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MONOGRAPH

VELIPARIB HYDROCHLORIDE

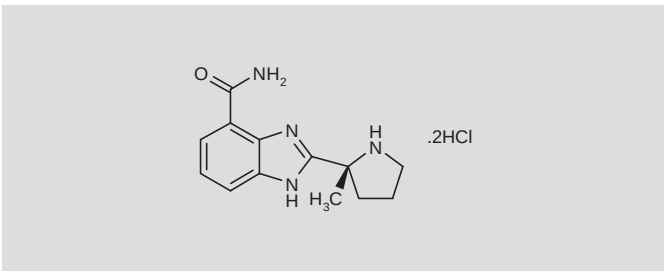
Poly(ADP-Ribose)Polymerase Inhibitor
Oncolytic

Prop INNM; USAN

A-861695
ABT-888
NSC-737664

2-[2(*R*)-Methylpyrrolidin-2-yl]-1*H*-benzimidazole-4-carboxamide dihydrochloride

InChI: 1S/C13H16N4O.2ClH/c1-13(6-3-7-15-13)12-16-9-5-2-4-8(11(14)18)10(9)17-12;;/h2,4-5,15H,3,6-7H2,1H3,(H2,14,18)(H,16,17);2*1H/t13-;;/m1../s1



$C_{13}H_{18}Cl_2N_4O$
Mol wt: 317.2140
CAS: 912445-05-7
CAS: 912444-00-9 (free base)
CAS: 912445-04-6 (racemic)
CAS: 912443-99-3 (racemic free base)
CAS: 912444-01-0 [(*S*)-enantiomer free base]
EN: 434374

SUMMARY

Veliparib hydrochloride (ABT-888) is an inhibitor of poly(ADP-ribose)polymerase 1 and 2 (PARP-1 and PARP-2). Both PARP-1 and PARP-2 are intimately involved in the repair of DNA damage. Hence, veliparib has been found to enhance the activity of multiple DNA-damaging agents, including temozolomide, cisplatin, carboplatin, cyclophosphamide and irinotecan, as well as radiation. Veliparib is an orally active drug that has been shown to cross the blood–brain barrier in preclinical studies. Clinical development of this drug is under way as an adjunct to chemotherapy for a variety of malignancies, e.g., high-grade gliomas, malignant melanoma, ovarian cancer, prostate cancer, lymphoproliferative disorders and breast cancer. It is also being studied clinically as a single agent in BRCA-mutated solid tumor patients, as well as a sensitizer to therapeutic ionizing radiation. The preclinical rationale and the current clinical status of this promising new drug are reviewed.

SYNTHESIS*

Alkylation of *N*-(benzyloxycarbonyl)proline methyl ester (I) with methyl iodide and NaHMDS in cold THF furnishes the protected 2-methylpyrrolidine methyl ester (II), which is hydrolyzed with LiOH in THF/H_2O/MeOH at 60 °C to give the carboxylic acid (III). Acid (III) is then coupled with 2,3-diaminobenzamide dihydrochloride (IV) by means of CDI in Pyr/DMF to provide amide (V). Subsequent cyclization of the 2-aminobenzenecarboxamide derivative (V) in refluxing AcOH leads to the benzimidazole derivative (VI). Racemic benzimidazole (VI) is resolved using chiral HPLC to provide the (*R*)-enantiomer (VII), which is finally deprotected by means of H_2 and Pd/C in MeOH (1, 2) and treated with HCl in MeOH (2). Scheme 1.

BACKGROUND

Poly(ADP-ribose)polymerase (PARP) is a nuclear enzyme that recognizes DNA damage and facilitates DNA repair. Activation of PARP-1 and PARP-2 enzymes is an essential step in the repair of DNA damage that results in the poly(ADP-ribosyl)ation of many nuclear target proteins (3-6). PARP activity is essential for the repair of single-strand DNA breaks through the base-excision repair pathways and is an important modulator of the nonhomologous end-joining and homologous recombination double-strand break repair pathways. Consequently, inhibition of PARP should enhance the effects of DNA-damaging agents, including alkylating agents, platinum compounds and topoisomerase inhibitors, and radiation therapy (7). It is noteworthy that PARP is ubiquitously expressed in almost all types of eukaryotic cells (8), and its activity may be increased in the nuclei of actively proliferating cells (7). Thus, the

