





ARTICLE



Translational Therapeutics

Combined PARP and HSP90 inhibition: preclinical and Phase 1 evaluation in patients with advanced solid tumours

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PURPOSE: PARP inhibitor resistance may be overcome by combinatorial strategies with agents that disrupt homologous recombination repair (HRR). Multiple HRR pathway components are HSP90 clients, so that HSP90 inhibition leads to abrogation of HRR and sensitisation to PARP inhibition. We performed *in vivo* preclinical studies of the HSP90 inhibitor onalespib with olaparib and conducted a Phase 1 combination study.

PATIENTS AND METHODS: Tolerability and efficacy studies were performed in patient-derived xenograft (PDX) models of ovarian cancer. Clinical safety, tolerability, steady-state pharmacokinetics and preliminary efficacy of olaparib and onalespib were evaluated using a standard 3 + 3 dose-escalation design.

RESULTS: Olaparib/onalespib exhibited anti-tumour activity against *BRCA1*-mutated PDX models with acquired PARPi resistance and PDX models with RB-pathway alterations (*CDKN2A* loss and *CCNE1* overexpression). Phase 1 evaluation revealed that dose levels up to olaparib 300 mg/onalespib 40 mg and olaparib 200 mg/onalespib 80 mg were safe without dose-limiting toxicities. Coadministration of olaparib and onalespib did not appear to affect the steady-state pharmacokinetics of either agent. There were no objective responses, but disease stabilisation ≥ 24 weeks was observed in 7/22 (32%) evaluable patients including patients with *BRCA*-mutated ovarian cancers and acquired PARPi resistance and patients with tumours harbouring RB-pathway alterations.

CONCLUSIONS: Combining onalespib and olaparib was feasible and demonstrated preliminary evidence of anti-tumour activity.

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INTRODUCTION

Homologous recombination repair (HRR) deficient tumours are exquisitely sensitive to PARP inhibitors (PARPis) and this synthetic lethal interaction is being exploited therapeutically across multiple cancer types [1]. However, acquired PARPi resistance occurs commonly, mainly due to secondary genetic or epigenetic events that restore HRR proficiency, and represents a rapidly emerging unmet medical need. Combination of PARPis with agents that induce “BRCAness”, i.e. agents which directly or indirectly inhibit HRR, is a promising strategy to overcome acquired PARPi resistance in HRR-deficient tumours as well as *de novo* PARPi resistance in HRR-proficient tumours [2, 3].

Heat-shock protein 90 (HSP90) is a molecular chaperone that facilitates the stability, maturation and function of a number of substrates (also referred to as HSP90 clients) that are involved in diverse cellular processes, including DNA repair [4–7]. Multiple components of the HRR pathway are HSP90 clients, including

BRCA1, *BRCA2* and *RAD51*, suggesting that inhibition of HSP90 may abrogate HRR and therefore sensitise cancer cells to PARPi [8, 9]. In this regard, we have previously demonstrated that HSP90 inhibition (HSP90i) in HRR-proficient high-grade serous ovarian cancer cell lines leads to downregulation of *BRCA1* and *RAD51*, compromise of HRR, increased DNA damage, and subsequent sensitisation to PARPis [10]. Importantly, *in vitro* synergism between HSP90i and PARPi was observed in HRR-proficient models even with sublethal concentrations of HSP90i [10]. Similar experiments have been conducted in HRR-proficient NCI-H1299 non-small cell lung cancer (NSCLC) cells, showing that HSP90i depletes *BRCA2*, *BRCA1* and *RAD51* and sensitises them to PARPi. HSP90 has also been implicated as a mechanism of acquired PARPi resistance in HRR-deficient cells. Specifically, in MDA-MB-436 triple-negative breast cancer cells harbouring a *BRCA1* BRCT domain mutation that were rendered PARPi resistant via exposure to graded concentrations of PARPi over time, resistance was

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associated with HSP90-mediated stabilisation of the BRCA1-mutant protein, without evidence for *BRCA1* reversion mutation [11]. In that setting, resistance was overcome with the application of an HSP90i, so that PARPi sensitivity was restored [11].

