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Miguel Villalona Calero

Baptist Health Medical Group; Miami Cancer Institute, miguelvil@baptisthealth.net

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Phase I Study of Veliparib on an Intermittent and Continuous Schedule in Combination with Carboplatin in Metastatic Breast Cancer: A Safety and [18F]-Fluorothymidine Positron Emission Tomography Biomarker Study

Robert Wesolowski,[a,b,†] Daniel G. Stover,[a,b,†] Maryam B. Lustberg,[a] Abigail Shoben,[b] Meng Zhao,[a] Ewa Mrozek,[c] Rachel M. Layman,[d] Erin Macrae,[e] Wenrui Duan,[b] Jun Zhang,[b] Nathan Hall,[b] Chadwick L. Wright,[b] Susan Gillespie,[a] Michael Berger,[a] Jeffrey J. Chalmers,[b] Alahdra Carey,[b] Priya Balasubramanian,[b] Brandon L. Miller,[b] Peter Amaya,[b] Eleni Andreopoulou,[f] Joseph Sparano,[g] Charles L. Shapiro,[h] Miguel Angel Villalona-Calero,[i] Susan Geyer,[j] Alice Chen,[k] Michael R. Grever,[b] Michael V. Knopp,[b] Bhuvaneswari Ramaswamy[a]

[a]Stefanie Spielman Comprehensive Breast Center, The Ohio State University, Columbus, Ohio, USA; [b]The Ohio State University Comprehensive Cancer Center, Columbus, Ohio, USA; [c]Mercy Health – St. Rita's Medical Center, Lima, Ohio, USA; [d]University of Texas MD Anderson Cancer Center, Houston, Texas, USA; [e]Columbus Oncology, Columbus, Ohio, USA; [f]Weill Cornell Medicine, New York, New York, USA; [g]Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, New York, USA; [h]Tisch Cancer Institute, Mt. Sinai Hospital, New York, New York, USA; [i]Miami Cancer Institute, Baptist Health South Florida, Miami, Florida, USA; [j]University of South Florida, Tampa, Florida, USA; [k]National Cancer Institute, Bethesda, Maryland, USA

[†]Contributed equally.

Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Metastatic breast cancer • Poly(ADP-ribose) polymerase inhibitors • Phase I clinical trials • Homologous recombination DNA repair • Fluorothymidine positron emission tomography scan

Abstract

Background. Poly(ADP-ribose) polymerase inhibitors (PARPis) are U.S. Food and Drug Administration (FDA) approved for treatment of *BRCA*-mutated metastatic breast cancer. Furthermore, the BROCADE studies demonstrated benefit of adding an oral PARPi, veliparib, to carboplatin and paclitaxel in patients with metastatic breast cancer harboring *BRCA* mutation. Given multiple possible dosing schedules and the potential benefit of this regimen for patients with defective DNA repair beyond *BRCA*, we sought to find the recommended phase II dose (RP2D) and schedule of veliparib in combination with carboplatin in patients with advanced breast cancer, either triple-negative (TNBC) or hormone receptor (HR)-positive, human epidermal growth receptor 2 (HER2) negative with defective Fanconi anemia (FA) DNA-repair pathway based on FA triple staining immunofluorescence assay.

Materials and Methods. Patients received escalating doses of veliparib on a 7-, 14-, or 21-day schedule with carboplatin every 3 weeks. Patients underwent [18]fluoro-3′-deoxythymidine (^{18}FLT) positron emission tomography (PET) imaging.

Results. Forty-four patients (39 TNBC, 5 HR positive/HER2 negative with a defective FA pathway) received a median of 5 cycles (range 1–36). Observed dose-limiting toxicities were grade (G) 4 thrombocytopenia (n = 4), G4 neutropenia (n = 1), and G3 akathisia (n = 1). Common grade 3–4 toxicities included thrombocytopenia, lymphopenia, neutropenia, anemia, and fatigue. Of the 43 patients evaluable for response, 18.6% achieved partial response and 48.8% had stable disease. Median progression-free survival was 18.3 weeks. RP2D of veliparib was established at 250 mg twice daily on days 1–21 along with carboplatin at area under the curve 5. Patients with partial response had a significant drop in maximum standard uptake value (SUV_{max}) of target lesions between baseline and early in cycle 1 based on ^{18}FLT-PET (day 7–21; p_{trend} = .006).

Conclusion. The combination of continuous dosing of veliparib and every-3-week carboplatin demonstrated activity and an acceptable toxicity profile. Decrease in SUV_{max} on ^{18}FLT-PET scan during the first cycle of this therapy can identify patients who are likely to have a response. ***The Oncologist*** 2020;25:1–12

Correspondence: Bhuvaneswari Ramaswamy, M.D., M.R.C.P., The Ohio State University Wexner Medical Center, James Comprehensive Cancer Center, 460 W. 10th Ave., Columbus, Ohio 43210, USA. Telephone: 614-366-4851; e-mail: bhuvaneswari.ramaswamy@osumc.edu Received January 16, 2020; accepted for publication May 14, 2020. http://dx.doi.org/10.1634/theoncologist.2020-0039

Implications for Practice: The BROCADE studies suggest that breast cancer patients with *BRCA* mutation benefit from addition of veliparib to carboplatin plus paclitaxel. This study demonstrates that a higher dose of veliparib is tolerable and active in combination with carboplatin alone. With growing interest in imaging-based early response assessment, the authors demonstrate that decrease in [18]fluoro-3′-deoxythymidine positron emission tomography (FLT-PET) SUV_{max} during cycle 1 of therapy is associated with response. Collectively, this study established a safety profile of veliparib and carboplatin in advanced breast cancer while also providing additional data on the potential for FLT-PET imaging modality in monitoring therapy response.

Introduction

Poly (ADP-ribose) polymerase (PARP) proteins sense single-strand DNA breaks, signal the presence of DNA damage, generate linear and branched poly(ADP-ribose) chains, recognize topoisomerase I cleavage complexes, and facilitate base excision repair (BER) [1–3]. PARP-1 and PARP-2 are considered the primary enzymes involved in the repair of single-stranded DNA breaks through the BER pathways [4], and enhanced PARP-1 expression and/or activity is one of the mechanisms by which tumor cells evade apoptosis caused by DNA-damaging agents [5, 6].

BRCA1 and *BRCA2* proteins are essential for homologous recombination repair, an error-free DNA double-strand break (DSB) repair pathway. Synthetic lethality due to defects in homologous recombination and BER that cooperate to repair DNA damage and dependence of non-homologous end joining (NHEJ) repair pathway is a popular hypothesis to account for the increased sensitivity of *BRCA1/2*-deficient cells to PARP inhibitors (PARPis) [7–9]. In preclinical studies, *BRCA*-deficient cells are more sensitive to platinum drugs than *BRCA*-proficient counterparts both in vitro and in vivo, and combining PARPis and platinum agents was shown to be synergistic [10, 11]. Germline or somatic mutations in *BRCA1* or *BRCA2* genes occur in approximately 25% of patients with triple-negative breast cancer (TNBC), which is more frequent compared with other breast cancer types. Clinical trials studying regimens containing platinum regimens have also demonstrated that patients with triple-negative breast cancer respond well to these agents [12–14].