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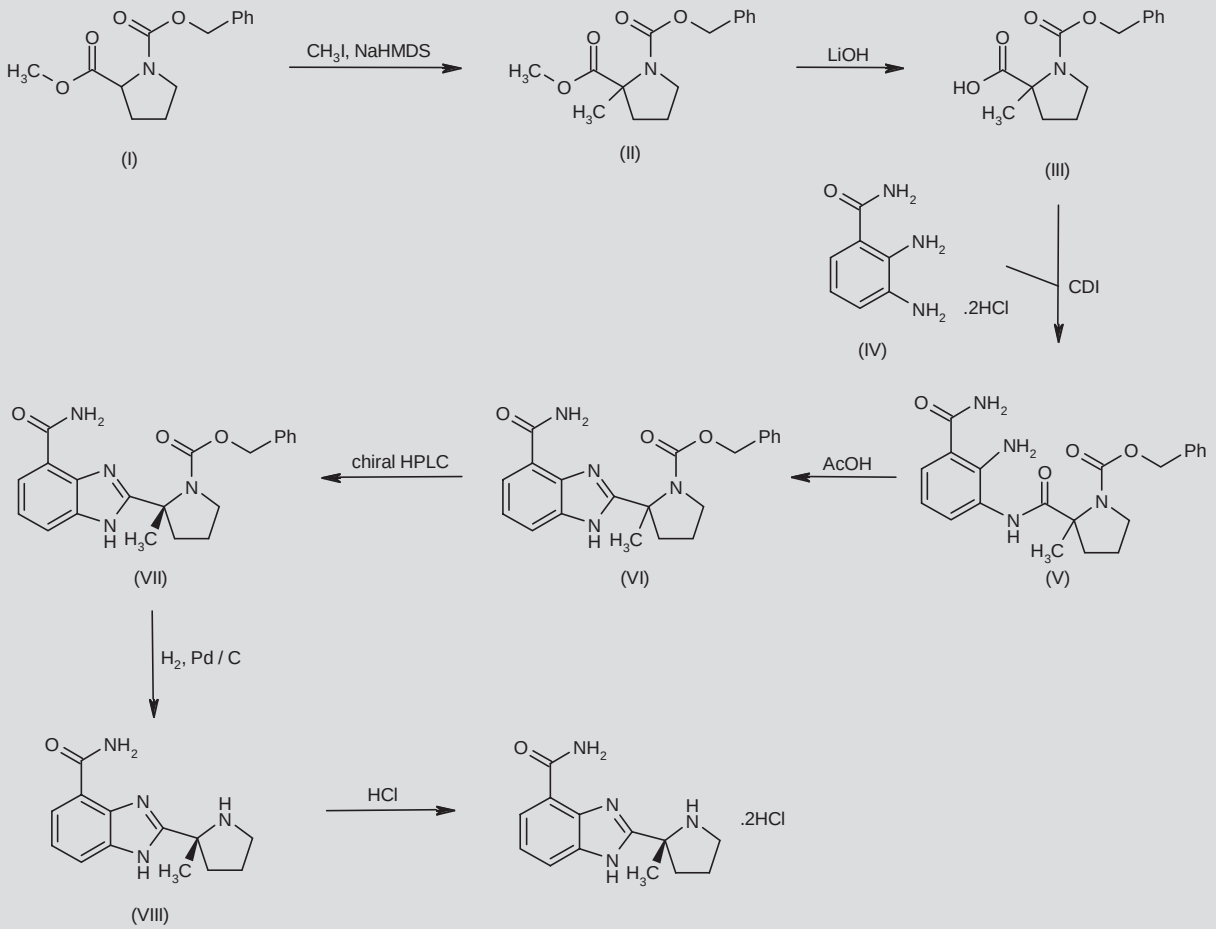
*Synthesis prepared by R. Castañer. Thomson Reuters, Provença 388, 08025 Barcelona, Spain.

Scheme 1. Synthesis of Veliparib Hydrochloride



clinical use of a PARP inhibitor in combination with cytotoxic chemotherapy may lead to an increase in myelosuppression. In this regard, enhanced PARP expression has been demonstrated in some tumor models, suggesting that a favorable therapeutic index may be achieved when applied clinically as an adjunct to DNA-damaging therapies (9-11). As an example, a controlled clinical trial of the i.v. PARP inhibitor BSI-201 (which is structurally different from veliparib) in combination with carboplatin and gemcitabine has shown dramatic results in triple-negative breast cancer (12).

Additionally, single-agent activity of PARP inhibitors has been reported for breast and ovarian tumors lacking *BRCA1* or *BRCA2* DNA double-strand repair mechanisms (13, 14). Relative to this, the concept of synthetic lethality, as reviewed by Iglehart and Silver, is relevant (15). This concept relates to the requisite mutation in two genes in order to result in cell death and can be applied to drug discovery. For example, *BRCA* mutations, which are deficient in a DNA repair pathway, can be exploited by inhibiting another pathway, i.e., base excision repair, which requires PARP. Hence, a variety of PARP inhibitors are being tested in this clinical setting, with the expectation of minimal toxicity. As proof of principal for this concept, three *BRCA* patient studies in breast (16, 17), ovarian (16, 18) and prostate cancer (16) have reported activity with the PARP inhibitor olaparib, an orally active drug structurally similar to veliparib.

Veliparib hydrochloride (ABT-888) is a novel, potent, orally active inhibitor of PARP (K_i = 5.2 and 2.9 nmol/L, respectively, for PARP-1 and PARP-2) (2). In vitro or in vivo application of veliparib inhibits the formation of PAR polymers. It has good oral bioavailability, crosses the blood–brain barrier and potentiates the activity of temozolomide, platinum agents, cyclophosphamide and radiation in syngeneic and xenograft tumor models (19-21). Here we summarize the results of preclinical and early clinical studies involving this promising new agent.