Onalespib (AT13387) is a synthetic, second-generation, non-ansamycin, small-molecule HSP90 inhibitor that exhibits a high affinity for the ATP-binding site at the N-terminal domain of HSP90 [12, 13]. Onalespib has been studied in advanced solid tumours both as monotherapy and in combination with targeted agents [12, 14, 15]. Here, we evaluated the synergism between HSP90i and PARPi *in vivo* by performing tolerability and efficacy studies of onalespib combined with olaparib in patient-derived xenograft (PDX) models of ovarian cancer and subsequently conducted a Phase 1 study of these agents in patients with advanced solid tumours.

METHODS

Preclinical studies using ovarian cancer patient-derived xenograft (PDX) models

A panel of clinically and molecularly characterised PDX mouse models of high-grade serous ovarian cancer (HGSOC) generated from patient ascites as previously reported [16] was selected for preclinical studies of olaparib in combination with onalespib. These models have been luciferized thereby providing the ability to follow the growth of tumours by non-invasive bioluminescent imaging (BLI) technology. All models have been characterised for HRR proficiency status utilising a RAD51 immunohistochemical assay developed in the Dana-Farber Cancer Institute (DFCI) Center for DNA Damage and Repair [17, 18].

Approximately 5×10^6 cells derived from ascites were injected intraperitoneally into 8-week-old female NSG mice. One to 2 weeks post implantation, upon establishment of tumour burden as measured by BLI, mice were randomised to vehicle, olaparib 100 mg/kg/day *p.o.* once daily for 28 days, onalespib (AT13387) 45 mg/kg/day intraperitoneally (*i.p.*) for 2 days (D1, D2) on/5 days off \times 4 weeks (*i.e.* days 1, 2, 8, 9, 15, 16, 22 and 23), and the combination of olaparib plus onalespib at the same doses and schedule of administration. Olaparib was formulated with 10% DMSO, 50% of 60% hydroxypropyl-beta-cyclodextrin (HP β CD) and 40% water, while onalespib was formulated with 17.5% HP β CD in water. Peritoneal disease dissemination in the mice was monitored serially once weekly for up to 6 weeks using a Xenogen IVIS-200 system (Xenogen). End of study mouse plasma CA125 levels was measured via a custom assay using BioScale's Acoustic Membrane Micro Particle technology as previously described [19].

Study design and treatment

This was a Phase 1, multicenter, open-label, investigator-initiated study of olaparib and onalespib (NCT02898207) sponsored by the National Cancer Institute (NCI) Cancer Therapy Evaluation Program (NCI-CTEP). Patients were enrolled at three different centres (Dana-Farber Cancer Institute, Boston, MA, Mayo Clinic, Rochester, MN and Mayo Clinic, AZ) in the United States through the Experimental Therapeutics Clinical Trials Network (ETCTN). A standard 3 + 3 design was employed, with dose escalation if 0/3 or 1/6 participants experienced dose-limiting toxicity (DLT). The primary objective of this study was to establish the maximum tolerated dose (MTDs) of olaparib and onalespib administered in combination in patients with advanced solid tumours. Secondary objectives were to determine the dose-limiting toxicity (DLT), safety, recommended Phase 2 doses (RP2D), plasma pharmacokinetics and preliminary anti-tumour activity of the olaparib and onalespib combination as assessed by RECIST 1.1.