Although *BRCA1/2* alterations are the most well-established biomarkers for response to PARPi and platinum chemotherapy, it is clear that a larger subset of non-*BRCA1/2* mutated TNBCs as well as some estrogen receptor–positive (ER+)/human epidermal growth receptor 2–negative (HER2−) breast cancers could also respond to these agents. *BRCA1/2* are part of the Fanconi anemia (FA) network of proteins that function in DNA-damage response to maintain genome integrity [8, 9, 15]. The FA network of proteins include around 19 members, many of which are mutated in FA syndrome, including *BRCA2*/FANCD1, but also additional interacting proteins involved in regulating DNA damage responses like ataxia telangiectasia, Rad3 related protein, and *BRCA1* [16, 17]. This suggests that tumors with dysfunction in any of the components of the FA network may also be particularly susceptible to PARP inhibition. The common hallmark of defective FA core complex, such as Fanconi Anemia Group F methylation, is lack of ubiquitination of FANCD2, leading to lack of FANCD2 foci in the nuclei of the tumor cells in S phase [17]. Studies provide evidence that link disruption of FA/*BRCA* cascade and sporadic cancers [18, 19] and an association between Fanconi complementaton group D2 (*FANCD2*) gene variants and sporadic breast cancer risk has been reported [20]. We hypothesized that a subset of breast tumors with defective DNA repair arising from loss of homologous recombination due to inactivation of the *BRCA*/FA pathway, so-called "*BRCA*ness," will be susceptible to treatment with platinum in combination with PARP inhibitor, similar to hereditary breast and ovarian cancers with *BRCA1/2* germ-line mutations [21]. Therefore, this provides a rationale to study combinations of platinum drugs with PARP inhibitors in patients with advanced triple-negative breast cancer and/or ER+/HER2− breast cancers with evidence of *BRCA*/FA pathway inactivation [22].

3′-deoxy-3′-[F-18] fluorothymidine (^{18}FLT) is a radiolabeled imaging agent with structural analog of the DNA constituent, thymidine [23]. The activity of this radiolabeling agent is dependent on cells undergoing DNA replication, and hence, the uptake of FLT is dependent on the proliferative rate of the cells. This is in contrast to a more conventional 5-fluorodeoxyglucose (FDG) positron emission tomography (PET) scan where uptake of FDG depends on high intake of glucose reflective of increased metabolic rate and the Warburg effect. Antiproliferative treatments that inhibit mitosis will result in marked decline in uptake of ^{18}FLT and hence can be used to measure response to therapy by using radioactively labeled ^{18}FLT as a contrast agent for PET scan (^{18}FLT-PET). Uptake of ^{18}FLT during therapy with PARP inhibitors and DNA-damaging agents lends itself well as an attractive imaging modality to study changes in DNA synthesis and early response to this therapy.

This multicenter phase I study (NCI8609), sponsored by the Cancer Therapy Evaluation Program (CTEP) at the National Cancer Institute (NCI), sought to assess the recommended phase II dose (RP2D) of veliparib on an intermittent (7- or 14-day) or continuous (21-day) schedule in combination with every-3-week schedule of carboplatin in patients with advanced breast cancer that was either triple negative or hormone receptor (HR) positive (estrogen and/or progesterone receptor positive), HER2 negative with defective FA pathway based on lack of FANCD2 foci in the nuclei of proliferating tumor cells detected by FA triple stain immunofluorescence (FATSI) assay. We report the primary endpoint of RP2D and schedule of veliparib in combination with carboplatin, and secondary endpoints of efficacy. We also report an exploratory endpoint of correlation of dose and schedule of veliparib and carboplatin on tumor proliferation and induction DNA damage in the tumor by analyzing ^{18}FLT PET scans and phosphorylated histone H2AX (γH2AX) as an indicator of DNA damage in circulating tumor cells (CTCs), respectively.

Materials and Methods

Patients

Patients eligible for the trial were adult women with metastatic or locally advanced inoperable breast cancer that fulfilled one of the three criteria: (a) negative for estrogen, progesterone, and HER2 receptors (based on American Society of Clinical Oncology/College of American Pathologists guidelines); (b) HR-positive (defined as ER and/or progesterone receptor positive), HER2-negative breast cancer that is deficient for the FA pathway based on the FATSI test (i.e., no FANCD2 foci in nuclei of 100 proliferating tumor cells); or (c) HER2-negative breast cancer with known germline *BRCA1/2* mutation. HR-positive/HER2-negative patients without known germline *BRCA* 1 or 2 mutation initially signed a screening consent for testing their archival tumors for FATSI and only proceeded with the therapeutic portion of the study if testing showed deficiency in FA pathway. Other eligibility criteria requirements included no more than three prior chemotherapy regimens for metastatic disease, at least 4 weeks from prior chemotherapy or radiation therapy, an Eastern Cooperative Oncology Group performance status of 0–2, and adequate bone marrow, renal, and hepatic function. Patients with treated central nervous system (CNS) metastasis were eligible. Prior platinum exposure was allowed. Exclusion criteria included prior therapy with veliparib or other PARP inhibitors for metastatic disease, uncontrolled intercurrent illness including ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, psychiatric illness/social situations that would limit compliance with study requirements, known human immunodeficiency virus infection, seizures, and uncontrolled CNS metastasis.

Ethics

All patients provided written informed consent. This study was approved by the local institutional review boards at Ohio State University (OSU) and each participating site and conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. This trial was registered with ClinicalTrials.gov on December 2, 2010, with identifier NCT01251874.

Study Design and Treatment

This was a multicenter, CTEP-sponsored, single-arm phase I trial of veliparib on an intermittent (7- or 14-day) or continuous (21-day) schedule given in combination with carboplatin in patients with advanced breast cancer. The study used a standard 3 + 3 dose escalation design. The primary objective was to determine the recommended phase II dose of veliparib in combination with carboplatin defined as the maximum tolerated dose (MTD) or the highest dose level (if MTD could not be determined). Other objectives included assessment of safety and tolerability and preliminary efficacy of the combination.

Veliparib was initiated at 50 mg, twice daily (b.i.d.), orally for 1–7 days of 21-day cycles (dose level 1 and 1A). If tolerated, the schedule of veliparib was escalated to days 1–14 of a 21-day cycle (dose levels 2–5) and then to continuous dosing (dose levels 6–7). Dose of carboplatin was held stable in all dose levels at area under the curve (AUC) of 5 mg/mL × minute (except for dose level I where the dose was AUC 6). Dose escalation of veliparib proceeded using a standard phase I dose escalation in cohorts of 3–6 patients for dose level (DL) 1–7.

Patients enrolled at Ohio State University underwent ^{18}FDG- and ^{18}FLT-PET/computed tomography (CT) scans, comprising 42/44 (95.5%) of all patients enrolled.