PRECLINICAL PHARMACOLOGY

Due to the role of PARP in DNA repair, PARP inhibition is an attractive target to potentiate the activity of known DNA-damaging agents (7). Veliparib is a novel oral PARP inhibitor. It has been studied in

preclinical models in order to gain insight into its activity as a PARP inhibitor, as well as its potential to synergize with chemotherapy and radiation in various cell lines and xenograft models. The section that follows summarizes many of the preclinical findings that have provided the basis for the clinical development of this drug to date.

PARP is activated by DNA damage, leading to ribosylation of various PAR proteins. Inhibition of PARP results in decreased PAR expression, which can be detected by Western blot analysis. To demonstrate such predicted changes in PAR expression with the application of veliparib, Donawho et al. treated B16F10 tumor-bearing mice with temozolomide with or without veliparib versus vehicle alone (19). Animals treated with veliparib alone or in combination with temozolomide showed a decrease in PAR protein on Western blot, demonstrating PARP inhibition in vivo. In these studies, veliparib was also found to inhibit the function of PARP-1 in human lung carcinoma NCI-H460 cell lines alone and in combination with radiotherapy. NCI-H460 cell lines were treated with veliparib alone or with radiotherapy. PAR protein levels were used as a marker for PARP-1 inhibition. Western blot of treated cell lines showed a decrease in PAR expression in cells treated with veliparib alone and in combination with radiotherapy (20). More recently, Palma et al. have shown changes in PAR expression as a function of differential PARP inhibition with combination therapy in a variety of tumor cell lines (human B-cell lymphoma DoHH2, small cell lung carcinoma NCI-H526, pancreas adenocarcinoma Capan-1 and carcinoma Calu-6) (21).

It is of interest to note that PARP inhibition can result in apoptosis. Albert et al. studied NCI-H460 cells using flow cytometry to measure the percentage of apoptosis (20). Cells were labeled with annexin V-FITC as a surrogate marker of apoptosis. The cells were treated 24 h before staining with radiation (3 Gy) alone or with veliparib and then irradiated. Cells treated with both veliparib and radiotherapy showed a 2.8-fold increase in apoptosis ($P = 0.00002$). Autophagy was also monitored in this group of treated cells. NCI-H460 cells were transfected with GFP-LC3 plasmid, a marker for autophagy. Cells treated with radiotherapy (3 Gy) alone showed a 2.4-fold increase compared to control, a 3.7-fold greater increase with veliparib alone and a 7.3-fold increase for the combination treatment. This demonstrates in vitro induction of apoptosis and autophagy in cells treated with veliparib and suggests a synergistic effect for veliparib used in combination with radiotherapy. In the context of these studies in NCI-H460 lung cancer cells, veliparib inhibition of PARP-1 was shown to induce histone γ-H2AX, a marker of double-strand DNA breaks. NCI-H460 cells were treated with radiotherapy (5 Gy) with or without veliparib. Labeling techniques (using γ-H2AX antibody) demonstrated increased double-strand breaks, indicating an increase in DNA damage due to PARP inhibition and a decrease in DNA repair.

Veliparib and radiotherapy effects on angiogenesis in vitro were studied in human umbilical vein endothelial cells (HUVEC) and in vivo in mice bearing NCI-H460 tumor xenografts. In vitro studies showed a reduction in the ability of endothelial cells to form capillary-like tubule structures necessary for angiogenesis when treated with combination therapy. Mice bearing NCI-H460 tumor xenografts were treated with daily veliparib 25 mg/kg followed by 2-Gy fractions of radiotherapy for a total dose of 10 Gy versus radiotherapy alone. Tissue samples fixed with antibodies to Von Willebrand factor were used as a stain for blood vessels. Combination therapy induced a statistically significant reduction in blood vessels per high-powered field compared to control and single-agent therapy (20).

Other veliparib studies in combination with radiation have been performed with the human colon cancer cell line HCT 116, which is characterized by sensitivity to radiation. Veliparib (25 mg/kg/day) potentiated fractionated radiation (2 Gy/day x 10 days), with a median survival of 36 days versus 23 days ($P < 0.036$) compared with radiation alone. At a dose of 12.5 mg/kg/day veliparib did not enhance median survival, but interestingly, this treatment group did have one cure, defined as no palpable tumor. Overall, veliparib showed a dose–response in combination with radiation ($P = 0.0165$) (19).

