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Phase Ib study of HSP90 inhibitor, onalespib (AT13387), in combination with paclitaxel in patients with advanced triple-negative breast cancer

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Abstract

Background: Heat shock protein 90 (HSP90) is a molecular chaperone required for stabilization of client proteins over-activated in triple-negative breast cancer (TNBC). Over-expression of HSP90 client proteins has been implicated in paclitaxel resistance. Onalespib (AT13387) is a potent inhibitor of HSP90 that could improve paclitaxel efficacy when administered in combination.

Design: This phase Ib trial administered onalespib with paclitaxel in patients with advanced TNBC to assess safety and establish a recommended phase II dose (RP2D).

Objectives: The primary objectives were determining the dose-limiting toxicities and maximum tolerated dose of combination therapy. Secondary objectives included pharmacokinetic (PK) analysis and determination of overall response rate (ORR), duration of response (DOR), and progression-free survival (PFS).

Methods: Patients with advanced TNBC were treated with standard dose intravenous paclitaxel in combination with intravenous onalespib at doses ranging from 120 to 260 mg/m^2 administered on days 1, 8, and 15 of a 28-day cycle using a standard 3 + 3 design. A total of 15 patients were enrolled to dose expansion cohort at RP2D to confirm safety profile.

Results: Thirty-one patients were enrolled in the study, of which over 90% had received prior taxane therapy. Paclitaxel was given for metastatic disease in 23% of patients. Adverse events (AEs) included anemia (grade 3: 20%), lymphopenia (grade 3: 17%), and neutropenia (grade 3: 33%, grade 4: 4%). The most frequent grade ⩾3 non-hematologic AE was diarrhea (7%). The established RP2D was 260 mg/m^2 onalespib when given with paclitaxel at 80 mg/m^2. PK analysis revealed a modest drug interaction profile for onalespib in the combination regimen. ORR was 20%. Three patients achieved complete responses, all of whom had received prior taxane therapy. Median DOR was 5.6 months; median PFS was 2.9 months.

Conclusion: Combination treatment with onalespib and paclitaxel had an acceptable toxicity profile and RP2D was determined to be 260 mg/m^2 of onalespib. Combination therapy showed antitumor activity in patients with advanced TNBC.

Trial registration: Onalespib and paclitaxel in treating patients with advanced TNBC https://clinicaltrials.gov/ct2/show/NCT02474173.

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Plain language summary

Phase 1b study of HSP90 inhibitor called onalespib in combination with paclitaxel in patients with advanced triple-negative breast cancer

This Phase 1b study demonstrated that treatment with a combination of onalespib and paclitaxel was reasonably well tolerated by most patients. Onalespib at 260 mg/m^2 given intravenously on days 1, 8 and 15 on 28-day cycles in combination with standard dose and schedule of paclitaxel was established as the recommended phase 2 dose for further clinical development. Despite minor drug-drug interactions between these 2 agents, onalespib did not alter paclitaxel exposure and paclitaxel did not affect exposure to onalespib. While onalespib with paclitaxel combination therapy did not yield durable objective responses or prolonged progression-free survival, there were several patients with long-lasting benefit from this combination including patients who previously experienced progression on taxane therapy.

Keywords: AT13387, heat shock protein, HSP90 inhibitor, onalespib, paclitaxel, phase I clinical trial, triple-negative breast cancer

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Introduction

Triple-negative breast cancer (TNBC) is a heterogeneous type of breast cancer that does not express estrogen receptors (ERs) or progesterone receptors (PRs) and lacks amplification of the *HER2/neu* gene.[1] This subtype represents approximately 15% of all diagnosed breast cancer cases and is associated with poor prognosis, including a high rate of early disease relapse and short overall survival of about 18–25 months in patients diagnosed with metastatic disease.[2] The majority of TNBC cases are high grade and characterized by heterogeneous molecular expression patterns such as basal-like, mesenchymal, immunomodulatory, or luminal androgen receptor subtypes.[3] TNBCs are also more likely to carry gene mutations that confer aggressive solid tumor behavior, such as alterations in *p53*.[4–7] Most patients diagnosed with TNBC are treated with conventional cytotoxic chemotherapy based on the aggressive characteristics of this disease and the limited number of effective targeted therapies. There is an unmet need for new therapeutic options that address the distinct genomic and molecular processes that drive TNBC, especially in patients with advanced disease.[8,9]

Molecular chaperones are a family of proteins that play an important role in supporting the folding and assembly of other proteins into tertiary structures required for their biologic activity.[10] Heat shock proteins (HSPs) are a family of molecular chaperones contributing to cellular differentiation, growth, and survival.[11] HSP expression is stimulated by cellular trauma such as oxidative stress and elevated temperatures.[12] In neoplastic cells, HSPs are highly expressed and accumulate in the form of multi-chaperone complexes that help malignant cells counteract genetic alteration and preserve protein homeostasis.[13] HSPs are divided into two categories according to molecular size: large molecular size HSPs (e.g. HSP100, HSP90, HSP70) and small molecular size HSPs (molecular size $\leqslant$30 kDa).[12,14] Numerous studies have reported that the overexpression of HSP70 and HSP90 in breast cancer is associated with poor prognosis.[15,16] Preclinical studies in TNBC models have demonstrated that HSP90 inhibitors have antitumor effects and the combination of HSP90 inhibitors with taxanes result in significantly greater tumor regression compared to either agent alone.[17–22] Onalespib (AT13387) is a novel, highly potent, non-ansamycin small molecule inhibitor of HSP90 proteins with a dissociation constant (Kd) of 0.71 nmol.[23] It interacts with the N-terminal domain of HSP90 that contains the adenosine triphosphate binding site. High water solubility is a unique feature of onalespib, resulting in a more favorable safety profile as compared to other HSP90 inhibitors.[24,25]

Taxanes, such as paclitaxel and docetaxel, are some of the most active chemotherapeutic agents utilized to treat breast cancer. Unfortunately, in the metastatic setting, only about 40–50% of breast cancer patients achieve an objective response to first- or second-line treatment with paclitaxel alone. Patients who benefit from this therapy typically experience disease progression after a median of 5–9 months of treatment.[26] It has been demonstrated that over-expression of HSP90 in TNBC is associated with resistance to tubulin polymerizing agents, including paclitaxel.[18] In addition, many client proteins of HSP90 have previously been implicated in paclitaxel resistance (such as cRaf) and are known to be highly expressed in malignant cells (e.g. Epidermal Growth Factor Receptor [EGFR], Anaplastic Lymphoma Kinase [ALK], c-Kit).[27] Given the additive effect of combining taxane therapy with HSP90 inhibitors in murine models of TNBC, there is rationale to study this combination therapy in patients with advanced TNBC.

