h after dosing; Activity rebounded by 24 h. Repeated dosing over 4 days produced ≥75% decreases in PARP activity with ≈50% decreases sustained 24 h after the last dose associated with increased tumour exposure to AG14447 (*ibid*.) These experiments suggested that rucaparib would exhibit a definable PK/PD relationship for PARP inhibition and antitumour activity. However, the PK/PD relationship wasn't defined preclinically[13] and a plan was made to assess it in Phase 0/1 clinical trials with AG014699 (CO-338).

A clinical rather than a preclinical strategy was pursued to assess the PK/PD relationship for PARP inhibition by rucaparib using an analytically-validated PARP expression:activity assay in cancer patients [70]. Based on preclinical data, a PARP Inhibitory Dose (PID) was defined as 'the dose of rucaparib at which PARP activity in peripheral blood lymphocytes (PBLs) was reduced to less than 50% of the baseline value after the first dose [12]. Rucaparib produced a strong PK/PD relationship in patient peripheral blood lymphocytes and tumour tissues in the clinic in combination with TMZ in melanoma patients [12]; reviewed by Clift, Coupe and Middleton (Chap. 22) in this book volume). Interestingly, rucaparib may impact chemosensitivity by increasing tumour blood perfusion by a vasoactive mechanism that isn't fully understood [71, 72]. In summary, PD activity in the clinic provided an early signal of confidence in the mechanism of rucaparib and the selection of an appropriate dose for its use in Phase 2 clinical oncology trials.

### *9.2.5 AZD-2281 (Olaparib) Studies*

The synthesis and initial pharmacology of KU59436 (AZD-2281) and related analogues have been reported [14]. KuDos Pharmaceuticals Ltd. sought to develop an orally bioavailable PARPi with favourable pharmaceutical properties. In addition to its high potency for PARP-1 (Table 9.1), AZD-2281 (now called olaparib) possessed good physicochemical attributes and was advanced for further analysis after demonstrating the greatest levels of oral absorption in a murine *in vivo* pharmacokinetic screen (*ibid*.) Olaparib markedly enhanced chemosensitivity to methyl methanesulfonate (MMS) *in vitro* (Fig. 9.11, Panel a) concomitant with decreasing PARP activity (Fig. 9.11, Panel b). This translated into marked enhancement of