Using ionizing radiation and veliparib in combination, Albert et al. demonstrated improved tumor growth delay and tolerability in NCI-H460 cell lines. Mice were treated with veliparib 25 mg/kg i.p. for 5 days followed by irradiation with 2-Gy fractions 1 h following administration of drug for a total treatment dose of 10 Gy. Combination therapy induced tumor growth delay of 13.5 days as compared to 1 day for the veliparib group and 7 days for the radiotherapy group ($P = 0.045$). The tolerability of the combination therapy was assessed by monitoring mouse weight. Combination veliparib plus radiotherapy was associated with a lower overall weight loss compared to radiation alone (15% vs. 10%), despite a greater decrease in tumor size in the group treated with the combination. This suggests that combination therapy was well tolerated. Tumor growth delay was further evaluated in NCI-H460 tumor xenograft models in mice by staining tissue sections with Ki67 and TUNEL, stains for apoptosis versus decreased proliferation. Mice treated with combination therapy had a decreased Ki67 index (2.6-fold compared to radiation alone and > 7-fold compared to untreated controls; $P = 0.005$ for combination versus radiation alone). TUNEL staining showed a 65% increase, suggesting increased apoptosis when veliparib was added to radiotherapy ($P = 0.02$) and a 7-fold increase for combination therapy versus untreated control (20).

Many studies involving the combination of PARP inhibitors and chemotherapy have focused on temozolomide. In part, this is due to the fact that temozolomide is not as myelosuppressive as most cytotoxic DNA-damaging agents. It would be anticipated that any given PARP inhibitor would increase myelosuppression in normal hematopoietic tissue, as base excision repair is also operational in normal cells. Temozolomide has become the standard of care for high-grade gliomas and is used extensively in other subsets of primary brain tumors. Additionally, as an analogue of dacarbazine, temozolomide is also being studied in malignant melanoma. There are at least two known major resistance mechanisms for temozolomide. High levels of O^6-methylguanine-DNA methyltransferase (MGMT) have been shown to be a significant resistance mechanism for temozolomide that has prognostic significance in brain tumor patients (22). The gene locus for the production of the enzyme is *MGMT*. If *MGMT* is methylated, levels of enzyme production are low, conferring temozolomide sensitivity; if *MGMT* is unmethylated, enzyme levels are high, conferring resistance by reversing temozolomide-induced alkylation. Strikingly, only 7% of the alkylation of temozolomide involves the O^6 position of guanine. Far more common (i.e., approximately 70%) is alkylation of the N^7 position of gua-

nine and (9%) the N^3 position of adenine (23). These sites of methylation relate to base excision pathways, for which PARP activity is required (24). Consistent with these observations, PARP has been shown to be directly related to temozolomide resistance (25, 26). Thus, the combination of veliparib (which crosses the blood–brain barrier) and temozolomide has been pursued aggressively as an approach to overcoming temozolomide resistance in both the laboratory and the clinic.

As temozolomide resistance results from increased MGMT activity in concert with mismatch repair (MMR) system mutations, Horton et al. (27) studied the relative importance of MGMT activity, MMR deficiency, nonhomologous end-joining (NHEJ) and PARP activity in veliparib-induced potentiation of temozolomide. Leukemia cells that were MMR-proficient and -deficient, with varying MGMT activity, as well as primary leukemia samples, were evaluated to determine the temozolomide IC_{50} alone and in combination with veliparib. Results demonstrated that veliparib effectively inhibited PARP activity and enhanced temozolomide-induced growth inhibition in most leukemia cells. Furthermore, veliparib potentiation was most effective in MMR-deficient cells with low MGMT activity (with a potentiation factor [PF] of 21); veliparib also potentiated temozolomide activity in MMR-deficient cells with elevated MGMT activity. Unexpectedly, veliparib also enhanced temozolomide activity in MMR-proficient cells (PF = 3-7). Potentiation by veliparib was found to be unrelated to NHEJ activity. Other findings included veliparib potentiation of temozolomide (PF = 2-5) in two of four acute myeloid leukemia patient samples, but little potentiation in primary acute lymphoblastic leukemia samples. Collectively, these studies demonstrated that veliparib potentiation of temozolomide was most pronounced in MMR-deficient cells with low MGMT activity; however, neither MMR proficiency nor MGMT overexpression completely abrogated veliparib-induced potentiation of temozolomide.

Other preclinical studies with veliparib and temozolomide suggest efficacy in several tumor types. Veliparib was shown to have broad-spectrum activity in combination with temozolomide in B-cell lymphoma, small cell lung cancer (SCLC), non-small cell lung cancer (NSCLC), ovarian, breast, pancreatic and prostate cancer models. Most responded to single-agent temozolomide as initial treatment, but temozolomide was ineffective by the second cycle. Combination therapy produced sustained activity, even in tumors that had shown resistance to temozolomide (21).

Donawho et al. administered veliparib (in rat equivalent doses) in combination with temozolomide in an orthotopic rat glioma model. This resulted in a significant reduction in tumor volume of 63%, which was 44% better than with temozolomide alone ($P < 0.005$). Maximum efficacy for veliparib with temozolomide was evaluated by magnetic resonance imaging (MRI) at 14 days (19).