Here, we report the results of a phase Ib clinical trial of HSP90 inhibitor onalespib administered in combination with paclitaxel in patients with triple-negative or hormone receptor low, HER2-negative breast cancer.[17–22]

Methods

Study design

This multicenter phase Ib study evaluated the combination of onalespib and paclitaxel in patients with advanced (defined as inoperable or metastatic) TNBC. TNBC was defined in this study as being hormone receptor negative (ER $<$ 1%, PR $<$ 1%) or low (ER $<$ 10%, PR $<$ 10%) and HER2-negative. Patients were recruited to this study from four academic institutions (The Ohio State University, University of Pittsburgh, University of Kentucky, and Thomas Jefferson University) beginning in April 2016 and ending in September 2019. The primary objectives were to determine the recommended phase II dose (RP2D) of this combination therapy and its toxicity profile based on the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v. 5.0. Secondary objectives included determination of the pharmacokinetic (PK) effects of each agent on the other, overall response rate (ORR), duration of response (DOR), and progression-free survival (PFS) based on the Response Evaluation Criteria in Solid Tumors (RECIST) v. 1.1.[28] The study protocol was approved by the Institutional Review Board (IRB) at each of the participating institutions as well as the NCI Central IRB under common study number NCI9876. This study protocol followed the Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice (ICH-GCP) guidelines and complied with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2013 Checklist (Supplemental Figure 1). Written informed consent was obtained from all patients. The study was sponsored by the NCI and registered at ClinicalTrials.gov (NCT02474173). Astex Pharmaceuticals, Inc. provided onalespib for this study.

This study followed a standard 3 + 3 phase I dose escalation trial design. Subjects who completed cycle 1 of therapy and received at least two of the three doses of onalespib and paclitaxel in cycle 1 comprised the dose-limiting toxicity (DLT) population. Patients who did not complete the DLT period for reasons other than toxicities were replaced. Cohorts of three patients were accrued to a given dose level (DL) and evaluated for DLTs during the designated observation period. If no DLTs were encountered at a DL, the next higher dose cohort was enrolled. If one DLT was encountered in a three-patient cohort, three more patients were accrued to that DL (six total) for further DLT evaluation. The MTD was defined as the highest DL at which there was 0 DLTs in the first three patients enrolled or less than or equal to one DLT in the first six patients enrolled. The RP2D was defined as (1) the MTD, or (2) doses of the combination therapy below the MTD, if, in the opinion of the investigators, lower doses were better tolerated, safer, and demonstrated acceptable PK. Once the RP2D was established, a total of 15 patients were enrolled at that DL to confirm the safety profile.

Patients

Patients who were 18 years of age or older and had histologically confirmed, measurable, or non-measurable, advanced TNBC were enrolled in this study. TNBC was defined in this study as breast cancer that had $<$10% expression of ER and PR based on immunohistochemical (IHC) staining and had no over-expression of HER2 (i.e. 0 or 1+ on IHC) based on the most recent American Society of Clinical Oncology and College of American Pathologists (ASCO/CAP)

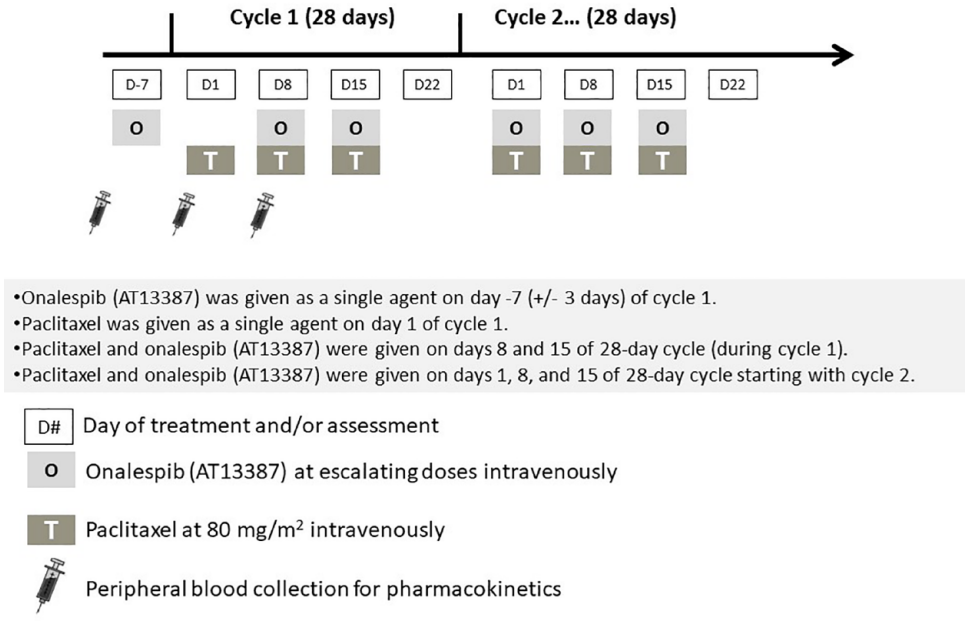

Figure 1. Study schema and dosing schedule.

guidelines. Patients were required to have an Eastern Cooperative Oncology Group performance score ⩽2, a life expectancy ⩾12 weeks, and adequate bone marrow, hepatic, and renal function. Patients were required to have response-evaluable disease but were not required to have measurable lesions. Additionally, eligible patients were allowed to have received any number of previous chemotherapies or endocrine-based treatment regimens, including prior taxane therapy for metastatic disease.

Patients were excluded from this study if they were experiencing more than grade 1 neuropathy, had a history of disease progression on taxane therapy within 3 months prior to study enrollment, or had untreated brain metastases. Patients with previously treated and stable brain metastases were eligible if they no longer required steroids, had completed radiation or surgical therapy more than 2 weeks prior to the first dose of the study regimen, and had no seizures or worsening neurologic symptoms. Other exclusion criteria included prior exposure to onalespib or other HSP90 inhibitors, history of HIV infection and receipt of highly active antiretroviral therapy, radiation therapy within 2 weeks prior to the first dose of the study regimen, and receipt of any other investigational agents within 4 weeks or five half-lives (whichever is longer) prior to the first dose of the study regimen.

Study treatment

Onalespib and paclitaxel were administered by intravenous infusion (IV) over 1 h on days 1, 8, and 15 of a 28-day cycle. The onalespib dose was escalated in cohorts of three to six patients while paclitaxel was administered at a standard dose of 80 mg/m^2. The starting dose of onalespib was 120 mg/m^2 IV over 1 h in DL1. If DL1 was associated with an unacceptable rate of DLTs, patients could be enrolled at an onalespib dose of 100 mg/m^2 (DL-1). Otherwise, the dose of onalespib was escalated up to 260 mg/m^2 in DLs 2–4 based on standard phase I trial design. The highest dose of onalespib was chosen to be 260 mg/m^2 based on the maximum tolerated dose declared in *a prior* single-agent study.[29] To study the effect of each agent on the exposure of the other, patients received single-agent onalespib on day –7 and single-agent paclitaxel on day 1 in cycle 1 only (Figure 1).