Clinical Assessments

Dose-limiting toxicity (DLT) was defined as a significant adverse event occurring in the first cycle and fulfilling one of the following criteria: grade ≥ 3 nonhematologic toxicity (excluding alopecia, nausea, vomiting, diarrhea, and tumor pain in patients that have not received optimal treatment with antiemetics, antidiarrheal agents, or analgesics), reversible electrolyte abnormalities of grade ≥ 3 unable to be corrected within 24 hours, grade 4 thrombocytopenia, febrile neutropenia, grade 4 neutropenia lasting for 7 days or more, or grade 5 toxicity. The MTD and the RP2D was defined as the highest dose at which no more than one out of six patients experienced a DLT. Adverse events were graded according to NCI Common Toxicity Criteria (version 3.0). Patients came off study for disease progression, treatment delay of more than 3 weeks, unacceptable toxicity, or consent withdrawal. Treatment could be delayed for up to 3 weeks to allow resolution of toxicities, and a patient could have up to two dose reductions of veliparib and/or carboplatin, alone or concurrent, to manage toxicity. Dose reductions of veliparib and/or carboplatin (depending on attribution) were recommended for grade 3 or 4 febrile neutropenia, grade 4 thrombocytopenia, grade 4 neutropenia that lasted for 7 or more days, and any grade 3–4 nonhematological toxicity. Tumor responses were based on blinded radiologist reader assessment of ^{18}FDG-PET/low-dose diagnostic CT scans obtained at baseline and every three cycles (9 weeks) thereafter according to the modified response evaluation criteria in solid tumors (mRECIST), version 1.1. A dedicated and blinded radiologist performed tumor assessments at baseline and after every three cycles thereafter.

Tumor Tissue Screening: FATSI Assay

Eligible patients with HR-positive, HER2-negative breast cancer consented to have their formalin-fixed, paraffin embedded tumor tissue screened for FA functional deficiency using the FATSI test [24]. The FATSI test uses a triple stain with Ki-67, 4′,6-diamidino-2-phenylindole (DAPI), and FANCD2 to identify FANCD2 foci in the nuclei of proliferating neoplastic cells. The assay was performed in a Clinical Laboratory Improvement Amendments (CLIA)–certified laboratory [24]. A negative FATSI test (i.e., absence of FANCD2 in the nucleus of 100 proliferating cells) would identify patients whose tumors were deficient in FA pathway [25]. For patients with TNBC, the FATSI test was performed as a potential biomarker for response to PARPi therapy.

Gamma H2Ax Assay

CTCs were collected at baseline (day 1 and 3 of cycle 1) and serially (day 1, 7, and 14 of cycle 2), on day 1 of every 3 cycles, and at progression, using negative selection technology based on immunomagnetic tagging and removal of CD45+ cells [26, 27]. We measured formation of γH2Ax in the CTCs using

Table 1. Patient demographics

Characteristics	Patients (n = 44), n (%)
Age, median (range), years	58 (31–77)
ECOG PS	
0–1	39 (89)
2	5 (11)
ER/PR status	
ER/PR−	39 (89)
ER+ and/or PR+	5 (11)
No. of metastatic sites	
1–3	38 (86)
>3	6 (14)
No. of prior chemo regimens (metastatic)	
0–1	29 (66)
2	7 (16)
3	8 (18)
Prior platinum exposure	8 (18)
BRCA1/2 mutation	7 (16)
Defect FA pathway	9 (20)

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; ER, estrogen receptor; FA, Fanconi anemia; PR, progesterone receptor.

an antibody targeting the phospho-histone (Clone JBW301, Millipore, Darmstadt, Germany cat no. 05-636) [28]. This primary mouse antibody was subsequently counterstained with a goat, anti-mouse immunoglobulin G conjugated to Alexa Fluor 594 (Invitrogen, Carlsbad, California, A-11005).

FLT-PET to Assess Varying Dose Schedules of PARP Inhibitor

We performed ^{18}FLT-PET/CT imaging at 4 time-points, baseline, cycle 1, day 7 and 14 (for cohorts receiving veliparib on days 1–7 and days 1–14 of every cycle) or cycle 1, day 14 and 21 (for cohorts treated with veliparib on days 1–21 of every cycle), and after cycle 3, day 1 to assess change in the uptake of FLT between patients with and without response. The imaging pharmaceuticals were commercially produced according to Good Manufacturing Practices (GMP) and under the Nuclear Regulatory Commission (NRC) license from Cardinal Health, which has been FDA/NRC approved to produce these PET radiotracers. The average ^{18}FLT dose used was 10 mCi with an average FLT injection-to-scan time of 63 minutes. Patients were imaged using Philips Gemini TF 64 PET/CT system (Philips, Amsterdam, The Netherlands) with a whole-body acquisition (either vertex of the head to the toes at 90 seconds per bed or vertex of the head to mid-thighs at 90 seconds per bed followed by mid-thighs to toes at 60 seconds per bed). PET data were reconstructed using time of flight with a 144 × 144 matrix, 4 mm isometric voxel size, standard reconstruction with 3 iterations and 33 subsets. Target lesions were identified by a blinded reader on the baseline ^{18}FDG-PET/CT enabled by simultaneous PET and CT review. Lesions were track-matched with the ^{18}FDG-PET/CT and semiquantitatively assessed using 3D region of interest (ROI) placement in a matched, blinded fashion. For quantitative assessment of tumor FDG and FLT uptake, ROIs were placed over tissues with FDG/FLT activity and SUV measurements determined. Maximum SUV was calculated from the activity concentration in the tumor ROIs. Comparison between ^{18}FLT-PET and ^{18}FDG-PET/CT was prespecified in the study protocol.

Statistical Analysis

The safety population included patients who received at least one dose of study drug. Adverse events were summarized descriptively by attribution of study therapy (unlikely, probably, likely, and definitely related) and grade using Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. Laboratory variables were summarized using mean change in value from baseline to scheduled time points for each dose level group and 95% confidence interval. Laboratory values were also categorized according to their CTCAE version 3.0 toxicity grade and tabulated by worst on-study toxicity grade and dose level group. Progression-free survival was estimated using Kaplan-Meier methods. All analyses conducted were using Stata for Windows and R version 3.4.1.

Results

Patient Demographics

Between December 2010 and April 2013, 44 patients with metastatic or locally advanced inoperable breast cancer were enrolled from The Ohio State University Comprehensive Cancer Center (OSUCCC) and Montefiore Medical Center and received a median 5 cycles (range 1–36). Patients received up to three lines of prior chemotherapy regimens for metastatic disease. Forty-two of 44 (95.5%) patients were enrolled at OSUCCC, and these patients underwent ^{18}FLT-PET/CT scans in addition to standard-of-care ^{18}FDG-PET/CT scans. The baseline characteristics of the patients are outlined in Table 1. Thirty-nine patients had TNBC and five patients had HR-positive/HER2-negative metastatic breast cancer (MBC) with functional deficiency of FA pathway based on negative FATSI assay. Thirty-four TNBC tumors were tested for FA deficiency using the FATSI test. Of these, four (11.8%) were found to have functional deficiency of FA pathway.

Dose-Limiting Toxicities and Safety

All patients were evaluable for toxicity from the time of their first treatment with veliparib. Three patients were enrolled on dose level 1 (veliparib 50 mg b.i.d. for 7 days) with carboplatin at AUC 6 every 21 days. One patient developed grade (G) 4 thrombocytopenia, and the dose level expanded to six patients. Two more patients in this dose level developed DLTs (G4 thrombocytopenia and G4 neutropenia). The protocol was subsequently amended, and the carboplatin dose was reduced to AUC 5 for all subsequent dose levels. No DLTs were observed in the three patients subsequently enrolled to dose level 1A with veliparib 50 mg b.i.d. for 7 days and carboplatin AUC 5. Patients were then enrolled on escalating doses of veliparib for 14 days, and MTD was not reached at dose level 5 (Table 2). After further discussions with the NCI, the veliparib schedule was changed to continuous dosing and two dose levels were planned with this schedule. No DLTs were observed at the highest planned dose of veliparib (250 mg b.i.d. for 21 days)

Table 2. Summary of dose levels and dose-limiting toxicities

Dose level	Carboplatin dose (AUC)	Veliparib dose/schedule (days in each 21-day cycle)	*n*	Dose-limiting toxicities
1	6	50 mg b.i.d. (1–7)	7	G4 thrombocytopenia (*n* = 2) G4 neutropenia (*n* = 1)
1A	5	50 mg b.i.d. (1–7)	3	None
2	5	50 mg b.i.d. (1–14)	6	G4 thrombocytopenia (*n* = 1)
3	5	100 mg b.i.d. (1–14)	3	None
4	5	150 mg b.i.d. (1–14)	6	G3 akathisia (*n* = 1)
5	5	200 mg b.i.d. (1–14)	6	G4 thrombocytopenia (*n* = 1)
6	5	200 mg b.i.d. (1–21)	7	G4 thrombocytopenia (*n* = 1)
7	5	250 mg b.i.d. (1–21)	6	None

Abbreviations: AUC, area under the curve; G, grade.