Palma et al. evaluated the efficacy of veliparib in combination with temozolomide in the orthotopic and metastatic setting. Human prostate carcinoma PC-3M-Luc and human breast carcinoma MDA-MB-231-Luc tumors were implanted ectopically in the brain and intratibially. Both showed initial sensitivity to temozolomide monotherapy, but combination therapy showed prolonged tumor regression ($P \leq 0.0001$), with 2 of 10 histological cures in the breast cancer cell line. There was no additional efficacy for temozolomide monotherapy for the second treatment, but the combination of veliparib and temozolomide maintained regression for 61 days versus 42 days ($P \leq 0.05$), as well as a significant survival advantage. Efficacy in brain metastasis was evaluated by ectopically implanting MDA-MB-231-Luc cells in mice and monitoring tumor growth. Mice were treated with temozolomide alone or temozolomide plus veliparib. Resistance to monotherapy occurred following the first cycle of temozolomide alone, but combination therapy showed a response through the third cycle. This resulted in a marked survival benefit for combination therapy compared to temozolomide monotherapy ($P \leq 0.0001$). Additionally, efficacy in bony metastases was evaluated in intratibial lesions in mice ectopically injected with MDA-MB-231-Luc cells and PC-3M-Luc cells. Again, tumors were resistant to monotherapy upon administration of the second cycle. Veliparib in combination with temozolomide showed activity in the setting of resistance to monotherapy with temozolomide and temozolomide in combination with zoledronic acid. Some mice injected intratibially with MDA-MB-231-Luc cells developed lung metastases with response to both temozolomide and combination therapy, which was sustained beyond the development of regrowth of intratibial tumors. This suggests a potential difference in tumor response based on tumor location (21).

With regard to other chemotherapeutic agents, veliparib potentiated the activity of platinum-based agents, e.g., cisplatin and carboplatin, in a human mammary MX-1 xenograft model. The xenograft was derived from a 29-year-old female with breast cancer and a known *BRCA1* mutation. Veliparib was combined with cisplatin and carboplatin versus platinum monotherapy. The combination of cisplatin and veliparib showed a statistically significant complete response rate ($P = 0.049$) versus cisplatin monotherapy. Maximum effect in dose–response studies was seen at 5 mg/kg/day veliparib. Combination with carboplatin also showed potentiation (19).

Veliparib in combination with cyclophosphamide was evaluated in t(14;18) MX-1 and DoHH2 xenograft models. In the MX-1 model, veliparib 25 mg/kg/day, but not at the lower dose of 12.5 mg/kg/day, enhanced the efficacy of cyclophosphamide 12.5 mg/kg/day. In the DoHH2 model, veliparib did not potentiate the activity of cyclophosphamide (19).

PHARMACOKINETICS AND METABOLISM

The pharmacokinetics of veliparib have been evaluated in CD-1 mice, rats, dogs and monkeys. The plasma clearance values for veliparib were between 0.6 and 4.1 L/h.kg, with a moderate volume of distribution (V_{ss} = 2.0-3.1 L/kg) and terminal elimination half-life ($t_{1/2}$) of 1.2-2.7 h. The oral bioavailability from a solution formulation was 56-92%. Veliparib has been demonstrated to cross the blood–brain barrier in preclinical studies. To evaluate its central nervous system (CNS) penetration, the pharmacokinetic profile was evaluated in tumor-bearing rats with drug concentrations measured in plasma, brain and tumor tissues. Following multiple doses of veliparib (50 mg/kg/day), the concentration of drug 2 h after dosing (near C_{max}) was 1.36 ± 0.16 µg/mL, 0.72 ± 12 µg/g and 3.00 ± 16 µg/g, respectively, in plasma, brain and tumor tissues (19).

Significantly, in a nonhuman primate model cerebrospinal fluid (CSF) penetration of veliparib was 57 ± 7% (mean ± SD) (28). The peak plasma drug concentration was 0.62 ± 0.18 µM. Plasma

and CSF $AUC_{0-\infty}$ values were 3.7 ± 1.7 and 2.1 ± 0.8 μM/h, respectively.

SAFETY

In general, veliparib has a tendency to aggravate the side effects of any given chemotherapeutic agent with which it is paired. Thus, in studies with temozolomide (29, 30) and topotecan (31), side effects have included increased myelosuppression and nausea. Detailed results from single-agent studies, e.g., in *BRCA* patients, have not been reported to date. It is our general impression derived from discussions with investigators (derived from early experience with veliparib) that the drug is generally well tolerated as a single agent, with no obvious side effects.

CLINICAL STUDIES

Veliparib has shown promise in preclinical studies and is currently under clinical investigation. It is of interest to note that veliparib was the first agent to enter the Food and Drug Administration (FDA) Phase 0 program (32, 33). Good bioavailability was demonstrated, with a $t_{1/2}$ of several hours and clearance 24 h after dosing. PARP inhibition of > 85% lasting up to 24 h after administration of veliparib was noted (33). In the context of this phase 0 study, tumor biopsies were obtained at time points after veliparib administration. Results demonstrated increases in the expression of PARP-1 in the majority of tumor samples (32), possibly due to a compensatory or feedback mechanism. These results serve as a platform for ongoing studies, as they highlight the importance of dosing and pharmacodynamic evaluation. In this context, current phase I and II studies have focused on determining dosing schedules, dose-limiting toxicities and pharmacokinetics. This section will review the current clinical data for veliparib as a single agent and in combination with chemotherapy.