Safety and definition of DLTs

Safety evaluations included physical examination, vital signs, collection of adverse events (AEs), 12-lead electrocardiogram, and laboratory assessments. AEs were characterized by type, incidence, severity, and relationship to study drug, and graded by the NCI CTCAE v. 5.0.

The DLT assessment period started on day 7 of cycle 1 and was completed on day 1 of cycle 2.

For patients to be evaluable for DLT assessment, they were required to have completed at least two of the three doses of onalespib and paclitaxel in cycle 1. The following toxicities were considered DLTs if they were assessed as at least possibly related to study therapy: grade 4 neutropenia lasting for more than or equal to 7 days in duration, grade $\geqslant 3$ neutropenia complicated by a fever, grade 4 thrombocytopenia, grade 3 thrombocytopenia complicated by bleeding, and grade $\geqslant 3$ non-hematologic AEs. Exceptions for the non-hematologic AEs included: grade $\geqslant 3$ nausea, vomiting, or diarrhea that resolved to grade $\leqslant 2$ within 48 h (with or without medical intervention or prophylaxis); grade 3 fatigue if it resolved to grade $\leqslant 2$ within 14 days; transient (<14 days) increase in liver function tests of more than or equal to one grade in severity compared to baseline levels in patients with baseline liver metastases; and grade 3 maculopapular rash if symptoms were easily managed with supportive care and without evidence of superinfection or limitations in self-care.

PK analyses

Serial blood samples for PK analysis of onalespib were measured on day 7 of cycle 1, while those for paclitaxel were collected on day 1 of cycle 1. Blood samples for analysis of combined therapy PK were collected on day 8 of the first cycle, which was the first time both agents were given together (Figure 1). Samples were collected at the following time points: pre-dose (within 15 min), immediately prior to end of infusion (EOI) (within 5 min), 0.5 h after EOI (± 5 min), 1 h after EOI (± 5 min), 2 h after EOI (± 5 min), and 4, 6, 8, and 24 h after EOI. Given multiple collections, the 8-h time point was skipped in cycle 1, day 8 for patient convenience.

PK samples were prepared for mass analysis and detection of paclitaxel and onalespib using a Thermo TSQ Quantiva mass spectrometer equipped with a heated electrospray ionization source, as previously described.[29,30] Separate methods were created and validated for each compound. A 50 µL human plasma sample and 5 µL of an internal standard, 750 nM paclitaxel-d5 for the paclitaxel assay, or 500 nM palbociclib for the onalespib assay were combined for sample processing. Further details can be found in Supplemental Material. The quantitation transition was used for the calculated concentration (nM) results.

PK noncompartmental analysis was performed with individual paclitaxel and onalespib plasma concentration–time profiles for patients 1–22 using Phoenix32 software (version 8.2.0.4383; Certara, Princeton, NJ, USA). PK parameters were determined for onalespib alone during day 7 (± 3 days); for paclitaxel alone during cycle 1, day 1; and the combination of the two in cycle 1, day 8 (Figure 1). Plasma model type was selected with uniform weighting and all area under the curve (AUC) values, representing plasma concentration of a drug over time after dosage, were calculated using the linear-up log-down trapezoidal method. The slope of the terminal phase was manually defined for individual onalespib profiles and defined at 4–24 h for paclitaxel. Maximum plasma concentration (C_{max}) values were obtained directly from the measured values.

Antitumor activity

Tumor response in all known or suspected disease sites was assessed by computed tomography or magnetic resonance imaging every 8 weeks until disease progression, death, or end of treatment. Responses and progression were determined using RECIST v. 1.1 criteria.

Statistical analyses

Patients who received at least one dose of study therapy comprised the safety population. AEs were summarized using descriptive statistics. Maximum grade (CTCAE v. 5.0), frequency, and perceived attribution of AEs were summarized across DLs. Defined efficacy endpoints of ORR, DOR, and PFS were calculated in an exploratory manner. ORR was defined as the percentage of evaluable patients achieving partial or complete response. DOR was calculated as the interval from the first assessment demonstrating a partial or complete response to the development of disease progression. PFS was calculated as the interval from study enrollment to first documented disease progression or death from any cause (whichever occurred first). Median DOR and PFS were estimated using the Kaplan–Meier method. Given the small sample size, no formal hypothesis testing for clinical efficacy was performed and all results were summarized using descriptive statistics only. Changes in onalespib and paclitaxel PK were analyzed using a Wilcoxon matched-pairs signed-rank test. All other statistical analyses were completed using either SAS 9.4 (SAS Institute Inc., Cary, NC, USA) or Stata

16.1 (StataCorp, LLC, College Station, TX, USA).

Results

Patients

Between April 2016 and September 2019, 31 patients were enrolled in the study: 5 at DL1, 3 at DL2, 7 at DL3, and 16 at DL4. In DL1, one patient provided informed consent for the study and was registered but was later found to be ineligible due to *a prior* episode of severe hypersensitivity to paclitaxel leading to the discontinuation of this agent. This patient did not receive any study therapy and was excluded from the safety, DLT, and efficacy analysis. Another patient at DL1 was found to have new brain metastases during the DLT assessment period in cycle 1 and was replaced. One patient in DL3 and one patient in DL4 had clinical evidence of disease progression during the DLT assessment period in cycle 1 and were replaced. Table 1 summarizes the demographic characteristics of study patients. The median age was 56 (range 29–74). Ninety percent of patients were Caucasian, while 7% and 3% were Asian and African American, respectively. Premenopausal women represented 39% of the study population. Seventy-one percent of study patients had TNBC at the time of diagnosis, while 19% were initially diagnosed with hormone receptor-positive, HER2-negative breast cancer that subsequently changed to the triple-negative phenotype. Ten percent of patients had weakly hormone receptor-positive (defined as $\geqslant$1%, but $<$10% expression of ER and/or PR), HER2-negative breast cancer. In this study, 94% of patients had previously received taxane therapy, nearly a quarter (23%) for metastatic disease. The median number of prior treatments for metastatic disease was 2 (range 0–8). The median number of unique cytotoxic chemotherapies received for metastatic disease was 1 (range 0–8).

DLT assessment and RP2D

Table 2 summarizes the DLs and DLTs. Patients received onalespib at 120, 150, 200, or 260 mg/m^2 in combination with paclitaxel at 80 mg/m^2 in DLs 1–4, respectively, on days 1, 8, and 15 of 28-day cycles. Of note, onalespib was given on day –7 as a single agent and omitted on day 1 of cycle 1 to study the effect of onalespib on paclitaxel exposure and vice versa. One DLT (persistent grade 3 nausea, vomiting, and abdominal

Table 1. Patient demographics.