Table 3. Overall number of patients with toxicity grade 2 or above

Toxicity	Max of G2, *n* (%)	Max of G3, *n* (%)	Max of G4, *n* (%)
Fatigue	31 (70)	4 (9)	0 (0)
Akathisia	0 (0)	1 (2)	0 (0)
Vomiting	10 (23)	3 (7)	0 (0)
Diarrhea	8 (18)	1 (2)	0 (0)
Dysesthesia	0 (0)	1 (2)	0(0)
Epistaxis	0 (0)	1 (2)	0 (0)
Respiratory, thoracic, and mediastinal disorders	1 (2)	1 (2)	0 (0)
Headache	11 (25)	3 (7)	0 (0)
Hypoglycemia	1 (2)	1 (2)	0 (0)
Thrombocytopenia	14 (32)	15 (34)	9 (20)
Lymphopenia	20 (45)	10 (23)	1 (2)
Neutropenia	20 (45)	8 (18)	2 (5)
Leukopenia	28 (64)	5 (11)	3 (7)
Anemia	30 (68)	8 (18)	0 (0)

Abbreviation: G, grade.

in combination with carboplatin on day 1 (dose level 7). Dose escalation, number of patients in each cohort, and DLTs are outlined in Table 2. To better assess tolerability, we compared the toxicity data between cycle 1 and cycles 2 and 3 within each dose level, and no new DLT was observed in cycle 2 or 3 as compared with cycle 1. We did not observe any DLT on dose level 7, but four out of the six patients eventually required dose reductions of carboplatin and/or veliparib for thrombocytopenia or nausea. Therefore, the RP2D of veliparib in combination with carboplatin (AUC5 every 3 weeks) was determined to be 250 mg b.i.d. on a continuous schedule in dose level 7 (the highest dose level).

Fifty percent (*n* = 22) of patients required dose reductions of either one or both agents primarily for myelosuppression (in particular, thrombocytopenia). Thirty-three (75%) patients experienced at least one or more grade 3 or 4 toxicities, which were attributable to study treatment (Table 3). The most common and clinically significant grade 3–4 toxicity events were hematologic and included thrombocytopenia, neutropenia, and anemia. Among nonhematologic toxicities that were G3 or higher, the most common were fatigue and vomiting (Table 3). No grade 5 toxicities were reported. Reasons for discontinuation of study therapy included disease progression (*n* = 40), patient withdrawal (*n* = 1), adverse events (prolonged neutropenia; *n* = 1), and death due to disease progression on study (*n* = 1).

Efficacy

Of 44 patients, 1 patient on DL 4 withdrew from the study after receiving only two cycles and was therefore not evaluable for response. Of the remaining 43 evaluable patients, 18.6% had a partial response (PR; *n* = 8); 48.8% had stable disease (SD; *n* = 21) as best response (Fig. 1A). Of 21 patients with SD, 10 (23.3%) had SD >24 weeks, providing a clinical benefit rate (CBR) of 41.9%. The median progression-free survival (PFS) for all patients who received at least one cycle of therapy was 18.3 weeks (95% confidence interval [CI]: 10.9–22.0 weeks), and there was no significant difference in PFS across veliparib dosing schedule (log-rank *p* = .87; Fig. 1B). Among the eight patients with PR, median duration of response was 28.3 weeks (95% CI: 15.4–60.1 weeks). Median overall survival was 62.6 weeks (95% CI: 33.9–87.1 weeks; Fig. 1C).

Deficiency in Homologous Recombination DNA Repair and Response

Of the nine patients with FATSI demonstrating defective FA pathway, 22.2% achieved PR (*n* = 2), 55.6% had SD (*n* = 5),