There are currently 29 active veliparib clinical trials. Of these, 23 are National Cancer Institute (NCI)-sponsored. Based on preclinical and clinical data, several trials are focused on combinations in breast and ovarian cancers with known *BRCA1* and *BRCA2* mutations, but a broader array of tumor types are also being evaluated. Current phase I trials are focused on the safety and tolerability of veliparib in combination with cytotoxic agents, including temozolomide, carboplatin, cyclophosphamide, gemcitabine, doxorubicin and whole-brain irradiation. Ongoing clinical trials in hematological malignancies, melanoma, glioblastoma multiforme, colorectal cancer and treatment-refractory tumors are focused on combination therapy. Additionally, there is an ongoing single-agent veliparib study sponsored by the Cancer Therapy Evaluation Program (CTEP). Current clinical trials with veliparib can also be reviewed on the U.S. National Institutes of Health (NIH) website (www.ClinicalTrials.gov).

Topotecan is currently FDA-approved for the treatment of SCLC, metastatic ovarian cancer and cervical cancer. Topotecan causes DNA damage by binding to topoisomerase 1 in the S phase of the cell cycle. Topotecan stabilizes the cleavable complex and prevents re-ligation of the cleaved DNA strand, leading to DNA strand breaks that activate the DNA damage signaling pathway (34). Activation of this pathway can be detected by an immunofluorescence assay that quantifies γ-H2AX foci in peripheral mononuclear cells (PBMCs) and/or tumor biopsies when compared pre- and post-treatment (35). PARP inhibition by veliparib is hypothesized to potentiate the antineoplastic activity of topotecan by preventing repair of double-strand DNA breaks. This has led to further investigation of veliparib and topotecan in combination in clinical trials in patients with advanced malignancies.

A recently published abstract by Ji et al. (35) evaluated the pharmacodynamics of veliparib and topotecan alone or in combination through quantitative detection of PARP activity and γ-H2AX foci in PBMCs and tumor samples in 24 patients with refractory solid tumors and lymphoma. Five dosing schedules were created and patients were evaluated pre- and post-treatment for response by PAR expression and γ-H2AX. Phosphorylation of histone γ-H2AX indicates activation of the DNA damage signaling pathway. In this study, γ-H2AX and PAR expression were quantified by an immunofluorescence assay and ELISA, respectively, in PBMCs. There was a 50% reduction in PAR in PBMCs in 13 of 18 patients. Reduction of PAR was highest at dose level –2 (veliparib 10 mg p.o. every 12 h on days 2-5) and topotecan (0.75 mg/m^2/day i.v. on days 1-5). The addition of topotecan did not appear to alter the ability of veliparib to reduce PAR levels when given in combination. Topotecan alone was able to induce elevated levels of γ-H2AX at two dose levels (0.9 and 1.2 mg/m^2/day alone). Veliparib in combination with topotecan demonstrated increased levels of γ-H2AX at a topotecan dose demonstrating potentiated DNA damage. Three of seven patients had increased γ-H2AX on topotecan 0.6 mg/m^2/day i.v. on days 1-5 and three of three patients had increased γ-H2AX on topotecan 0.75 mg/m^2/day i.v. on days 1-5 with the same veliparib dose (10 mg p.o. every 12 h on days 2-5). The upregulation of γ-H2AX also corresponded to a statistically significant reduction in PAR, suggesting a corresponding increase in PARP inhibition. At dose level –3 (topotecan 0.6 mg/m^2/day on days 1-5 and veliparib 10 mg p.o. every 12 h on days 2-5), three of seven patients who had increased γ-H2AX (γ-H2AX responders) had a corresponding decrease in mean PAR expression of 97.8 ± 5.5% versus 52.3 ± 28.1% in patients who did not respond (i.e., did not have an increase in γ-H2AX). The results of this phase I trial suggest that veliparib retains the ability to inhibit PARP in combination with topotecan and the DNA damage caused by topotecan is potentiated by the addition of veliparib. In a similar study, the combination of veliparib and topotecan at 0.75 mg/m^2/day x 5 produced dose-limiting toxicity (DLT) of (grade 4) neutropenia and thrombocytopenia necessitating dose de-escalation to 0.6 mg/m^2/day x 5 (31).