[12] Karen Maegley (Pfizer Inc.), personal communication

[13] Nicola Curtin (NCIR, UK), personal communication

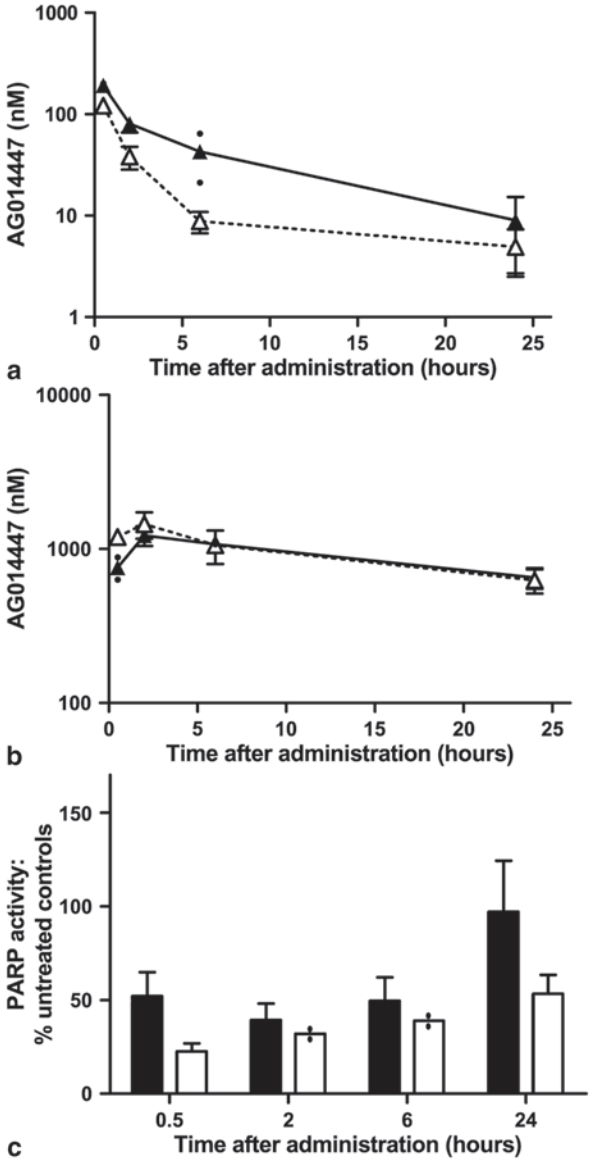



**Fig. 9.10** Rucaparib (AG014699 CO-338) pharmacokinetics and PARP-1 inhibition in an *in vivo* model of neuroblastoma. The concentration of the rucaparib parent compound, AG014447, is shown in **a** plasma or **b** NB-1691 xenograft homogenates from tumour-bearing mice, following a single (*solid symbols and lines*) or the last of four daily doses (*open symbols/dotted lines*) of rucaparib (1 mg/kg i.p.) and harvested at the indicated times after administration. Corresponding PARP-1 activity in the NB-1691 xenograft homogenates is shown in **c**, following a single (*filled columns*) or the last of four daily doses (*open columns*) of rucaparib. Data are the mean (± SD) from three tumour-bearing mice per time point. Where data from < three mice were available for a given time point, mean and actual data points are shown. (Reprinted with permission [70])

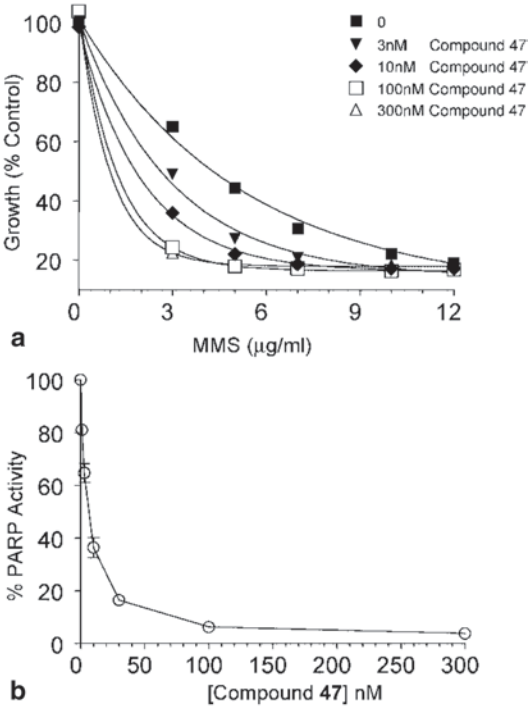



**Fig. 9.11** Potentiation of methyl methanesulphonate (*MMS*) cell killing of cultured SW620 cells in combination with compound 47 (olaparib, KU59436, AZD-2281). **a** Increasing concentrations of MMS were coincubated with or without the PARP inhibitor at single concentrations ranging from 1 to 300 nM. **b** PARP activity in SW620 tumour cell lysates treated with increasing nM concentrations of compound 47. PARP activity was quantified by the use of a PAR formation assay. (Reprinted with permission [14])

chemosensitivity in combination with TMZ in established SW620 xenografts *in vivo* (Fig. 9.12); The combination of 10 mg/kg olaparib (PO, daily x 5) and 50 mg TMZ was well-tolerated and produced sustained tumour regressions. These results are consistent with those expected from a potent PARPi drug candidate.

Assessment of the preclinical chemosensitising activities of olaparib were not undertaken in combination with cytotoxic agents as was done initially for rucaparib and veliparib. Preclinical trials of olaparib have focused on testing its activity in molecularly-defined HRR repair-proficient and HRR repair-deficient models. In contrast to earlier PARPi, olaparib has been developed from the start of its clinical development program with the goal of inducing synthetic lethality to benefit cancer patients.