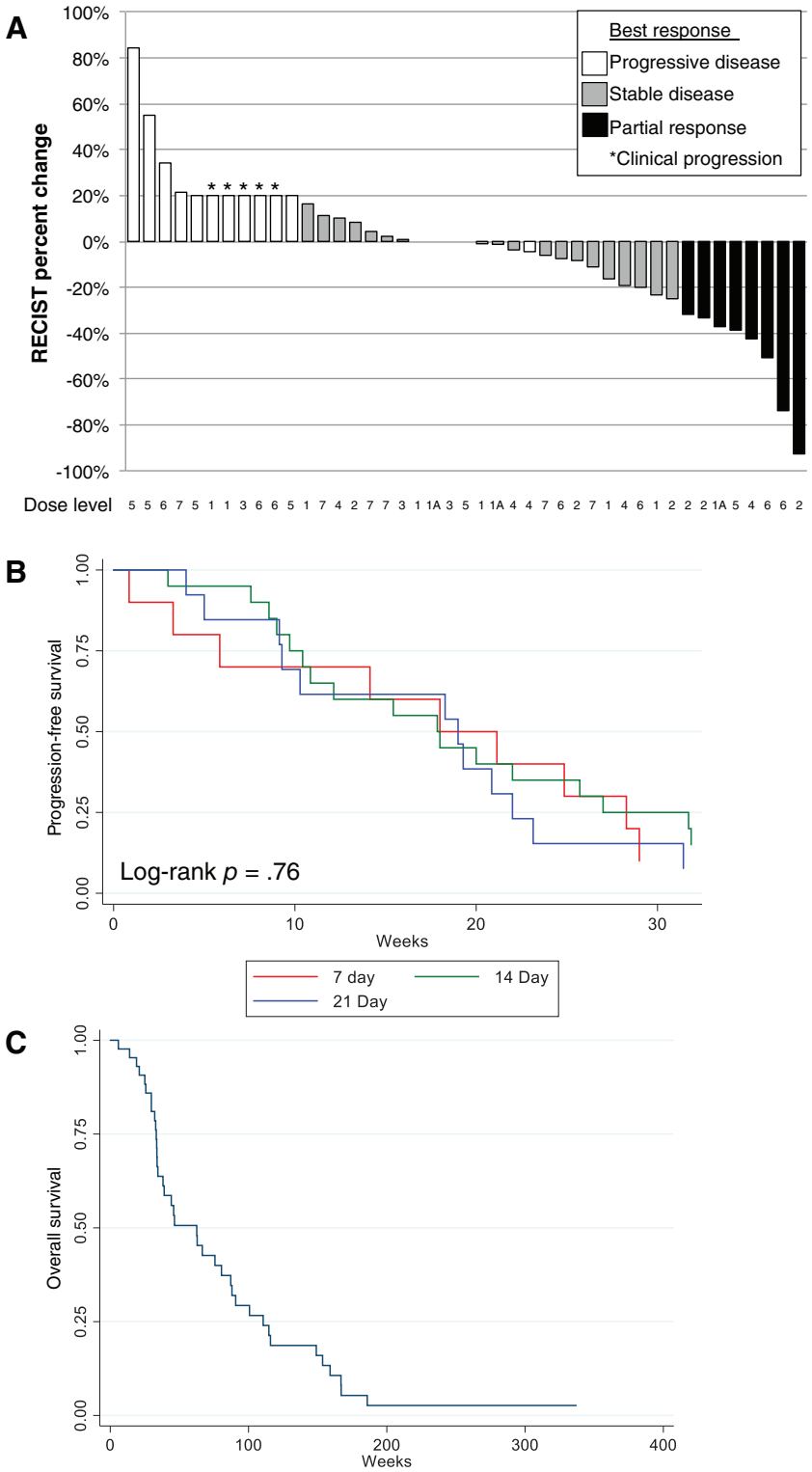

Figure 1. Efficacy and outcomes. **(A):** Waterfall plot of best response with RECIST percent change indicated on y-axis. Patients with clinical progression prior to cycle 3 are included at 20% fold change and indicated by asterisk (*). Best response indicated by color of bars: progressive disease, white; stable disease, grey; partial response, black. **(B):** Progression-free survival from study entry by veliparib dosing schedule. Median progression-free survival for all patients who received at least 1 cycle of therapy was 18.3 weeks. Veliparib dosing indicated by line color: 7-day dosing, red; 14-day dosing, green; 21-day dosing, blue. **(C):** Overall survival from study entry. Median overall survival was 62.6 weeks.

and 22.2% (*n* = 2) had primary progression. Four of the five patients with stable disease showed disease stabilization for >24 weeks (44.4%). Among the seven patients with known *BRCA1/2* mutation, 28.6% (*n* = 2) had PR, 71.4% (*n* = 5) had SD, and 42.9% (*n* = 3) had SD >6 months. When patients with tumors deficient in FA pathway based on FATSI testing and *BRCA1/2* mutations were analyzed together (*n* = 16), 25% had a PR (*n* = 4) and 62.5 % had stable disease

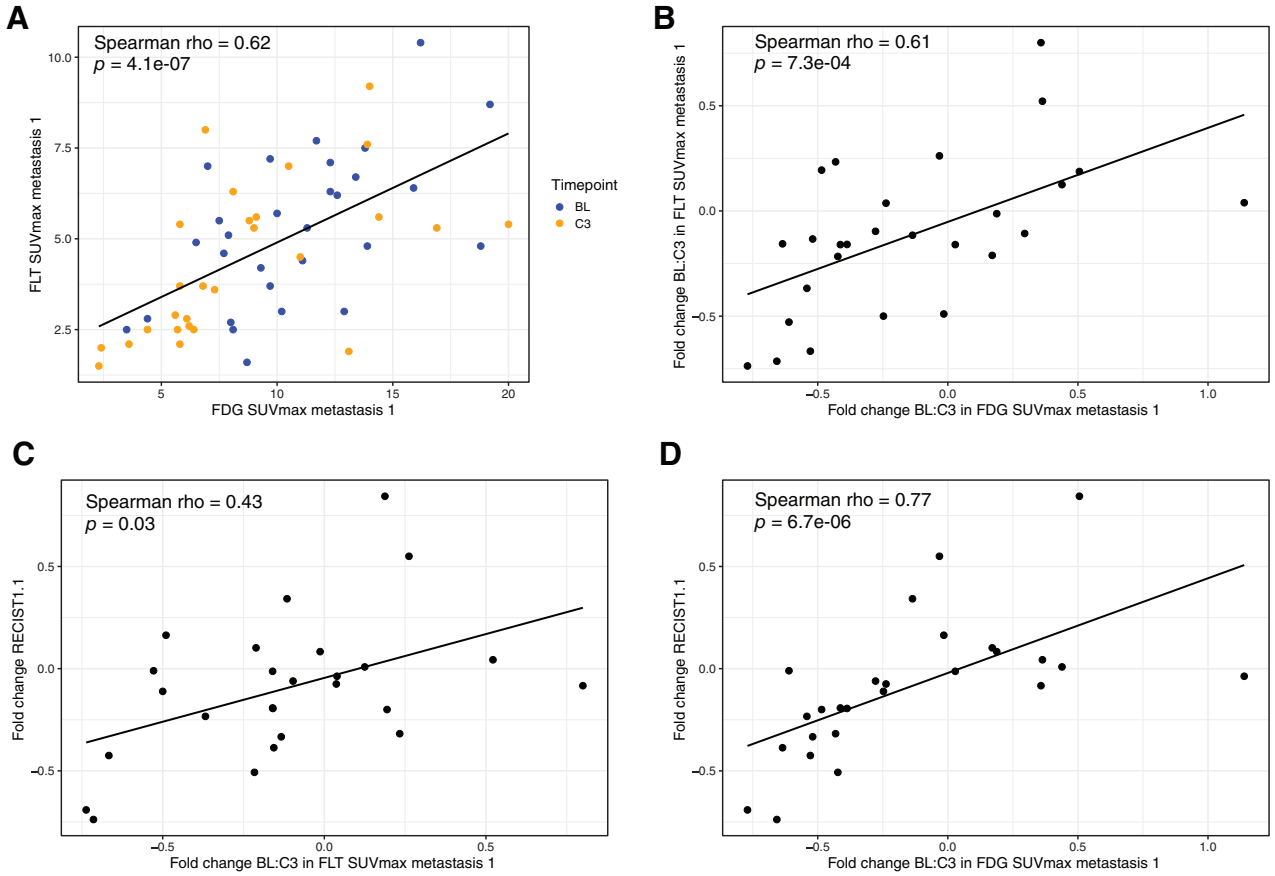

Figure 2. ^{18}FLT-PET imaging: correlation with ^{18}FDG-PET and RECIST measurement. **(A):** Scatter plot of ^{18}FLT-PET SUV_{max} of target lesion versus ^{18}FDG-PET SUV_{max} of the same target lesion at baseline ("BL"; indicated in blue) and cycle 3 day 1 ("C3"; indicated in orange). Line of best fit indicated. **(B-D):** Scatter plot of fold change from BL to C3 of ^{18}FLT-PET SUV_{max} of target lesion versus ^{18}FDG-PET SUV_{max} of the same target lesion **(B)**, ^{18}FLT-PET SUV_{max} of target lesion versus RECISTv1.1 **(C)**, and ^{18}FDG-PET SUV_{max} of target lesion versus RECISTv1.1 measurement **(D)**. For all comparisons, correlation evaluated by Spearman's rank correlation coefficient with *p* value indicated.
Abbreviations: BL, baseline; C3, cycle 3 day 1; FDG, 5-fluorodeoxyglucose; FLT, fluoro-3′-deoxythymidine; SUV_{max}, maximum standard uptake value.

(SD >6 months occurred in 43.8% of patients). One patient with *BRCA1* mutation achieved durable partial response to study therapy and received a total of 95 cycles of treatment. The patient was taken off the study after experiencing treatment-related thrombocytopenia (despite dose reductions and multiple delays of carboplatin dosing) and was subsequently diagnosed with myelodysplastic syndrome. The myelodysplastic syndrome was assessed as possibly related to study therapy.