Variable	Number
Total	31
Age (median, range)	56 (29–74)
Race	
Caucasian	28 (90%)
Asian	2 (7%)
African American	1 (3%)
ECOG status	
0	13 (42%)
1	17 (55%)
2	1 (3%)
Menopausal status	
Pre-menopausal	12 (39%)
Post-menopausal	19 (61%)
Hormone receptor status	
De novo triple-negative	22 (71%)
Switched to triple-negative[a]	6 (19%)
Hormone receptor low	3 (10%)
Prior lines of any systemic therapy for metastatic disease[b]	
Median	2
Range	0–8
Patients with no prior treatment for metastatic disease	5 (16%)
Prior lines of cytotoxic chemotherapy for metastatic disease	
Median	1
Range	0–8
Prior taxanes	
Prior taxane in any setting	29 (94%)
Prior taxane for metastatic disease	7 (23%)
Prior paclitaxel	27 (87%)
Prior docetaxel	2 (6%)

[a]Patients initially diagnosed with hormone receptor-positive breast cancer which subsequently switched to triple-negative phenotype.
[b]Six patients received endocrine therapy for weakly hormone receptor-positive breast cancer; three patients received palbociclib in combination with endocrine therapy, two patients received pembrolizumab, one patient received veliparib, one patient received everolimus, and one patient received romidepsin in combination with other agents.
ECOG, Eastern Cooperative Oncology Group.

Table 2. Summary of DLs and DLTs.

DL	Dose of onalespib[a] (mg/m^2)	Number of patients	DLTs
–1	100	0	Not applicable
1	120	5[b]	None
2	150	3	None
3	200	7[c]	Grade 3 nausea, vomiting, and abdominal pain
4	260	16[d]	None

[a]Onalespib was given on days 1, 8, and 15 of 28-day cycles, except for cycle 1, when onalespib was given on days-7, 8, and 15. Paclitaxel was administered at a standard dose of 80 mg/m^2 on days 1, 8, and 15 of 28-day cycles.
[b]One patient in DL1 signed consented to the study and was registered but was later found ineligible and did not receive treatment. One additional patient did not complete DLT assessment period due to discovery of brain metastases and needed to be replaced.
[c]One patient in DL3 did not complete DLT assessment period due to evidence of disease progression and had to be replaced.
[d]One patient in DL4 did not complete DLT assessment period due to evidence of disease progression and needed to be replaced.
DL, dose level; DLT, dose-limiting toxicity; ECOG, Eastern Cooperative Oncology Group.

pain) occurred within a six-patient cohort at the 200 mg/m^2 dose of onalespib (DL3). No other DLTs were found. DL4 was declared as the MTD of onalespib and selected as the RP2D in combination with the standard dose and schedule of paclitaxel. DL4 was expanded to a total of 15 DLT-evaluable patients with no additional DLTs noted. DL-1 (100 mg/m^2 of onalespib) was not utilized as none of the patients enrolled in DL1 experienced DLTs requiring dose reduction.

Safety

Table 3 summarizes adverse reactions that were attributed as possibly treatment related and occurred in at least 10% of evaluable study patients. The most common adverse effects were hematologic, and they included anemia (all grades: 83%; grade 3: 20%), lymphopenia (all grades: 66%; grade 3: 17%), and neutropenia (all grades: 63%; grade 3: 33%; grade 4: 4%). The most frequent non-hematologic AEs were diarrhea (all grades: 73%; grade 3: 7%), nausea (all grades: 57%; grade 3: 10%), fatigue (all grades: 40%; grade 3: 7%), vomiting (all grades: 37%; grade 3: 10%), and dry mouth (all grades: 37%; grade 3: 0%). The only grade 4 toxicities were one case of grade 4 neutropenia and one case of grade 4 leukopenia, both of which resolved within 7 days. There were no grade 5 AEs. The reason for study discontinuation was progressive disease in 26 of the 30 evaluable study patients. Only three patients discontinued study therapy for toxicities, including one patient who was taken off due to persistent grade 3 neutropenia despite multiple dose reductions and a rise in transaminase levels with stable radiographic assessment after 22 cycles of study therapy. One patient discontinued treatment due to persistent grade 3 thrombocytopenia despite dose adjustments after 12 cycles of study therapy. One additional patient requested to be removed from the study for grade 3 fatigue, grade 2 vomiting, and grade 2 diarrhea after cycle 3, day 15. Supplemental Table 1 summarizes all graded serious AEs that were possibly attributed to study therapy. Supplemental Table 2 lists the most frequent AEs regardless of attribution based on DL.

PK analyses

From 22 patients, 351 and 308 plasma concentration observations were available for onalespib and paclitaxel PK, respectively. These represent 88 concentration-*versus*-time profiles. Following the enrollment of 22 patients, the study protocol was amended to discontinue further blood collection for PK analysis since enough samples were already collected and to minimize patient inconvenience. Figure 2 displays the mean onalespib and paclitaxel concentration-versus-time profiles for all 22 patients by cohort. One outlier profile (patient 4, DL1) displayed significant interpatient variability at each EOI time point for both compounds; therefore, the EOI data points for this patient were removed from the data set.

Table 3. Summary of AEs that occurred in at least 10% of evaluable study patients.

Adverse event	Grade 1–2		Grade ⩾3		Total	
	N	Percent	*N*	Percent	*N*	Percent
Anemia	19	63	6	20	25	83
Diarrhea	20	66	2	7	22	73
Leukopenia	15	50	7	23	22	73
Lymphopenia	15	50	5	17	20	66
Neutropenia	8	27	11	37	19	63
Nausea	14	47	3	10	17	57
Fatigue	10	33	2	7	12	40
Vomiting	8	27	3	10	11	37
Dry mouth	11	37		0	11	37
Thrombocytopenia	10	33	1	3	11	37
Anorexia	7	23		0	7	23
Rash maculo-papular	7	23		0	7	23
Pain	5	17		0	5	17
Mucositis oral	5	17		0	5	17
Dysgeusia	5	17		0	5	17
Headache	5	17		0	5	17
Peripheral sensory neuropathy	5	17		0	5	17
Pruritus	5	17		0	5	17
Myalgia	4	13		0	4	13
Urinary tract infection	2	7	2	7	4	13
Aspartate aminotransferase increased	3	10		0	3	10
Weight loss	3	10		0	3	10
Hypokalemia	3	10		0	3	10
Insomnia	3	10		0	3	10

Additionally, outlier onalespib data for patient 2 (DL1), day 7 at 24 h and paclitaxel data for patient 20 (DL4), day 8 at 1 h were removed following failure to estimate the elimination phase kinetics. For both compounds, maximum concentrations were achieved at the end of the 1 h IV infusion and then declined rapidly with the observed clearance (CL) being independent of dose.