Seminal publications by Farmer et al. [44] and Bryant *et.* al. [43] demonstrated hypersensitivity to PARPi in *BRCA1*- and *BRCA2*-deficient tumour models, respectively. Cell-based studies with olaparib were conducted in *BRCA1*- and *BRCA2*-

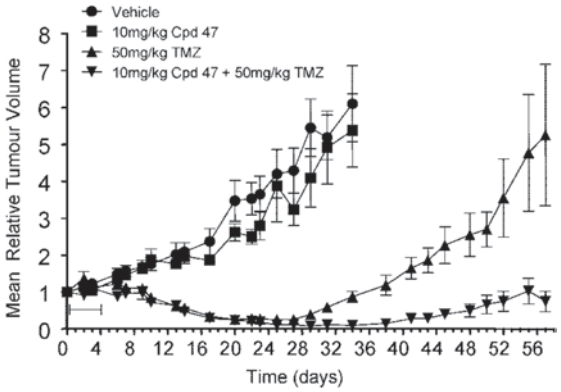



**Fig. 9.12** Antitumour efficacy of compound 47 (KU59436, AZD-2281) in combination with temozolomide (*TMZ*) in an SW620 tumour model. Mice were orally dosed with compound 47 (KU59436, AZD-2281) once daily for 5 consecutive days (days 0–4 are indicated by a *short line*), and KU59436 was administered 45 min before TMZ. Tumour volumes are shown relative to the initial tumour volume prior to dosing. Error *bars* represent SEM. (Reprinted with permission [14])

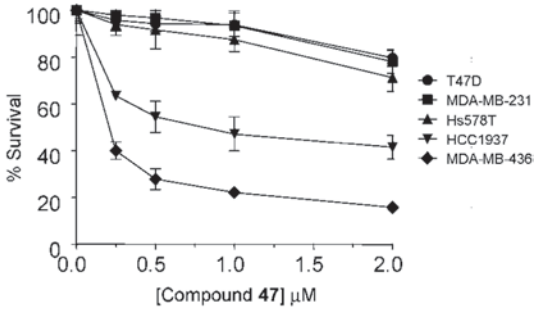



**Fig. 9.13** Cell survival (measured as a survival fraction) of breast cancer cell lines treated with PARP inhibitor 47 (olaparib, KU59436, AZD-2281). The *BRCA1*- and *BRCA2*-proficient lines were Hs578T, MDA-MB-231, and T47D, whereas the *BRCA1*-deficient lines were MDA-MB-436 and HCC1937. (Reprinted with permission [14])

deficient cell lines *in vitro* demonstrating preferential hypersensitivity to PARP inhibition in the *BRCA*-deficient lines, MDA-MD-436 and HCC1937 (Fig. 9.13). These findings have been replicated and extended by others. Preclinical monotherapy and platinum-based combination regimens with olaparib have subsequently been reported in *BRCA1/BRCA2*-deficient breast cancer models to induce synthetic lethality [73, 74] and form the basis for ongoing Phase 2/3 clinical trials with olaparib in cancer patients with *BRCA*-deficiency [18, 53, 54]. In summary, preclinical PD results and prominent antitumour activity in *BRCA*-deficient models provided confidence in the mechanism of olaparib to move it forward into clinical trials in ovarian and breast cancer patients with *BRCA*-deficiency.

Puzzling cell-based data with AZD-2281 were recently reported; AZD-2281 did not inhibit the *in vitro* growth of *BRCA1*-deficient HCC1937 breast cancer cells when exposed to drug concentrations that clearly decrease PARP activity [75]. The basis for this aberration is unclear. Further studies did not show chemosensitising effects of AZD-2281 on the *in vitro* activity of melphalan, doxorubicin, or vincristine across a panel of pediatric tumours (*ibid*).