^{18}FLT-PET Imaging: Correlation with ^{18}FDG-PET and RECIST Measurement

^{18}FLT-PET imaging was obtained successfully in all patients treated at OSU (42/44 total patients) with the proliferative whole-body mapping revealing expected uptake in the bone marrow, spleen, and liver (supplemental online Fig. 1). There were no toxicities attributable to administration of ^{18}FLT. The use of two distinct PET radiotracers in this study facilitates evaluation of distinct biological processes in cancer cells, specifically, metabolic activity (^{18}FDG-PET) and proliferation (^{18}FLT-PET). We first evaluated the correlation between the two tracers (Fig. 2A). Evaluating the primary target lesion at both baseline (BL) and first planned imaging (cycle 3 day 1 [C3]), we show that there is overall good correlation between maximum standard uptake value (SUV_{max}) of ^{18}FDG- and ^{18}FLT-PET (Spearman's rho = 0.62, p = 4.1e-07). We evaluated BL and C3 time points independently and demonstrated similar correlations (supplemental online Fig. 2A, 2B).

To investigate dynamic changes over time on therapy, we evaluated the association of fold change from BL to first planned imaging (C3) for ^{18}FLT-PET, ^{18}FDG-PET, and RECISTv1.1 (Fig. 2B–2D). ^{18}FLT-PET and ^{18}FDG-PET primary target lesion SUV_{max} fold change from BL to C3 showed similar correlation to the simple SUV_{max} values (Spearman's rho = 0.61, p = 7.3e-04). When comparing RECISTv1.1 measurements BL:C3 fold change with the PET metrics, ^{18}FLT-PET primary target lesion SUV_{max} fold change showed lower correlation (Spearman's rho = 0.43, p = .03) than ^{18}FDG-PET primary target lesion SUV_{max} (Spearman's rho = 0.77, p = 6.7e-06). Of note, both patients not enrolled at OSU had stable disease as best response.

Serial ^{18}FLT-PET Imaging and Association with Response

We performed ^{18}FLT-PET scan at four time points: (a) baseline (BL); (b) cycle 1 day 7 (time point 1 [T1]); (c) day 14 for

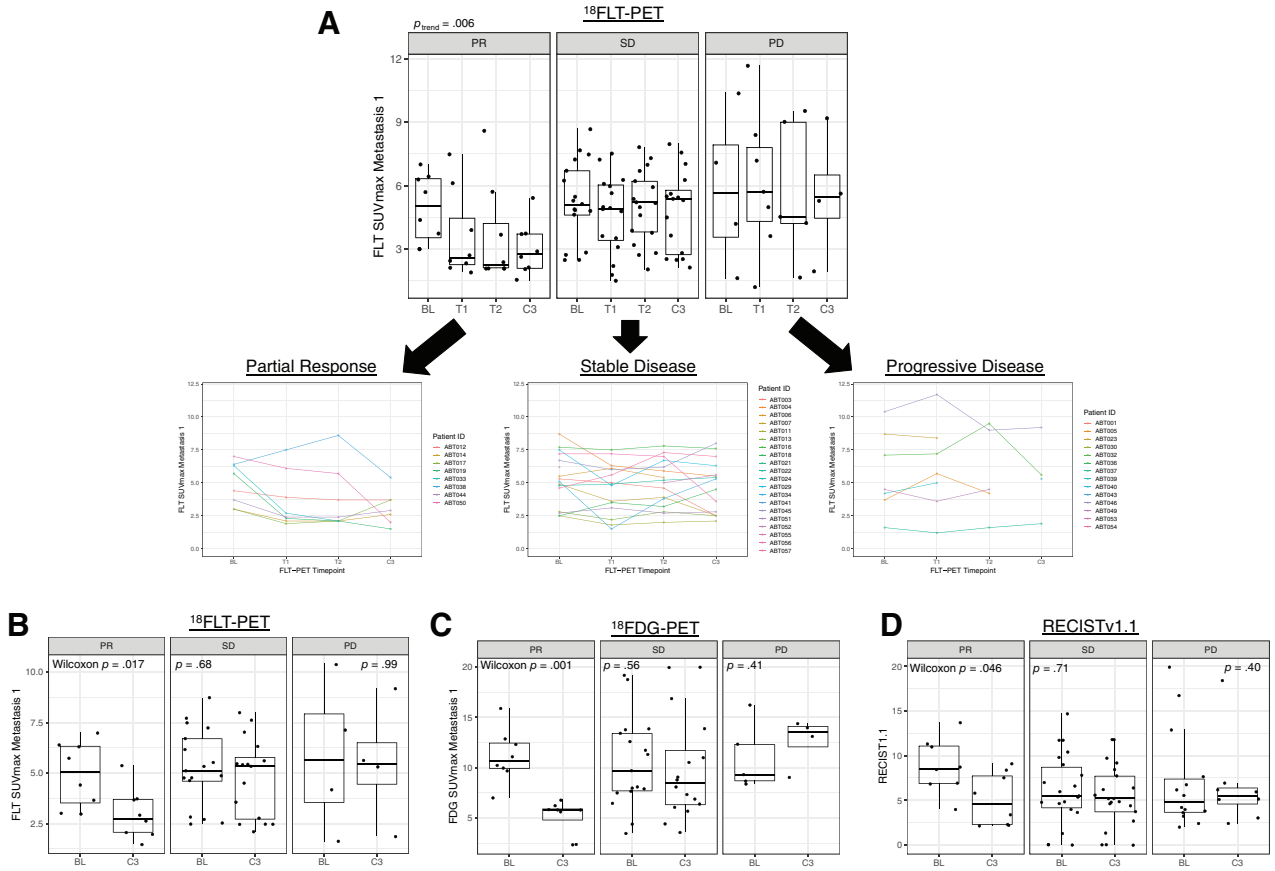

Figure 3. Early change in ^{18}FLT-PET SUV_{max}. **(A):** Top panel shows ^{18}FLT-PET SUV_{max} of target lesion from baseline ("BL"), to day 7 ("T1") to day 14 or 21 timepoint ("T2"), to cycle 3 day 1 timepoint ("C3"). Patients are grouped by best response, partial response (PR), stable disease (SD), and progressive disease (PD). The linear trend across 4 time-points in responders versus others (P_{trend}) indicates was a trend towards the responders having an early drop in ^{18}FLT uptake. **Bottom panel** shows individual patient level change at the four time points. **(B-D):** Baseline ("BL") versus cycle 3 day 1 ("C3") ^{18}FLT-PET SUV_{max} of target lesion **(B)**, ^{18}FDG-PET **(C)**, and RECISTv1.1 **(D)** grouped by response. *p*-value indicates Wilcoxon signed-rank test for each patient's paired BL and C3 sample, by response grouping.