During cycle 0 (C0), olaparib was administered alone twice daily for 1 week (days 1–7). To test the hypothesis that onalespib sensitises to olaparib by inhibiting HRR, three optional biopsies were planned, one pretreatment/baseline, one after C0 when olaparib was administered alone and one after the combination of olaparib/onalespib with the expectation that there will be the induction of BRCA1 and RAD51 foci after administration of olaparib alone and reduced formation of these foci and downregulation of expression of HRR pathway genes after the combination of olaparib/onalespib. All biopsies were optional but, eventually, no patient agreed to undergo these biopsies. Cycle 1 and beyond, olaparib continued to be administered twice daily (days 1–28) and onalespib was administered IV for 2 consecutive days on/5 days off \times 3 weeks of a 4-week cycle (*i.e.* days 1, 2, 8, 9, 15, 16 of a 28-day cycle). Per

the dose-escalation schema, doses of olaparib ranging from 50 to 300 mg orally twice daily were combined with doses of onalespib ranging from 20 to 160 mg/m² (20-40-80-120-160) IV using a ping-pong strategy. Tumour assessment by RECIST 1.1 occurred every two cycles \pm 7 days. Protocol treatment continued indefinitely until progression, unacceptable toxicity, patient refusal, the intercurrent illness that prevented further administration of treatment, or general or specific changes in the participant's condition which rendered the participant unacceptable for further treatment in the opinion of the treating investigator.

The choice of the twice-weekly (days 1 and 2), *i.e.* consecutive-day dosing of onalespib, was supported by the tolerability and efficacy studies of olaparib/onalespib in our PDX models. Historically, many HSP90 inhibitors were developed with a twice-weekly intermittent schedule based on an initial depletion in HSP90 client protein expression with re-expression after approximately 72 h. Therefore, intermittent, twice-weekly dosing of onalespib was the chosen schedule for the initial clinical study in humans [20]. However, the alternative dosing schedule with daily administration for the first 2 days per week (QD \times 2/week, *i.e.*, consecutive-day dosing) demonstrated consistently greater tumour growth inhibition and intratumoral drug accumulation in several human xenograft models [21]. Consecutive-day dosing also allowed increased magnitude and duration of drug exposures in tumours, while the drug was cleared rapidly from the plasma, potentially improving the therapeutic window with sustained client protein depletion and minimising damage to normal tissues. Additional preclinical work demonstrated similar results with ganetespib, where consecutive-day dosing resulted in superior tumour xenograft regressions, accompanied by more sustained pharmacodynamic effects compared to intermittent dosing [22]. Finally, in a second Phase 1 study of onalespib, consecutive-day dosing was well tolerated with pharmacodynamic effects demonstrated among nine tumour biopsy pairs [12]. For these reasons, the daily administration of onalespib for the first 2 days per week (QD \times 2/week) was chosen. Nonetheless, it is important to acknowledge that the optimal schedule of onalespib (as well as of HSP90 inhibitors in general) remains unclear and future studies randomising between different schedules are needed to address this question.

The clinical trial was approved by the NCI Central Institutional Review Board (CIRB) and the US Food and Drug Administration (NCT02898207). All procedures involving human participants were carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from patients or guardians before enrolment in the study. This study was funded by the NCI-Cancer Therapy Evaluation Program (CTEP) and conducted under the auspices of the Experimental Therapeutics Clinical Trials Network (ETCTN). Dana-Farber/Harvard Cancer Center served as the lead site for the study, supported by NIH grant UM1 CA186709. The drugs were provided by AstraZeneca (olaparib) and Astex Pharmaceuticals (onalespib) via the NCI. AstraZeneca and Astex Pharmaceuticals had no role in study design, data collection, data analysis, data interpretation or writing of the report. All authors had full access to all the data in the study, were involved in writing the report, and approved the final version for submission. The first and last authors had final responsibility for the decision to submit for publication.

Eligibility

Eligibility criteria included histologically or cytologically confirmed malignancy that was metastatic or unresectable and for which standard curative or palliative measures did not exist or were no longer effective. Patients may have received any number of prior therapies, including prior PARPi therapy. Other eligibility criteria included age \geq 18 years, availability of a formalin-fixed, paraffin-embedded tumour specimen, ECOG performance status 0 or 1 (Karnofsky $>$ 60%), life expectancy of greater than 12 weeks, normal organ and marrow function, QTcF \leq 450 ms, ability to swallow tablets and no significant impairment in gastrointestinal absorption, and ability to understand and willingness to sign a written informed consent document. Women of child-bearing potential and men had to agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation, and for 3 months after the last dose of study drugs. Exclusion criteria included known active or history of brain metastases, known history of QT/QTc prolongation or Torsades de Pointes (TdP), and uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements. Pregnant and HIV-positive patients were excluded. Finally, participants receiving any