### *9.2.6 BMN 673 and MK-4827 (Niraparib) Studies*

*BMN 673.* The preclinical pharmacology and antitumour activities of BMN 673 have been published [19]. BMN 673 has been studied as a chemosensitiser in combination chemotherapy and as an inducer of synthetic lethality. This compound has been reported to be extremely potent with a $K_i$ of 0.57 to 1.2 nM against the PARP-1 enzyme and an $IC_{50}$ of 2.5 nM in a cell-based cytotoxicity assay (Table 9.1).

BMN 673 has been reported to chemosensitise xenograft tumours to TMZ, Cisplatin and SN-38 [19]. Chemosensitisation results were similar to those reported for other PARPi, demonstrating a consistency in sensitizing effects observed across PARP inhibitors. A key finding reported in these studies was that BMN 673 had superior potency to olaparib in side-by-side testing. A review of these potency studies will be discussed below.

BMN 673 selectively inhibits the clonogenic survival of *BRCA1/BRCA2*-deficient cancer cells *in vitro* (data not shown) and exerts dose-dependent (0.1 or 0.33 mg/kg/day) single agent activity against *BRCA1*-deficient MX-1 xenografts (Fig. 9.14, Panel A). BMN 673 (1 mg/kg single dose) markedly decreased tumour xenograft PARP activity with partial recovery after 24 h (Fig. 9.14, Panel B). BMN 673 also had preferential cytotoxicity against tumour cells harboring PTEN dysfunction (*ibid*.). Notably, when the same total daily dose of BMN 673 was divided into 2 BID doses, complete tumour regressions were observed vs. prolonged tumour growth delays, suggesting that sustained exposures to BMN 673 were most efficacious (*ibid*.). The preclinical PK profiles for orally-administered BMN 673 were not reported for OD vs. BID dosing regimens. However, based on PK properties reported for other agents given orally or by osmotic minipump in mice with fractionated doses [76–78, 27] lower peak-to-trough ratio would be expected on the BID vs. OD regimen, suggesting that sustained exposures are required to exert efficacy and may be associated with greater tolerance due to resulting decreased $C_{max}$.

Separate siRNA screening studies demonstrated that the cytotoxic activity of BMN 673 was enhanced when genes involved in HRR- or DSB-repair were silenced. Of note, the potency at which BMN 673 exerted its effects were reported to be consistently higher than those for other PARPi [19].

BMN 673 has undergone analyses of its physico-chemical, preclinical PK, metabolism, and safety profiles (*ibid*). These profiles, along with demonstration of preclinical antitumour efficacy associated with HRR-deficiency and in accordance with the hypothesized mechanism-of-action (decreased PARP activity, chemosensitisation of chemotherapy, etc.), have supported the progression of BMN 673 into clinical oncology trials.