Zhao et al. (36) studied veliparib in patients with acute leukemia in combination with topotecan with or without carboplatin. Plasma and bone marrow samples were obtained on day 1 of veliparib alone and on day 4 after the first dose of veliparib in combination with topotecan or topotecan plus carboplatin. Following a single oral dose of veliparib 10 mg, the maximum plasma concentration achieved was 269 ± 107 nM at 2.0 h (0.25-8.1 h). When combined with topotecan or carboplatin, there was no change in veliparib pharmacokinetics. After multiple doses, the maximum plasma concentration achieved was 413 ± 125 nM at 1.1 h (0.5-4.0 h). Veliparib was detected in marrow supernatant (122 ± 127 nM on day 1 and 162 ± 55 nM on day 4) and bone marrow cells (0.35 ± 0.17 nmol/10^6 cells on day 1 and 0.22 ± 0.30 nmol/10^6 cells on day 4).

Cyclophosphamide is an alkylating agent that prevents cell division by crossing DNA strands, causing DNA damage and decreasing DNA synthesis. Cyclophosphamide is FDA-approved for the treatment of breast and ovarian cancer, hematological malignancies, neuroblastoma and retinoblastoma. Combination of cyclophosphamide with PARP inhibitors is attractive given the ability of cyclophosphamide to cause DNA damage, as well as the growing body of evidence for PARP inhibition in the treatment of breast cancer in patients with *BRCA1* and *BRCA2* mutations. Two phase I clinical trials were recently published at ASCO 2010 that combined veliparib with p.o. or i.v. cyclophosphamide.

The phase I dose-escalation clinical trial by Tan et al. (37) determined the MTD, DLT and pharmacokinetics of veliparib in combination with i.v. cyclophosphamide. Eighteen patients with advanced cancer and performance status ≤ 2 were enrolled. Five dosing schedules were evaluated for veliparib and cyclophosphamide (10/450, 20/450, 50/600 and 50/750 mg). Cycle length was 21 days, with a mean number of 4 cycles administered. Cyclophosphamide was given on day 3 and veliparib was given every 12 h on days 1-4. Patients were monitored for drug-related toxicities, which included mainly grade 1 and 2 fatigue (28%), neutropenia (11%), thrombocytopenia (6%) and vomiting (6%). One DLT occurred in one patient with NHL who developed grade 2 thrombocytopenia that caused a delay of > 2 weeks in starting cycle 2 veliparib/cyclophosphamide (50/450 mg). PARP inhibition was measured in PBMCs by ELISA. The study showed a 50% reduction in PAR in 11 of 18 patients, demonstrating PARP inhibition when veliparib is used in combination with cyclophosphamide. The MTD was not presented in the abstract, as patients were still accruing at higher dose levels at the time of publication. Pharmacokinetic evaluation showed that veliparib reached steady-state plasma concentrations on day 3, and the AUC (2.71 ± 0.8 μg.h/mL vs. 2.68 ± 1.2 μg.h/mL) and clearance (19.6 ± 4.5 L/h vs. 21.6 ± 7.5 L/h) of veliparib were not altered for cycle 1 or 2, suggesting that the addition of cyclophosphamide on day 3 did not alter veliparib pharmacokinetics. The AUC and clearance of cyclophosphamide were also not altered by veliparib. The study was not powered to assess response, although the authors reported that 4 of 18 patients (1 male breast cancer, 1 bladder cancer, 1 NSCLC and 1 colon cancer) had stable disease for > 8 weeks. The preliminary results of this study (updated at the time of presentation) showed that veliparib (200 mg p.o. every 12 h on days 1-4) in combination with cyclophosphamide (750 mg/m^2 i.v. on day 3) is well tolerated.

Metronomic cyclophosphamide is an oral form of cyclophosphamide recently evaluated in a phase I clinical trial in combination with veliparib (38). The potential for a combination oral-only therapy is attractive due to ease of administration. Eighteen patients were enrolled on 6 dosing schedules. The primary objective was to determine safety, tolerability and the MTD of combination therapy, assess the pharmacokinetics of veliparib and evaluate PARP inhibition in PBMCs and tumor samples. PARP inhibition was demonstrated by reduction in PAR on ELISA in PBMCs. The patient population included adults with advanced solid tumors and lymphomas: ovarian (n = 5), breast (n = 6), gastrointestinal (n = 3), NHL (n = 2), sarcoma (n = 1) and melanoma (n = 1). The authors reported drug-related toxicities, including three patients with grade 3 or 4 lymphopenia and two patients with grade 2 neutropenia. PARP inhibition was demonstrated by a reduction in PAR levels of ≥ 50% in 16 of 18 patients. Stable disease was seen in two male patients with *BRCA*$^+$ breast cancer for eight cycles and partial response was seen in three patients, two with *BRCA*$^+$ ovarian cancer and one with *BRCA* triple-negative breast cancer. The results demonstrated good overall tolerability for the combination oral regimen. MTD had not yet been reached. The results of these studies suggest that veliparib is well tolerated in combination with cyclophosphamide both p.o. and i.v., and have set the stage for phase II combination trials.