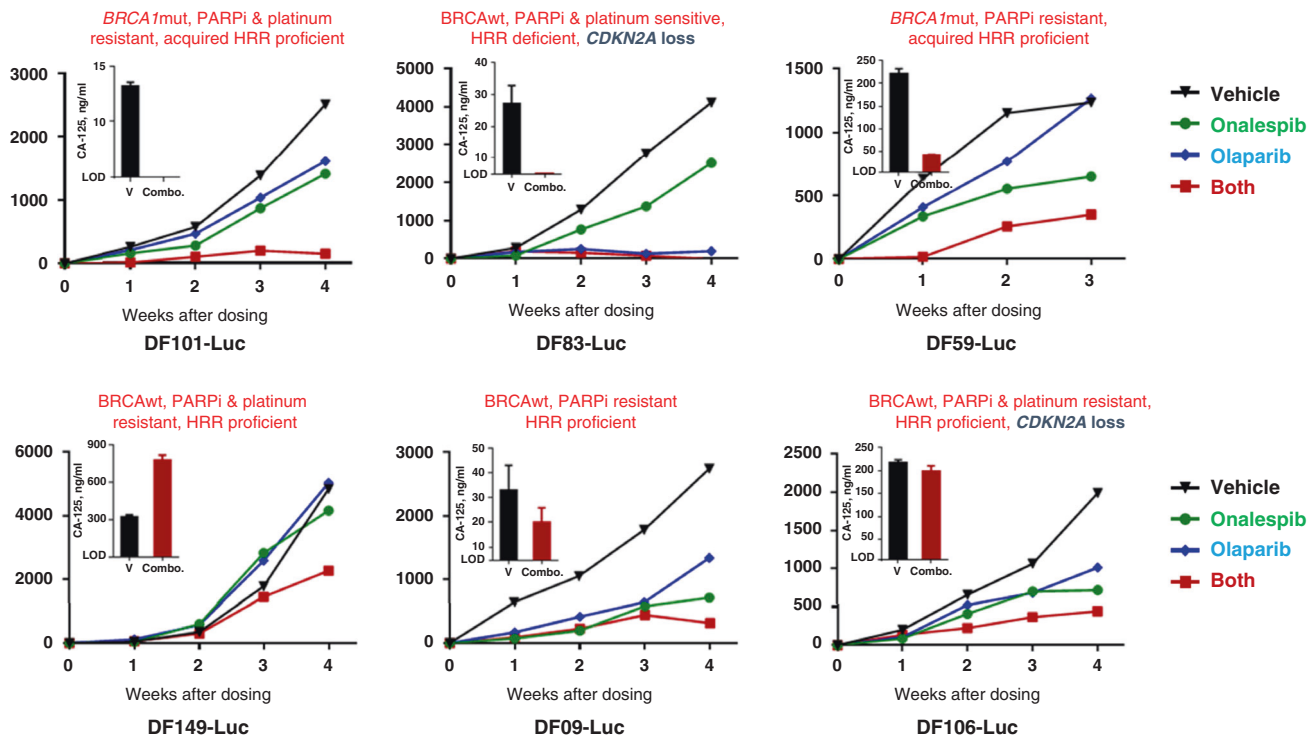


Fig. 1 Olaparib and onalespib combination in ovarian PDX models. NSG mice bearing luciferized PDXs were treated with vehicle, olaparib (100 mg/kg, daily), onalespib (45 mg/kg 2×/week) or their combination for 4 weeks with $n = 1$ per group per model. Tumour growth was monitored by weekly bioluminescence imaging. Fold change in the bioluminescence from day 0 is shown. Inlay bar graph shows CA125 in mouse plasma at the end of treatment performed in triplicate.

medications or substances that are strong inhibitors or inducers of CYP3A4 or moderate inhibitors of CYP3A4 or medications with a known risk to prolong the QT interval or inducing TdP were ineligible. All patients had to undergo a pre-study ophthalmologic exam by an ophthalmologist that included visual acuity testing, slit-lamp examination, and fundoscopic examination.