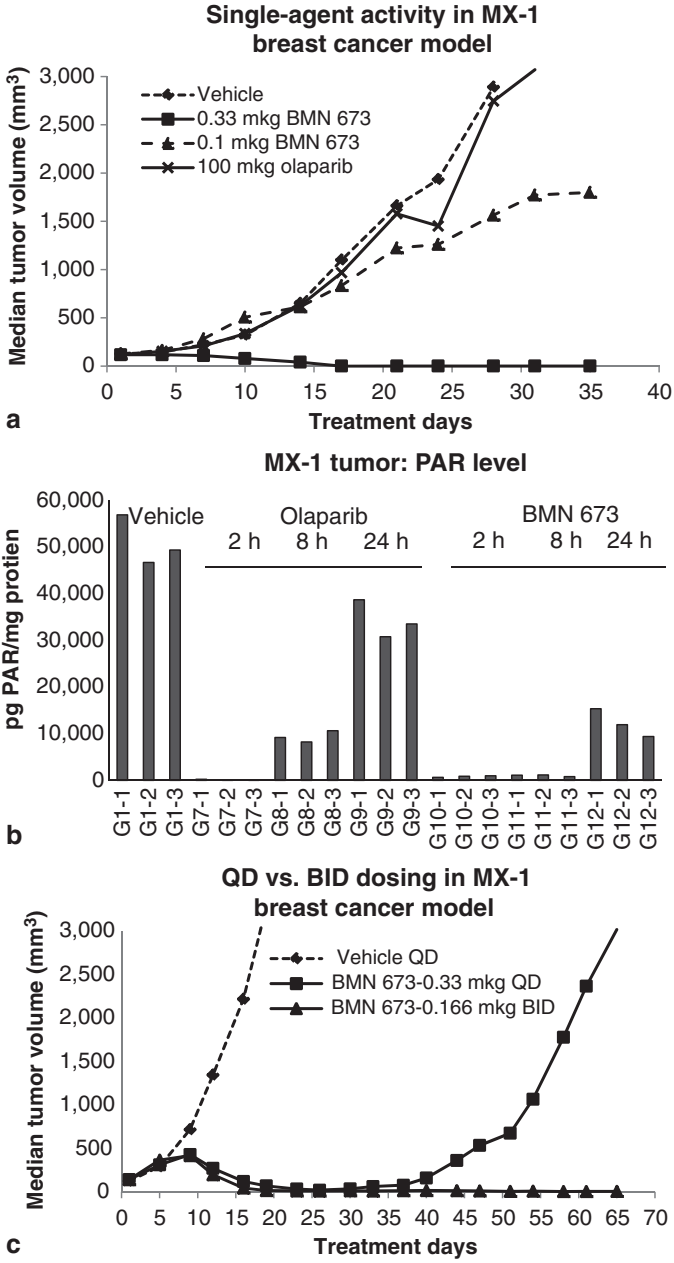



**Fig. 9.14** BMN 673 exhibits antitumour activity against *BRCA1*-deficient MX-1 xenografts in mice. **a** MX-1 human mammary xenografts were inoculated SC in female nu/nu mice. When tumours reached a mean volume of ≈150 mm$^3$, mice were randomized and treated orally OD for 28 consecutive days with vehicle, 0.33 or 1.0 mg/kg/day BMN 673, or 100 mg/kg/day olaparib. Median tumour volumes were plotted against days of treatment (first day of treatment is defined as day 1). **b** Inhibition of PARP activity was measured by measuring PAR levels 2, 8 and 24 h after a single oral dose of 1 mg/kg BMN 673 or 100 mg/kg olaparib. Each bar in the graph represents an individual tumour. **c** BMN 673 was more effective in mouse xenograft studies when administered at 0.165 mg/kg/BID vs. 0.33 mg/kg OD. Median tumour volumes were plotted against days of treatment (first day of treatment is defined as day 1). Used with permission [19]

### 9.2.7 MK-4827 (Niraparib)

The synthesis and initial pharmacology of niraparib have been published [56]. Niraparib has affinity for PARP 1 and 2 inhibition ($IC_{50}$ = 3.8 and 2.1 nM, respectively) and inhibits the proliferation of cancer cells with mutant *BRCA1* and *BRCA2* with $IC_{50}$ values in the 10–100 nM range *in vitro*. Niraparib demonstrated efficacy as a single agent in a xenograft model of *BRCA1*-deficient cancer. Niraparib has also been reported to act as a preclinical radiosensitiser [79, 80] and has entered into clinical oncology trials.

## 9.3 Other Potential PARPi Mechanisms

It is of great interest to understand the unique properties of each PARPi with so many undergoing drug development. Relatively few studies have been published for each agent by their respective companies or in studies comparing the activities of PARPi in standardised, side-by-side testing.