A descriptive analysis was performed to define systemic exposure (AUC_{inf}), drug clearance (CL), maximum concentration (C_{max}), terminal half-life ($t_{1/2}$), and volume of distribution of the terminal phase (V_z). Table 4 presents a summary of the calculated PK parameters. The overall ranges of concentrations and PK parameter values for both onalespib and paclitaxel were similar to previously reported studies.[25,31,32] When administered as a single agent

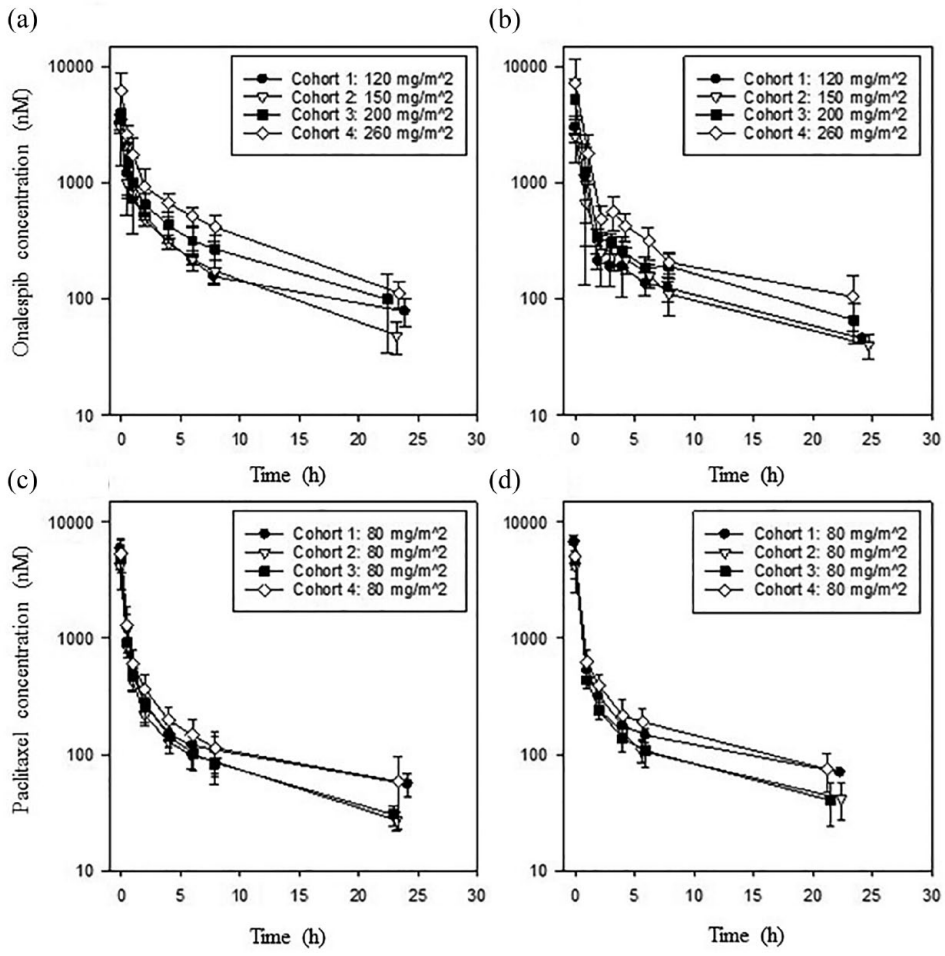

Figure 2. PK profiles of onalespib and paclitaxel from study patients. Mean semi-logarithmic onalespib plasma concentration-versus-time profiles for onalespib dosing levels (a) without paclitaxel, or (b) with 80 mg/m^2 paclitaxel. Paclitaxel dosing level profiles for 80 mg/m^2 paclitaxel (c) without onalespib, or (d) with onalespib at escalated dosing intervals for each cohort. Data are represented as mean plus standard deviation (SD) values for each DL.
DL, dose level; PK, pharmacokinetics.

on cycle 1, day –7, onalespib AUC_{inf} ranged from 4.30 to 22.7 μM × h. Mean AUC_{inf} increased proportionally across DLs, ranging from 6.72 ± 0.688 μM × h at DL1 to 15.2 ± 3.84 μM × h at DL4. Mean C_{max} showed a similar trend, increasing from 2.80 ± 0.844 μM at DL1 to 5.36 ± 2.80 μM at DL4. In contrast, when administered as a single agent on cycle 1, day 1, paclitaxel mean AUC_{inf} and mean C_{max} were relatively stable at the 80 mg/m^2 dose. Mean AUC_{inf} ranged from 5.59 ± 0.185 to 7.75 ± 1.75 μM × h. Mean C_{max} ranged from 3.87 ± 2.47 to 4.68 ± 1.82 μM.

To assess the effects of onalespib on paclitaxel, comparison of paclitaxel PK was made between cycle 1, day 1 (administration of paclitaxel alone) and cycle 1, day 8 (paclitaxel administered in combination with onalespib). Paclitaxel administration at 80 mg/m^2 was unaffected by coadministration with onalespib, as demonstrated in Table 4, where mean C_{max}, AUC_{inf}, CL, V_z, and $t_{1/2}$ were similar at both time points. Additionally, dose escalation of onalespib did not have any obvious effect on paclitaxel PK parameters or its concentration–time profile. Specifically, paclitaxel mean AUC_{inf} in cycle 1, day 8 ranged from 6.50 ± 0.575 to 8.72 ± 2.54 μM × h and mean C_{max}, ranged from 2.99 ± 3.59 to 4.23 ± 2.18 μM, across all four DLs, demonstrating consistency of paclitaxel exposure irrespective of onalespib dose.

Table 4. Mean PK parameters of onalespib and paclitaxel by DL.