Pharmacokinetic (PK) evaluation

The PK study was designed to assess the effect of onalespib on the steady-state PKs of olaparib and if the PKs of onalespib were affected by the concurrent administration of olaparib. PK sampling was performed in all patients enrolled in the dose escalation to define the plasma concentration-time profile of olaparib over the dosing interval for the day 7 dose when given alone in cycle 0. Pharmacokinetic samples were also collected over a 24-h interval on days 1 and 15 of cycle 1 to define the plasma profiles for olaparib and onalespib. Blood samples were collected in 4 mL plastic Vacutainer tubes with spray-dried K2EDTA (Becton, Dickinson and Co., Franklin Lakes, NJ) and promptly centrifuged to harvest the plasma which was stored in cryovials at -80°C . The concentrations of olaparib and onalespib in the plasma were determined independently using analytical methods based upon reversed-phase high-performance liquid chromatography with tandem mass spectrometric detection. The analytical methods were validated and applied to the routine analysis of study samples as recommended in the current U.S. Food and Drug Administration Guidance for Industry (<https://www.fda.gov/media/70858/download>). At the lowest concentration (free base equivalent) included in the calibration curves, which was 1.0 ng/mL for olaparib and 2.5 ng/mL for onalespib, interday accuracy was within 1.0% of the nominal concentration and the precision was $\leq 3.4\%$ for both analytes. Interday accuracy ranged from 95.5 to 103.5% and the precision ranged from 2.9 to 6.8% for all other calibration standards for both analytes. Time points were determined as the difference between the blood sample collection time and the time that olaparib was taken or the starting time of the onalespib infusion. The plasma concentration (free base equivalent)-time data were analysed by noncompartmental methods using WinNonlin Professional version 5.0.1 (Pharsight Corp, Mountain View, CA). Pharmacokinetic parameters are reported as the geometric mean (geometric %CV) of the values for individual patients at each dose level. GraphPad Prism for Windows,

version 8.3.0 (GraphPad Software, La Jolla, CA) was used for the statistical comparison of mean pharmacokinetic parameters using the paired or unpaired two-tailed t test, as appropriate, after logarithmic transformation of the data. $P < 0.05$ was the criterion for statistical significance.

Targeted next-generation sequencing of archival EOC

Tumour DNA from archival, formalin-fixed tissues was analysed at the Dana-Farber Cancer Institute (DFCI)/Brigham and Women's Hospital (BWH) Center for Advanced Molecular Diagnostics (CAMD) using targeted panel next-generation sequencing (Oncopanel) covering exons of 447 cancer-associated genes, plus intronic regions of genes involved in somatic rearrangements [23–25].

Statistical analysis

Activity was measured as an objective response using RECIST 1.1. Safety data were described by the number and proportion of patients who had treatment-related adverse events using CTCAE v5.0. Patient characteristics and adverse event frequencies were summarised using descriptive statistics.

RESULTS

Olaparib/onalespib in HGSOc PDX models

Before embarking on the clinical trial of olaparib plus onalespib, we assessed the tolerability and efficacy of this combination in HGSOc PDX models. We initially performed tolerability studies. As shown in Supplement Fig. S1, doses of olaparib 100 mg/kg/day p. o. daily \times 3 weeks and onalespib 45 mg/kg i.p. for 2 days (D1, D2) on/5 days off \times 3 weeks (i.e. days 1, 2, 8, 9, 15, 16) were well tolerated without weight loss in the mice. Efficacy studies revealed that the combination of onalespib and olaparib induced inhibition of tumour growth in a variety of ovarian high-grade serous PDX models, including (i) *BRCA1/2* wild-type (wt), PARPi-resistant, HRR-proficient models (DF149, DF09, DF106, Fig. 1, bottom panel); (ii) the *BRCA1/2* wild-type, PARPi and platinum-sensitive, HRR-deficient DF83 model (Fig. 1, top panel) and (iii) *BRCA1*-mutated,

Table 1. Clinicopathologic characteristics of patients enrolled in the study.