### 9.3.1 Potency and Antitumour Efficacy Studies

Cellular viability and clonogenic assays conducted in triple negative breast cancer cell lines *in vitro* demonstrated a trend for potency for inhibition of cellular growth that generally correlated with the ability of respective agents to induce $G_2$/M arrest and induction of DNA-damage markers [81]. In addition, each agent decreased PARP activity *in vitro* at the pharmacologic concentration of 1 μM except for BSI-201 which has been excluded as a bonafide catalytic PARPi [82–84]. Shen et al. [19] recently reported that BMN 673 is significantly more potent than other PARP inhibitors. Notably, the potency of BMN 673 as monotherapy in *BRCA1*- or *BRCA2*-deficient tumour cells *in vitro* was greater than that of the other PARP inhibitors, and demonstrated antitumour activity *in vivo* (Fig. 9.13). *In vivo*, pM to nM concentrations of BMN 673 were reported to induce λ-H2AX formation linking this activity to PARP inhibition. The discrepancy between differences in potency observed for BMN 673 in *in vitro* vs. *in vivo* studies may be due to pharmacokinetic or metabolic ADME profiles, or may indicate additional mechanisms-of-action.

### 9.3.2 Additional Mechanisms-of-Action of PARPi

PARP maintains genomic stability and homeostasis through a variety of mechanisms. PARPi's may exert effects independently of inhibiting PARP catalytic activity as previously discussed [2, 3]. In side-by-side testing, it has been shown that

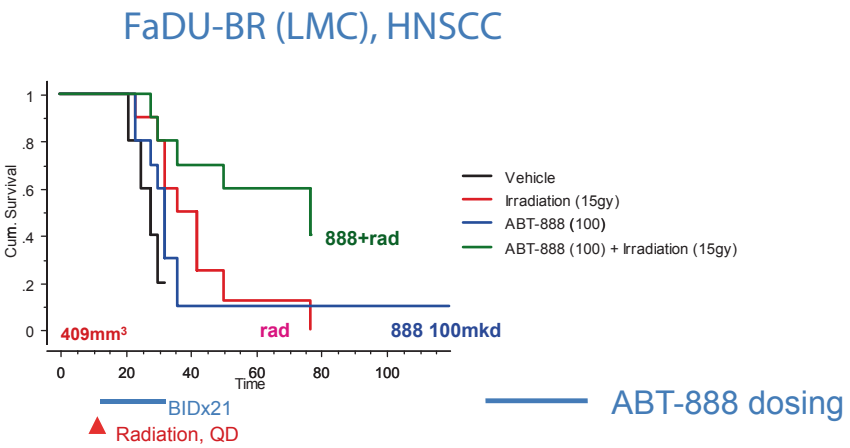
intracellular signaling pathways can be uniquely affected by exposure to PARPi. Examples of experimental results include: (1) rucaparib (≤2.5 μM) decreased the phosphorylation of Stat3 in MDA-MB-468 and MDA-MB-231 tumour cells but olaparib and veliparib had no measurable effect [81] and (2) rucaparib and olaparib increased the phosphorylation levels of Akt and/or ERKs in MDA-MB-468 and Cal-51 cells whereas veliparib had no effect (*ibid.)*. While these studies are a small sampling of the extensive amount of ongoing investigations, they fuel the contention that PARP inhibitors may have unique non-PARP catalytic activity-related mechanisms-of-action that may be exploited to improve cancer therapy.

The concept of DNA-PARP-trapping as an additional mechanism-of-action by which a PARPi may induce antitumour activity has been proposed repair [41, 42]. This was first postulated in very elegant studies demonstrating repair of nicked plasmid DNA using PARP-depleted and replete nuclear extracts in the presence or absence of substrate (NAD) or inhibitor (3AB) [20]. Catalytic inhibition of PARP activity remains very important but may not be the only mechanism for the anti-tumor activity of PARP inhibitors. Trapped PARP-DNA complexes induced by PARP inhibitors may engender more cytotoxic activity than unrepaired SSBs caused by PARP inactivation [85]. Moreover, the patterns of potency for PARP-trapping are different than patterns correlated with catalytic inhibitors. These findings explore as well as underscore additional repair mechanism(s) and genetic background(s) may be amenable to the PARP-trapping properties of PARP inhibitors.