Abbreviations: BL, baseline; C3, cycle 3 day 1; FDG, 5-fluorodeoxyglucose; FLT, fluoro-3′-deoxythymidine; PD, progressive disease; PET, positron emission tomography; PR, partial response; SD, stable disease; SUV_{max}, maximum standard uptake value; T1, time point 1; T2, time point 2.

cohorts receiving veliparib on days 1–14 (time point 2 [T2]) of every cycle or (b) cycle 1, day 14 (time point 1 [T1]); (c) day 21 (time point 2 [T2]) for cohorts treated with veliparib on days 1–21 of every cycle; and (d) after cycle 3 (C3) to assess change in the uptake of ^{18}FLT between patients with and without response. The change in SUV_{max} on ^{18}FLT-PET between baseline and follow-up scans did not depend on dose or schedule of veliparib (n = 24). Comparing the linear trend across four time points in responders versus others, there was a statistically significant drop in ^{18}FLT uptake in the responders (p = .006), whereas there was no trend in patients who achieved stable disease or had progressive disease (PD) as best response (Fig. 3A, top panel). Among responders, patients had a rapid decrease in ^{18}FLT uptake by T1 (cycle 1 day 7 or day 14) with little change to T2 (cycle 1 day 14 or day 21) or C3 (Fig. 3A, bottom panel). Nonresponders showed little change in ^{18}FLT uptake across time points. As an exploratory analysis, we also evaluated fold change in ^{18}FLT for each of six possible pairs: (a) BL:T1; (b) BL:T2; (c) BL:C3; (d) T1:T2; (e) T1:T3; (f) T2:T3 (supplemental online Fig. 2C, 2D). For responders (PR) versus nonresponders (SD + PD), fold change from baseline to T1, T2, or C3 were all associated with response (nominal $p < .05$) but not after multiple test correction (all false discovery rate adjusted $p > .05$), whereas fold change from T1 or T2 showed no association (supplemental online Fig. 2C). For clinical benefit versus not, there was no significant association between fold change and clinical benefit (supplemental online Fig. 2D). We then evaluated ^{18}FLT-PET SUV_{max} at BL versus C3, compared with ^{18}FDG-PET SUV_{max} and RECISTv1.1 total measurement (Fig. 3B, 3D). At C3, all three metrics demonstrated significant drop among responders, whereas those with stable disease or progressive disease did not show a significant decrease by any metric.

Circulating Tumor Cells

Peripheral blood for CTCs were obtained serially in 36 patients enrolled at OSU, with 32 patients having at least three serial samples. Although CTC values did not have any correlation with response groups, gamma H2Ax in CTCs at baseline showed higher trend among those with a PR (p = .02), and these values tended to be numerically higher during cycle 2 in

this group (p = .08), suggesting higher induction of DNA damage (supplemental online Fig. 3).

Discussion

Preclinical studies have shown that PARP1 inhibitors potentiate cytotoxicity when combined with platinum chemotherapy agents (cisplatin or carboplatin), which induce DNA damage through adducts and cross-linking [29]. Veliparib (ABT-888) is an efficient oral PARP inhibitor that targets PARP1 and PARP2, the primary enzymes involved in DNA repair [30]. Specifically, veliparib inhibits both baseline and cytotoxic-induced PARP activity in in vivo tumor models and thus provides evidence of the ability of veliparib to target PARP [31, 32]. Single-agent PARP inhibitor was approved for patients with *BRCA*-deficient hereditary advanced ovarian cancers and breast cancers [33, 34]. To date, combination of PARP inhibitors and other agents are tested in clinical trials, but none of them have yet received FDA approval. Neoadjuvant studies in breast cancer have shown that platinum agents are highly effective in triple-negative cancers, particularly the *BRCA*-associated tumors. This is consistent with preclinical *BRCA* mutant models demonstrating the combination of veliparib and carboplatin being more effective than either drugs alone or combination of cisplatin plus veliparib [35]. Recently, the I-SPY 2 Trial showed that veliparib–carboplatin added to standard therapy resulted in higher rates of pathological complete response than standard therapy alone in TNBC [12, 36, 37]. These data clearly point out that in tumors with defective homologous recombination, DNA-damaging agents in combination with PARP inhibition are highly effective and show promise as future therapies in the clinic. The BrighTNess trial tested addition of veliparib to carboplatin or carboplatin alone to neoadjuvant chemotherapy in patients with stage II–III triple-negative breast cancer (not selected for *BRCA1/2* mutation) and showed that addition of carboplatin but not veliparib resulted in higher pathologic complete response rate [36]. However, both these studies used a low dose of veliparib at 50 mg p.o. twice daily on a continuous schedule along with carboplatin in the early breast cancer setting.

Our multi-institutional phase I study demonstrated that veliparib in combination with carboplatin was well tolerated. Veliparib at 250 mg daily on a continuous schedule given along with carboplatin at AUC of 5 on day 1 of a 21-day cycles was the RP2D and schedule based on 3 + 3 dose escalation schema. This was the highest planned dose (250 mg b.i.d. daily), and we did not do further dose escalations because most patients on this dose level required dose reductions during later cycles owing to toxicities. Patients tolerated the combination well overall, and the most common grade 3 or higher toxicities were hematologic, such as neutropenia, anemia, and thrombocytopenia. The most common nonhematologic toxicities were fatigue and vomiting. Our RP2D was higher than that established by the California Consortium Trial (NCT01149083) in patients with *BRCA* mutation–associated MBC, where the RP2D of veliparib with carboplatin was established at 150 mg b.i.d. (continuous dosing). The DLTs and toxicities reported in this study were similar to ours, with grade 3 thrombocytopenia and neutropenia being the most frequent [38]. Another phase I study of veliparib combined with cisplatin and vinorelbine in advanced TNBC and/or *BRCA* mutation–associated cancer established 300 mg b.i.d. (days 1–14 on a 21-day cycle) as the RP2D. This study also reported hematological toxicities as the most frequent DLTs [39].

Our trial is unique in not only focusing on all TNBC but also including HR-positive patients with defective FA pathway. In addition, our phase I study investigated three different schedules including days 1–7, days 1–14, and continuous treatment of veliparib. Based on our study, in the metastatic setting, patients are able to tolerate a higher dose of veliparib (250 mg daily) on a continuous schedule with carboplatin every 3 weeks provided that the dose of carboplatin is kept at an AUC of 5.

The overall CBR in our patient population was 41.9% (CBR = PR + SD ≥6 months). Higher clinical benefit was seen in patients with germline *BRCA1/2* mutation (CBR 71.5%) and defective FA pathway (CBR 66.6%). This is similar to the CBR reported by other investigators. Somlo et al. reported a 51% CBR in their phase I study with veliparib and carboplatin in *BRCA* mutation carriers with MBC [38]. The phase II portion of this trial tested the efficacy of single-agent veliparib at 300 mg b.i.d. in germline *BRCA1/2* mutation carriers, with those progressing on single-agent veliparib treated with the combination carboplatin and veliparib at 150 mg b.i.d. Interestingly, the median PFS of the 30 patients treated with this combination after progression on single-agent veliparib was low at 1.8 months (95% CI: 1.4–2.3). This suggests that combining veliparib with a platinum agent earlier in the disease course may be a better strategy. Another study reported a 35% overall response rate in all patients (TNBC) but showed a 57% response rate among patients with *BRCA* mutation treated with the combination of cisplatin, vinorelbine, and veliparib [39]. A phase II trial of the combination of paclitaxel, carboplatin, and veliparib in *BRCA1/2* mutation carriers resulted in a 77.8% response rate (BROCADE) [40]. A subsequent phase III, double-blind, randomized study (BROCADE3) showed a nearly 2-month improvement in progression-free survival with the addition of veliparib (120 mg b.i.d. on days −2 to 5) to standard doses of carboplatin and paclitaxel (compared with placebo added to carboplatin and paclitaxel) in patients with HER2-negative metastatic breast cancer and germline *BRCA1/2* mutation [41].