	Overall (n = 28)	
	N	%
Gender		
Female	26	92.9%
Male	2	7.1%
Age		
Mean (SD)	61.5 (9.2)	
Median (IQR) [min, max]	65 (11.3) [45,78]	
Race		
White	25	89.3%
Asian	1	3.6%
Unknown	2	7.1%
Ethnicity		
Not Hispanic or Latino	28	100%
ECOG performance status		
0 = asymptomatic and fully active	17	60.7%
1 = symptomatic; fully ambulatory; restricted in physically strenuous activity	11	39.3%
Primary site of disease		
Ovary	18	64.3%
Uterus	5	17.9%
Colon	2	7.1%
Breast	1	3.6%
Melanoma	1	3.6%
Malignant solitary fibrous tumour	1	3.6%
Stage at diagnosis		
IC	1	3.6%
IIC	1	3.6%
III	3	10.7%
IIIC	5	17.9%
IV	14	50%
IVB	4	14.3%
Histology		
Well differentiated	2	7.1%
Moderately differentiated	2	7.1%
Poorly differentiated	15	53.6%
Grade cannot be assessed/unknown	9	32.1%
Lines of prior therapy		
Median [min, max]	5.5	1–15

PARPi-resistant DF101 and DF59 models with acquired HRR proficiency (Fig. 1, top panel). In five of these six models, treatment with olaparib/onalespib was associated with a reduction in the CA125 (DF101, DF83, DF59, DF09 and DF106) while in one model (DF149) CA125 increased (Fig. 1). The phenomenon whereby there is a decrease in tumour burden but the rise in the CA125 is not unusual as it is well described that, particularly in platinum-resistant disease, CA125 is not a reliable marker for assessment of response and progression [26].

The PARPi (olaparib)-resistant DF149, DF09, DF106, DF101 and DF59 models were determined to be HRR proficient using a RAD51-based IHC assay whereby freshly derived tumour cells from PDX-bearing mice were exposed to radiation and stained with an anti-RAD51 antibody. Consistent with the olaparib response, these five olaparib-resistant models exhibited DNA damage-induced

RAD51 foci suggestive of intact HRR. The presence of RAD51 foci in the *BRCA1*-mutant DF101 and DF59 models suggested that restoration of HRR had occurred thereby explaining the olaparib resistance of these models. Conversely, the olaparib- and platinum-sensitive DF83 model showed the absence of RAD51 foci, consistent with HRR deficiency (this model harbours RAD51C promoter hypermethylation) as previously described [18]. Of note, two of the models (DF83 and DF106) that responded to the combination of onalespib and olaparib, harboured *CDKN2A* loss while four models exhibited elevated *CCNE1* mRNA expression (DF106, DF09, DF149 and DF101) as previously described [18]; no models exhibited *CCNE1* amplification. Taken together, activity was observed in both HRR-deficient and -proficient models, *BRCA1*-wt and *BRCA1*-mutated models, as well as PARPi-sensitive and PARPi-resistant models.

Patient accrual

Between September 8, 2017 and November 12, 2019, a total of 28 patients were enrolled into the study; 26 patients were female (Table 1). In total, 18 patients had ovarian cancer, 5 uterine cancer, 2 colon cancer and the remaining 3 had breast cancer ($n = 1$), melanoma ($n = 1$) and malignant solitary fibrous tumour ($n = 1$). The median number of prior lines of therapy was 5.5 (range 1–15). Twenty-one patients were evaluable for DLT; 7 patients were not evaluable for DLT assessment and were replaced. Of these seven patients, two patients developed small bowel obstruction related to disease progression during cycle 0 and 1, two patients withdrew consent during cycle 1 because of advanced disease, two patients could not receive at least 75% of cycle 1 dosing because of advanced disease, and one patient assigned to dose level DL3 was erroneously treated with DL2. This patient was replaced but continued treatment on DL2, was followed for radiographic response and exhibited no DLT (Table 2).