For the sake of completeness, we briefly mention, BSI-201 because it was initially proposed to be a PARPi acting at the zinc fingers of the DNA binding domain rather than at the catalytic domain despite its structural resemblance to the benzamides [86]. BSI-201 does bind to the $Zn^{++}$ finger region of PARP [82] and is no longer considered to be a PARPi because further studies demonstrated that it does not inhibit the catalytic region of the PARP-1 enzyme [82–84]. Therefore, the mechanism of action for BSI-201 is not via inhibition of PARP activity. BSI-201 is highly reactive, forming covalent interactions with many cysteine-containing proteins. Furthermore, both BSI-201 and its nitroso metabolite, form protein adducts nonspecifically in tumour cells [83], which may be associated with preclinical cytotoxic effects.

### *9.3.3 Radiosensitisation by PARPi*

Since the first revelation that radiation cytotoxicity can be enhanced by PARPi (3AB) [7], there has been keen interest in exploring the utility of combining PARPi with radiation in cancer therapy. Briefly, the role(s) of PARP in radiation response and DNA damage mechanisms have been extensively reviewed in the literature [7, 87] as have the radiosensitising activities of PARPi in preclinical models in the literature [38] in the literature and in great detail by Fouillade et al in Chap. 11 in this book volume.




Survival advantage to 1cc Logrank (Mantle-Cox)

| *p* values *vs* | Veh | ABT-888 (100 mkd) | Irrad (15 Gy) |
|---|---|---|---|
| Irrad (15 gy) | **≤ 0.05** | NS | --- |
| ABT-888 (100 mkd) | NS | --- | NS |
| ABT-888 (100 mkd) + Irrad (15 gy) | **≤ 0.01** | **≤ 0.05** | **≤ 0.05** |

**Fig. 9.15** Survival analysis following treatment with veliparib ± irradiation in nude mice. Treatment started 14 days post inoculation of tumour cells. The average tumour size on day 14 was 409 ± 24 $mm^3$. The dose, route of administration and regimen are specified in the graphs [62]

Examples of the radiosensitising effects of veliparib have been well-documented in preclinical tumour models *in vivo* [27] and/or *in vitro* [1, 62, 88, 31]. A representative graph demonstrating the preclinical radiosensitising effects of veliparib is shown in Fig. 9.15 [62]. Radiosensitising results have also been reported for rucaparib [72, 89, 90], olaparib [91–93] and niriparib [94, 80]. In summary, there is a promising potential to improve radiotherapy when used in combination with PARPi. Readers are referred to Chap. 11 for a detailed review of this topic in this book volume.

## 9.4 Summary

There are strong and ample preclinical data to support the strategy of employing PARPi as chemo- and radiosensitisers to enhance cancer therapy. The earliest pharmacological studies with PARPi demonstrated inhibition of PARP-1 catalytic activity but the compounds had low potency and poor pharmaceutical properties [95]. The preclinical pharmacology of potent PARP inhibitors, such as rucaparib, olaparib, and veliparib, share a common mechanism of catalytic inhibition of PARP activity and

impairment of DNA damage repair [23]. To achieve optimal potency and pharmaceutical properties for the desired clinical compounds, companies have successfully integrated x-ray crystallography, chemistry, biology, 'omics analyses, pharmacokinetic (ADME), and safety evaluations into their preclinical testing programs.

Whilst promising, no PARP inhibitor has yet been approved for use in chemo- or radiotherapy regimens. A key goal of early phase clinical trials (in addition to traditional safety evaluations) is to conduct mechanistic studies to test whether a drug candidate inhibits PARP activity in a manner defined by a strong PK/PD relationship in human malignancies. It is generally unrealistic to expect to see clinical efficacy produced by PARPi used as chemosensitiser in patients with advanced malignancies exposed to multiple therapies. Therefore, PD biomarkers have been especially needed to guide development of PARP inhibitors, which are not cytotoxic *per se*. Using practical, analytically-qualified biomarker assays of PARP inhibition in PBLs and/or tumour biopsies, the measurement of PAR formation/PARP activity was initially established by the rucaparib and veliparib programs as a clinically-relevant PD biomarker; it was used to provide confidence-in-mechanism to help guide "Go, No Go" decisions for advancing compounds from Phase 1 to Phase 2 trials.