Onalespib dose (mg/m²)		N	C_{max} (μM)	AUC_{inf} (μM × h)	CL (L/h/m²)	V_z (L/m²)	$t_{1/2}$ (h)
Onalespib (AT13387) plasma PK							
Day 7 (no paclitaxel)							
DL1	120	3[a]	2.80 ± 0.844	6.72 ± 0.688	42.6 ± 4.99	344 ± 134	7.23 (3.0–7.6)
DL2	150	3	3.03 ± 0.353	7.09 ± 0.647	51.1 ± 4.37	443 ± 23.2	6.04 (5.3–6.6)
DL3	200	7	3.21 ± 2.56	10.0 ± 4.35	48.4 ± 29.2	522 ± 273	7.32 (6.1–11.7)
DL4	260	9	5.36 ± 2.80	15.2 ± 3.84	41.6 ± 10.0	427 ± 102	6.83 (6.4–8.9)
Day 8 (with 80 mg/m² paclitaxel)							
DL1	120	3[b]	2.74 ± 0.676	6.10 ± 0.244	47.9 ± 2.03	730 ± 121	10.5 (8.6–12.7)
DL2	150	3	2.41 ± 1.03	5.64 ± 2.32	64.3 ± 21.0	801 ± 263	8.55 (6.0–10.6)
DL3	200	7	4.64 ± 2.64	9.46 ± 2.24	51.4 ± 14.8	717 ± 282	9.62 (7.5–17.4)
DL4	260	9	5.53 ± 4.50	14.0 ± 4.66	45.2 ± 13.4	598 ± 208	9.11 (6.3–14.5)
Paclitaxel plasma PK							
Day 1 (no onalespib with 80 mg/m² paclitaxel)							
DL1	0	3[c]	3.87 ± 2.47	7.44 ± 1.54	12.6 ± 2.63	279 ± 41.9	13.7 (12.8–20.7)
DL2	0	3	4.17 ± 0.299	5.59 ± 0.185	16.8 ± 0.535	216 ± 19.3	9.18 (8.0–9.7)
DL3	0	7	4.45 ± 2.28	6.10 ± 2.24	15.3 ± 4.77	206 ± 89.8	9.06 (7.7–11.6)
DL4	0	9	4.68 ± 1.82	7.75 ± 1.75	11.9 ± 2.78	181 ± 46.0	10.6 (8.6–12.2)
Day 8 (with 80 mg/m² paclitaxel)							
DL1	120	3[c]	2.99 ± 3.59	7.97 ± 2.95	11.7 ± 5.18	245 ± 81.9	15.0 (13.1–15.4)
DL2	150	3	4.16 ± 0.234	6.50 ± 0.575	14.4 ± 1.36	226 ± 71.9	9.27 (8.8–15.8)
DL3	200	7	4.23 ± 2.18	6.47 ± 2.18	14.5 ± 4.59	213 ± 74.3	8.67 (7.9–18.3)
DL4	260	9[d]	3.89 ± 2.26	8.72 ± 2.54	10.7 ± 3.64	183 ± 69.9	10.7 (8.4–18.4)

Data are represented as geometric mean ± SD; $t_{1/2}$ is represented as median (range).
[a]One patient outlier EOI datapoint and one patient outlier PK model datapoint removed.
[b]One patient outlier EOI datapoint removed.
[c]One patient outlier EOI datapoint removed.
[d]One patient outlier PK model datapoint removed.
PK, pharmacokinetics.

Onalespib concentration–time profiles appeared multi-phasic following IV bolus administration of doses ranging from 120 to 260 mg/m². Onalespib exposure, as measured by AUC_{inf} and C_{max}, increased in an approximately dose-proportional manner, as outlined above. To determine the effects of paclitaxel on onalespib, a comparison of onalespib PK on cycle 1, day –7 (administration of onalespib alone) was made with cycle 1, day 8 (onalespib administered in combination with paclitaxel). As demonstrated in Table 4, no apparent changes in overall exposure (AUC_{inf} and C_{max}) of onalespib were observed in combination with paclitaxel. The $t_{1/2}$ of the terminal phase ranged from 3.0 to 17.4 h and mean $t_{1/2}$ was longer after combination with paclitaxel (8.55–10.5 h) than as a single agent only (6.04–7.32 h). Likewise, the terminal phase volume (V_z) of

Table 5. Summary of clinical efficacy of onalespib in combination with paclitaxel in women with TNBC.

Response parameter	*N* (%)
Objective response rate	
Complete response	3 (10%)
Partial response	3 (10%)
Objective response rate	6 (20%)
Stable disease	13 (43%)
Clinical benefit rate	19 (63%)
Progressive disease	11 (37%)
Median DOR	5.6 months (95% CI: 1.3 to not reached)
Median PFS	2.9 months (95% CI: 1.8–4.6)

onalespib increased in the presence of paclitaxel with mean V_z increasing from 37% to 112%. These changes were significant at onalespib doses of 260 mg/m^2 (V_z p value 0.0039, $t_{1/2}$ p value 0.0078) and 200 mg/m^2 ($t_{1/2}$ p value 0.0156) using a Wilcoxon matched-pairs signed-rank test.

Antitumor activity

Thirty of the enrolled patients were evaluable for response. Table 5 is a summary of the efficacy of the study combination. ORR was 20% (n=6) with 10% of patients experiencing a complete response. The clinical benefit rate (defined as overall response or stable disease for 6 months) was 63% (n=19). Thirty-seven percent of patients (n=11) experienced disease progression as their best response. Median PFS was 2.9 months [95% confidence interval (CI): 1.8–4.6] and median DOR was 5.6 months (95% CI: 1.3 to not reached). Figure 3 shows a waterfall plot of change in target lesions in study patients with measurable disease (n=19). Note that two of the three patients who experienced a complete response had non-measurable disease and were not included in the figure (one had biopsy-proven pulmonary metastases that were <1.0 cm in size and one had an unresectable chest wall recurrence that could not be reliably measured on imaging). Figure 4 shows a Kaplan–Meier curve of PFS. Supplemental Figure 2 is a swimmer plot summarizing the duration of time on study treatment.

Three patients who experienced a complete response had previously received paclitaxel: one for *de novo* metastatic disease, one in the neoadjuvant setting, and one as adjuvant treatment for positive nodal disease. The patient who received taxane therapy for *de novo* metastatic disease and subsequently developed unresectable chest wall metastases achieved a complete response, but soon progressed after deciding to discontinue study therapy following completion of three cycles due to grade 3 fatigue and intolerable grade 2 gastrointestinal toxicities. Another patient who had received neoadjuvant paclitaxel for TNBC and later developed lung metastases achieved a complete response to study therapy after receiving 36 cycles of paclitaxel and 12 cycles of onalespib (due to discontinuation of clinical drug development by the manufacturer). She experienced brain-only progression in May 2022 and underwent brain resection with stereotactic radiosurgery. The 13 patients who experienced stable disease all had a history of prior taxane exposure. Of the 11 patients with progressive disease, 2 were taxane-naïve.