Molina et al. presented preliminary results of an ongoing phase I study of veliparib in combination with temozolomide in 29 patients with nonhematological malignancies and metastatic melanoma (30). The combination was well tolerated, with the main toxicities to date of neutropenia and thrombocytopenia. The investigators reported a partial response in one patient with hepatocellular carcinoma who had previously failed sorafenib. Pharmacokinetic data were not reported at the time of presentation.

Isakoff et al. published an abstract for a phase II clinical trial that evaluated veliparib with temozolomide in patients with metastatic breast cancer (29). Temozolomide has very little activity in breast cancer alone. However, the preclinical data presented above demonstrated promise for combination therapy with temozolomide and veliparib due a synergistic effect seen in murine breast cancer models, as veliparib presumably inhibits base excision repair-mediated resistance. The combination of temozolomide and veliparib (19) is also an attractive combination, as both drugs cross the blood–brain barrier. This phase II trial enrolled 41 patients with metastatic breast cancer with triple-negative and *ER/HER2*$^+$ subtypes. The primary endpoint was overall response rate. Secondary endpoints included progression-free survival, overall survival, safety and toxicity. Patients were treated with temozolomide 150 mg/m^2 on days 1-5 and veliparib 40 mg p.o. every 12 h on days 1-7 of a 28-day cycle. The dose of veliparib was reduced to 30 mg b.i.d. after grade 4 thrombocytopenia was observed. The study was designed to assess disease progression every two cycles by repeat imaging using RECIST criteria. Two primary drug-related toxicities were seen: grade 3-4 thrombocytopenia and grade 2-3 neutropenia. The results of the trial were preliminarily reported in an abstract published at ASCO 2010 and updated at the meeting. Responses were only seen in *BRCA* carriers (n = 8): one complete response, two partial responses, two stable disease and three progressive disease. There were no responses in 33 non-*BRCA* patients. The authors also concluded that the combination of veliparib and temozolomide is reasonably well tolerated, with activity seen in several patients. The final efficacy results are still pending.

Zhao et al. have taken another approach to using veliparib, i.e., as a single agent (39). The authors recognized that *BRCA* gene deficiency is a predictor of response to PARP inhibitors. Thus, they wished to potentially take advantage of the fact that *BRCA* is one of several genes involved in the Fanconi anemia (FA) repair pathway. The hypothesis is that cancers with an absence of *FANCD2* foci formation, a surrogate marker for somatic functionality of the FA pathway, would be sensitive to PARP inhibition by veliparib. The concept is to extend the population of patients that might benefit from PARP inhibition, as the number of cancer patients with germ-line *BRCA* deficiency is low. Using an immunofluorescence-based method to

evaluate somatic functionality of the pathway (*FANCD2* foci formation) in paraffin-embedded tumor tissues, they hypothesized that they could identify patients more suitable for treatment with PARP inhibitors. Sixty-four patients have been enrolled so far; all have been screened for an absence of *FANCD2* foci formation. Of these, 20% were *FANCD2*-deficient and four patients have initiated treatment. This ongoing clinical trial will help determine if these patients are more susceptible to veliparib treatment.

At least three ongoing clinical studies have focused on the use of veliparib in the setting of CNS disease. There is a multi-institutional, company-sponsored study (M10-128) which combines veliparib with whole-brain irradiation in subjects with brain metastases, and is addressing safety, tolerability and pharmacokinetics. The Adult Brain Tumor Consortium (ABTC), a cooperative group sponsored by the NCI, launched a phase I/II trial (NABTT 0801) of temozolomide and veliparib in subjects with newly diagnosed glioblastoma. The use of temozolomide during and after irradiation is the standard approach to newly diagnosed glioblastoma (40), known as the Stupp regimen. In this study, veliparib is administered during radiotherapy with temozolomide and after radiotherapy with temozolomide. Another NCI-sponsored cooperative group, the Radiation Therapy Oncology Group (RTOG), has just opened a clinical trial (RTOG 0929) in patients with relapsed glioblastoma. Patients enrolled in this study will have progressed after the Stupp regimen (see above) and will be rechallenged with temozolomide in combination with veliparib.

CONCLUSIONS

As reviewed above, the orally active agent veliparib is one of several PARP inhibitors. It is known to cross the blood–brain barrier and appears to have a favorable toxicity profile as a single agent. In addition to other members of this drug class, it offers promise as an effective chemotherapy and/or radiation potentiator. Furthermore, in *BRCA*-deficient tumors these drugs, as single agents, have provided the first clinical example of a successful exploitation of tumor synthetic lethality. The notion of related DNA repair pathways (discussed above), which may also be sensitive to PARP inhibitors, requires further delineation with appropriate markers. Ultimately, the future of these exciting drugs will be rooted in the careful design of clinical studies with validated pharmacodynamics, as well as predictive and prognostic markers.

SOURCE

Abbott Laboratories, Inc. (US).

DISCLOSURES

H.I. Robins is the principal investigator on an investigator-initiated NCI-sponsored RTOG study (RTOG 0929) of veliparib and temozolomide in recurrent glioblastoma. The other authors declare no conflicts of interest.

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