The understanding of the mechanism(s) by which PARPi may enhance antitumour therapy has grown considerably over time. Induction of synthetic lethality in *BRCA-1/BRCA2*-deficient tumour models was a key advance that has spurred the development of PARPi for use as monotherapy [43, 44]. Olaparib was the first PARPi tested for ability to induce synthetic lethality and rapidly provided evidence of proof-of-concept in its Phase 1 trial [53]. The 2014 approval of olaparib in BRCA-deficient ovarian cancer has provided evidence to clinically validate the benefit of exploiting synthetic lethality to improve antitumour therapy. The promise of this approach has led to Discovery and Development efforts for other PARP inhibitors, such as BMN 673 and niraparib, and even for the original class of PARPi (veliparib, rucaparib) to exploit this mechanism. For example, rucaparib has now shown selective single agent cytotoxicity in HRR-deficient tumour cells lines *in vitro* and primary ovarian ascites cultures *ex vivo* [96]. In addition, investigators are rapidly exploring the mechanism(s) and antitumour activity(ies) associated with PARP inhibition in genetic backgrounds that are HRR-deficient and HRR-proficient independent of BRCA1/BRCA2-deficiency. Therefore, there is high hope that PARP inhibitors will broad gain regulatory approval in clinical oncology based on synthetic lethality.

Further, the concept of PARP-trapping and the complexity of the role(s) played by PARPs in cellular and DNA homeostasis/damage repair suggest that additional mechanisms of PARP inhibition or modulation may have therapeutic utility. The reported high potency of BMN 673 or niraparib for PARP-trapping vs. catalytic enzyme inhibition [42, 85] is intriguing and has opened new avenues of study.

Biomarkers are also needed to help select patients expected to respond to therapy particularly combination therapies. The lack of patient-selection biomarkers is a major deficit that has contributed to the lack of clinical registration of PARPi. Efforts to identify proteomic and/or genomic profiles associated with sensitivity are ongoing [34, 97] but have not yet translated into clinical therapeutic value. Additionally, a pharmacogenomic study was unable to identify polymorphisms in the PARP-1 gene associated with patient's response to anticancer therapy [98]. Success-

fully identifying and using patient-selection biomarkers to enrich for responders is of utmost importance in order to gain regulatory approval for PARPi in oncology. There is high need to focus translational research, from the preclinic to clinic, to develop analytically-validated patient-selection assays to identify the right patients who will respond to therapy. This is the goal of personalized therapy and promise provided by emerging clinical results in patients with *BRCA1/BRCA2* tumours who are undergoing treatment with PARPi.

In conclusion, preclinical research has provided a strong rationale for employing PARP inhibitors as monotherapy to induce synthetic lethality and as chemosensitisers (or radiosensitisers) to improve anticancer therapy. There is high need to focus translational research, from the preclinical setting to the clinic, to enrich for the patients most likely to respond to therapy. While PARPi combination therapy offers an attractive prospect to potentially broaden clinical benefit, to date, predicting the best combination for clinical benefit remains a challenge.

**Acknowledgements** We thank Dr. Karen Maegley (Pfizer Global Research and Development); Dr. Vincent Giranda and Dr. David Maag (AbbVie Oncology) for helpful review and critique of the chapter. The authors are also grateful for the contributions of so many dedicated scientists and clinician-scientists across academia and industry for their preclinical, translational and clinical research contributing to the development of PARP inhibitors for cancer therapy. Lastly and most importantly, to all the patients for their dedication and commitment to participate in clinical trials, without which, the future of pioneering therapeutic innovations will not be possible.

Views presented herein represent those of pharmaceutical scientists who have been responsible for designing preclinical translational strategies and executing experiments to develop PARPi as antitumour agents in industry. These views do not necessarily represent those of our respective organizations or development teams.

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