Discussion

The present study demonstrated that the combination of onalespib and paclitaxel was well-tolerated with hematologic toxicities comprising most of the adverse reactions seen. Most of the hematologic events were grade 1–2 in severity except for grade 3 neutropenia (which occurred in 33% of cases) and one case each of grade 4 neutropenia and leukopenia. The MTD and RP2D of onalespib combined with the standard dose of paclitaxel was declared to be the highest tested dose of 260 mg/m^2 IV on days 1, 8, and 15 of a 28-day cycle. This dose of onalespib was previously found to be the MTD when used as monotherapy on the same schedule.[33] PK analyses revealed a modest drug interaction profile for onalespib in the combination regimen, without an associated effect on paclitaxel exposure. The ORR was 20%, with three patients who had received prior taxane therapy achieving complete responses. Median PFS was 2.9 months which was less than the average of 5–9 months reported in the literature for patients treated with taxanes as the first- or second-line of therapy. However, the patient population in this study had received on median of two lines of prior therapies for metastatic disease (range 0–8).[26]

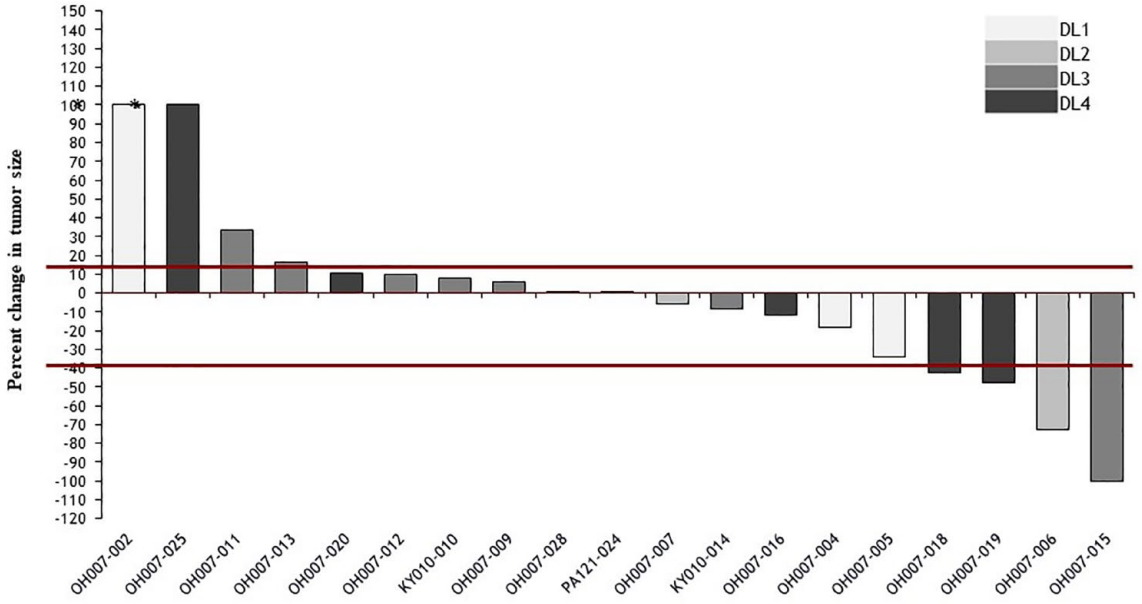

Figure 3. Waterfall plot. Maximum percentage change in tumor size for patients who were evaluable for responses. Patients with at least one post-treatment radiographic assessment were included. Positive values indicate tumor growth and negative values indicate tumor reduction.
RECIST, Response Evaluation Criteria in Solid Tumors.

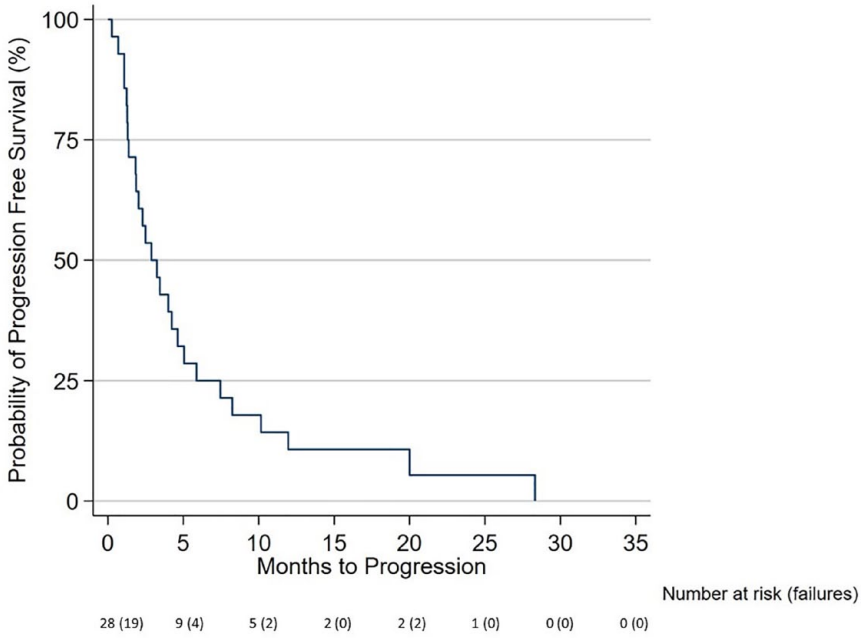

Figure 4. Kaplan–Meier curve depicting PFS of study subjects.
PFS, progression-free survival.

Patients with weakly hormone receptor-positive breast cancer were included in this trial based on prior studies that showed a disease trajectory and prognosis similar to conventionally defined TNBC (i.e. <1% expression of ER and PR).[34,35] Endocrine therapy has limited activity in patients

with weakly hormone receptor-positive breast cancer, and thus cytotoxic chemotherapy becomes an option sooner compared to strongly hormone receptor-positive disease.[36] Therefore, the present results could be applicable to a larger patient population.

Among AEs, grade 1–2 hematologic events were most common. One-third of patients experienced grade 3 neutropenia. The only grade 4 toxicities were one case each of neutropenia and leukopenia, both of which were non-sustained. Of the non-hematologic AEs, diarrhea was the most common, occurring in 73% of study participants (grade 1–2: 66%, grade 3: 7%). Diarrhea occurred early in onalespib therapy and tapered off following the first cycle. Most grade 3 events occurred at the beginning of study enrollment and the severity of this AE improved over time after increased recognition and the institution of more aggressive supportive therapy with loperamide. Overall, the combination regimen was reasonably well-tolerated by study participants and only three patients withdrew due to side effects.

The ORR of 20% and median PFS of 2.9 months were less than expected. However, 94% of patients enrolled in this study had received prior taxane therapy and 23% of patients had received taxane therapy for metastatic disease. All six patients who experienced a response to combination therapy had prior taxane exposure, including one patient who received prior taxane therapy for metastatic disease. It should also be noted that three patients experienced complete radiographic or clinical response. One patient who had previously received neoadjuvant paclitaxel for TNBC and subsequently developed metastatic pulmonary nodules was recurrence-free after receiving 36 cycles of paclitaxel and 12 cycles of onalespib prior to developing brain-only metastasis. A second patient who had previously received paclitaxel in the adjuvant setting experienced a complete response of a chest wall recurrence that had been deemed unresectable. This patient's disease extended across the chest and on to the back of their torso. A clinical response in this situation is notable.