The question then is whether sporadic cancers with defective homologous recombination would benefit from similar strategy, and furthermore, what would be the effective and yet tolerable chemotherapy to use in combination with PARP inhibitors. Phenotypic and mechanistic studies have shown that sporadic breast tumors with "*BRCA*ness" show inactivation of components of this pathway either through silencing of *BRCA* or dysfunction of other genes in the cascade of DNA repair pathway such as FA pathway [17]. Our phase I trial included patients with advanced TNBC (many of whom have the "*BRCA*ness" phenotype) and those with HR-positive advanced breast cancer with defective FA pathway to establish the dose, schedule, safety, and preliminary efficacy of combining veliparib with carboplatin. Our study has shown that combining carboplatin with veliparib is effective and well tolerated, particularly in tumors with DNA repair deficiency. In fact, we have preliminarily found in the current study that patients with *BRCA* 1 and 2 mutations or those that have tumors with FA pathway deficiency experienced greater responses (PR and SD of 25%

and 62.5% compared with 18% and 48%, respectively, in all study patients). Veliparib is the weakest PARP trapping agent in contrast to other PARP inhibitors such as talazoparib or olaparib. This likely results in less hematologic toxicities associated with use of veliparib but probably adversely affects its antitumor efficacy. It is not clear whether combination of platinum agents with other PARP inhibitors that have stronger PARP trapping properties would result in higher efficacy compared with similar combinations with veliparib.

A unique aspect of our study is the inclusion of functional imaging using ^{18}FLT-PET scans at early time points to noninvasively assess reduction in proliferation rate and compare this with ^{18}FDG-PET scans. Increased uptake of ^{18}FLT is seen in cells that express high levels of thymidine kinase 1, the key enzyme in the pyrimidine salvage pathway of DNA synthesis [42], hence correlating with increased cell proliferation. The SUV_{max} measurements on ^{18}FLT-PET-CT have been shown to correlate with response to therapy in breast cancer [23]. We performed ^{18}FLT-PET scans at four early time points (baseline, cycle 1 day 7 and 14, cycle 1 day 14 and 21, and cycle 3 day 1) as a tool to determine the impact of dose and schedule of veliparib on the proliferation rate of metastatic sites, and we found that the SUV_{max} on FLT-PET scan did not vary with dose or schedule (7 vs. 14 vs. 21 days) of veliparib. We demonstrated that among responders, drop in ^{8}FLT uptake is rapid in many patients—within 7 days—implicating a potential early imaging marker of response. Three other studies in metastatic and primary breast cancer have demonstrated that changes in FLT uptake in the primary or metastatic sites after one cycle of chemotherapy correlated with best response (metastatic setting) or neoadjuvant chemotherapy response (primary setting) [43–45]. The largest study, performed in the neoadjuvant setting, suggested that ^{18}FLT-PET had robust correlation with tissue KI67 staining after chemotherapy but more modest correlation with KI67 at baseline or overall tumor response [45]. By performing ^{18}FLT-PET imaging along with ^{18}FDG-PET imaging, this rich data set also allowed comparison of PET radiotracers—we found that although ^{18}FLT-PET and ^{18}FDG-PET were overall correlated, there are differences, and we are currently evaluating whether these modalities may be complementary. Our study has demonstrated that performing serial ^{18}FLT-PET scans is feasible without adverse effects and can be a noninvasive tool to assess early response and proliferation rates.

We also obtained CTC at multiple time points to assess the impact of dose and schedule of veliparib on the induction of γH2Ax as a marker of DNA damage. Phosphorylation of histone H2Ax on serine 139 (γH2AX) occurs at sites flanking DNA DSBs and provides a measure of the number of DSBs within a cell [46]. Although the dose and schedule of veliparib did not affect the induction of γH2Ax, we did find that the responders had a numerically higher baseline γH2Ax in CTCs and there was evidence of further induction of γH2Ax in these patients at the end of cycle 2. This is not surprising as baseline levels of γH2Ax in breast tumors have been associated with triple-negative breast cancer and worse prognosis indicating higher proliferative rates [47]. Larger studies need to be done to confirm the use of higher γH2AX in CTCs as a biomarker of response to DNA-damaging agents and PARPis.

Conclusion

Our phase I, dose-finding study of varying dose and schedule of veliparib along with carboplatin identified 250 mg b.i.d. daily as the recommended phase II dose and demonstrated safety and tolerability in patients with sporadic and *BRCA*-mutated TNBC and in patients with HR-positive MBC who had a functional deficiency of FA pathway as detected by FATSI assay. Furthermore, our study showed that use of novel functional FLT-PET imaging as a tool to assess reduction in proliferative rate in the tumor and early response is feasible. The single-agent PARP inhibitors olaparib and talazoparib are approved for the management of *BRCA*-mutated MBC. Our study provides rationale to study platinum/PARPi combination in other tumor subtypes as well.

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Author Contributions

Conception/design: Robert Wesolowski, Michael R. Grever, Michael V. Knopp, Bhuvaneswari Ramaswamy

Provision of study material or patients: Maryam B. Lustberg, Ewa Mrozek, Rachel M. Layman, Erin Macrae, Eleni Andreopoulou, Joseph Sparano, Charles L. Shapiro, Miguel Angel Villalona-Calero, Susan Geyer, Alice Chen

Collection and/or assembly of data: Daniel G. Stover, Abigail Shoben, Meng Zhao

Data analysis and interpretation: Daniel G. Stover, Abigail Shoben, Meng Zhao, Wenrui Duan, Jun Zhang, Nathan Hall, Chadwick L. Wright, Susan Gillespie, Michael Berger, Jeffrey J. Chalmers, Priya Balasubramanian, Brandon L. Miller, Peter Amaya

Manuscript writing: Robert Wesolowski, Daniel G. Stover, Maryam B. Lustberg, Abigail Shoben, Meng Zhao, Ewa Mrozek, Rachel M. Layman, Erin Macrae, Wenrui Duan, Jun Zhang, Nathan Hall, Chadwick L. Wright, Susan Gillespie, Michael Berger, Jeffrey J. Chalmers, Alahdra Carey, Priya Balasubramanian, Brandon L. Miller, Peter Amaya, Eleni Andreopoulou, Joseph Sparano, Charles L. Shapiro, Miguel Angel Villalona-Calero, Susan Geyer, Alice Chen, Michael R. Grever, Michael V. Knopp, Bhuvaneswari Ramaswamy

Final approval of manuscript: Robert Wesolowski, Daniel G. Stover, Maryam B. Lustberg, Abigail Shoben, Meng Zhao, Ewa Mrozek, Rachel M. Layman, Erin Macrae, Wenrui Duan, Jun Zhang, Nathan Hall, Chadwick L. Wright, Susan Gillespie, Michael Berger, Jeffrey J. Chalmers, Alahdra Carey, Priya Balasubramanian, Brandon L. Miller, Peter Amaya, Eleni Andreopoulou, Joseph Sparano, Charles L. Shapiro, Miguel Angel Villalona-Calero, Susan Geyer, Alice Chen, Michael R. Grever, Michael V. Knopp, Bhuvaneswari Ramaswamy

Disclosures

Robert Wesolowski: Acerta, AstraZeneca (RF), PUMA, Pfizer (C/A); **Miguel Angel Villalona-Calero:** Merck (RF [institution]); **Bhuvaneswari Ramaswamy:** Pfizer (RF), Eisai, Pfizer (C/A). The other authors indicated no financial relationships.

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