The PK of onalespib and paclitaxel alone and in combination were evaluated in this trial to study the potential for drug–drug interactions. The PK profiles of paclitaxel showed similar overall exposure with or without coadministration of onalespib across all DLs of study drug (120–260 mg/m^2), indicating no obvious impact of onalespib on paclitaxel exposure and elimination. A modest drug–drug interaction profile was observed for onalespib in the combination regimen, with mean volume of distribution, V_z, increasing 37–112% when given in combination with paclitaxel compared to when onalespib was given alone. In addition, the half-life of onalespib ($t_{1/2}$) increased 31–46% with coadministration. These differences were significant at onalespib doses of 200 and 260 mg/m^2. Therefore, this drug–drug interaction profile should be considered in future studies of HSP90 inhibitors combined with other agents with common metabolism or transport pathways.

Drug–drug interactions altering V_z and $t_{1/2}$, independent of a change in CL, are most commonly mediated by transporters or by displacement from plasma proteins.[37] Both paclitaxel and onalespib are substrates of CYPP3A4, and they interact with the P-glycoprotein (P-gp) multidrug transporter – paclitaxel as a substrate for MDR1 P-gp[38,39] and onalespib as a substrate and moderate inhibitor of P-gp.[40] This suggests that the significant V_z changes observed in this study may be due to transporter interactions. In addition, with onalespib as a substrate for glucuronidation[25] and paclitaxel as a strong inhibitor of uridine diphosphate-glucoronosyltransferases,[41] interactions among these metabolizing enzymes may contribute to the observed increases in onalespib $t_{1/2}$. Additional contributing factors may include the physical increases in plasma volume that accompanied the intravenous paclitaxel administration approximately 1–1.5 h following infusion of onalespib. The observed drug interactions were mild overall and did not result in changes to overall onalespib exposure, clearance, or maximum achieved EOI concentrations. At the doses and dosing regimen used in this study, these interactions are likely not of clinical significance. In fact, preclinical studies utilizing onalespib demonstrated preferential inhibitor accumulation and retention within the tumor microenvironment *in vivo*. This coupled with rapid clearance from the plasma and normal tissues have allowed for decreased dosing intervals and associated systemic toxicity of onalespib, as well as other HSP90 inhibitors.[42–45]

HSP90 inhibitors have been attractive as a potential breast cancer treatment due to HSP90 overexpression being associated with paclitaxel resistance, poor prognosis, and worse recurrence-free survival in TNBC.[46–51] The first-generation

HSP90 inhibitors, including the geldanamycin analogs tanespimycin, alvespimycin, and retaspimycin, were evaluated in patients with advanced breast cancer with varying results.[52–56] Clinical activity was noted in HER2-positive metastatic breast cancer patients receiving HER2-targeting therapy, however these agents could not be fully developed due to a number of safety and pharmacological limitations. Second-generation HSP90 inhibitors were developed and had more preclinical antitumor activity and a more favorable safety profile.[57–60] In early-phase clinical trials, ganetespib, a second-generation HSP90 inhibitor, showed promising clinical activity in patients with metastatic TNBC. However, these agents have yet to exhibit sustained clinical benefit in larger trials. These previous studies demonstrate that HSP90 inhibitors may have limited probability of success as a single agent among unselected patients with TNBC. However, the present study demonstrates HSP90 inhibition may have efficacy in taxane-resistant TNBC populations when combined with taxane therapy. Additionally, these findings have implications not only for the treatment of TNBC but also possibly for HER2-positive breast cancer and non-small cell lung cancer where there have been marginally successful studies of HSP90 inhibitors in patients with previous paclitaxel resistance.[57,60,61] For instance, a phase I study by Jhaveri *et al.*[60] tested a second generation HSP90 inhibitor ganetespib in combination with weekly trastuzumab and paclitaxel in patients with treatment refractory HER2 overexpressing breast cancer. Of the nine patients enrolled in this trial (median of three prior lines of systemic anti-cancer therapies), two (22%) experienced partial response, and five (56%) had stable disease. Another phase I study enrolled 23 patients with metastatic lung cancer ($N=23$) to treatment with escalating doses of SNX-5422 (oral prodrug of a highly selective HSP90 inhibitor) in combination with standard dose and schedule of paclitaxel and carboplatin.[62] Of 18 response-evaluable patients, 33% experienced a partial response and 56% had stable disease. Patients who responded had tumors that were enriched with oncogenic driver gene mutations including *KRAS*, *EGFR*, and *HER2*, which are known to interact with HSP90. Despite the promising early clinical efficacy signal of HSP90 inhibitors and paclitaxel combinations, these were early phase studies that enrolled small numbers of patients with heterogenous and heavily pretreated cancers. Therefore, these results need to be interpreted with caution. A recently published phase III study in patients with metastatic adenocarcinoma of the lung after progression on one prior line of systemic therapy showed no progression-free and overall survival benefit of adding ganetespib to docetaxel. This trial was stopped early due to futility noted at a planned interim analysis.[63]

Our study has several strengths, including assessment of the novel combination of onalespib and paclitaxel in patients who have previously progressed on a taxane therapy, the inclusion of patients with weakly hormone receptor-positive, HER2-negative breast cancer, and a design that allowed bidirectional evaluation of effects of one agent on the PK of the other. A weakness of this study is the heterogeneity of patients with no limit to the number of prior therapies. Sixteen percent of study participants received the study treatment as a first-line therapy for metastatic disease with some patients having received up to eight prior lines of systemic therapy for metastatic disease (mean 2, range 0–8). However, this limitation does not diminish the value of this dose-finding study with safety and tolerability as the primary objectives.

Conclusion

This study demonstrated that treatment with a combination of onalespib and paclitaxel was reasonably well-tolerated by most patients. Hematologic toxicities responded well to dose modifications and supportive care measures. In the PK studies, there were some changes in $t_{1/2}$ and V_z of onalespib when given with paclitaxel, but ultimately onalespib did not alter paclitaxel exposure and paclitaxel did not affect exposure to onalespib. Although onalespib with paclitaxel combination therapy did not yield durable objective responses or prolonged PFS rates, there were several patients who derived long-lasting benefit from this combination including patients who previously experienced progression on taxane therapy. This subset of patients with TNBC may benefit from HSP90 inhibitors and further exploration of this agent class is warranted.

Disclaimer

SS is an Editorial Board Member of Therapeutic Advances in Medical Oncology and an author of this paper; therefore, the peer-review process was

managed by alternative members of the board and the submitting editor not been involved in the decision-making process.

Declarations

Ethics approval and consent to participate

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice (ICH-GCP) guidelines. This study was approved by each participating institution's Internal Review Board (IRB) as well as the National Cancer Institute Central IRB under common study number NCI9876. Written informed consent was obtained from all patients.

Consent for publication

Not applicable.

Author contributions

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Competing interests

The authors declare that there is no conflict of interest.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Supplemental material

Supplemental material for this article is available online.

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