Dose escalation and toxicities

Six dose levels were evaluated (Table 2). The intended starting dose (defined as dose level (DL) 0) was olaparib 200 mg p.o. twice daily and onalespib 20 mg/m² and was well tolerated without DLTs (0 DLTs out of three evaluable patients). The next dose level (DL1) of onalespib 40 mg/m² plus olaparib 200 mg b.i.d. was also well-tolerated without DLTs (0 DLTs among three evaluable patients). The next dose level (DL2) of onalespib 40 mg/m² plus olaparib 300 mg b.i.d. was also well-tolerated without DLTs (0 DLTs among three evaluable patients). However, when the onalespib was escalated to 80 mg/m² with olaparib 300 mg b.i.d. (DL3) there were 2 DLTs (both anaemia) in five evaluable patients. When olaparib was de-escalated back to 200 mg b.i.d. with onalespib 80 mg/m² (DL2a), this dose level was well-tolerated without DLTs (0 DLTs among three evaluable patients). The next dose level (DL3a) of olaparib 200 mg b.i.d. with onalespib 120 mg/m² did not complete evaluation (1 DLT (thrombocytopenia) among four evaluable patients) as enrolment was suspended due to discontinuation of further development of onalespib.

Treatment-related non-haematologic and haematologic toxicities that were Grade 3+ or occurred in ≥10% of all treated patients are listed in Table 3. There were no unexpected toxicities observed based on the known toxicities of olaparib and onalespib and no irreversible toxicities. Diarrhoea (expected toxicity of onalespib), nausea and anaemia (expected toxicities of both olaparib and onalespib) were the most common treatment-related toxicities, predominantly Grades 1 and 2. Grade 3 anaemia was observed in five patients (18%). There was only one Grade 4 toxicity (neutropenia) and no Grade 5 toxicities.

Pharmacokinetic (PK) studies

Mean values of selected steady-state pharmacokinetic parameters for olaparib and onalespib are presented in Tables 4 and 5,

Table 2. Dose levels explored, number of patients on each dose level, and whether DLTs were observed.

Dose level	Cycle 0 dose (olaparib p.o. b.i.d.)	Cycle 1 + dose (olaparib p.o. b.i.d.; onalespib i.v. on days 1, 2, 8, 9, 15, and 16)	Total patients	Evaluable patients for DLT ^a	DLTs (aetiology)
DL0	Olaparib 200 mg	Olaparib 200 mg; onalespib 20 mg/m ²	3	3	0
DL1	Olaparib 200 mg	Olaparib 200 mg; onalespib 40 mg/m ²	4	3	0
DL2	Olaparib 300 mg	Olaparib 300 mg; onalespib 40 mg/m ²	6	3	0
DL3	Olaparib 300 mg	Olaparib 300 mg; onalespib 80 mg/m ²	7	5	2 (both Gr3 anaemia)
DL2a	Olaparib 200 mg	Olaparib 200 mg; onalespib 80 mg/m ²	3	3	0
DL3a	Olaparib 200 mg	Olaparib 200 mg; onalespib 120 mg/m ²	5	4	1 (Gr3 thrombocytopenia)

^aSeven patients were not evaluable for DLT assessment and were replaced. Of these seven patients, two patients developed small bowel obstruction related to disease progression during cycle 0 and 1 (DL1 and DL2), two patients withdrew consent during cycle 1 because of advanced disease (DL2 and DL3), two patients could not receive at least 75% of cycle 1 dosing because of advanced disease (DL2 and DL3a), and one patient assigned to dose level DL3 was erroneously treated with DL2 (this patient was replaced but remained on treatment on DL2, was followed for radiographic response and exhibited no DLT).

Table 3. Treatment-related toxicities that are grade 3+ or occurring in ≥ 10% of patients (n = 28).

Toxicity	Maximum grade		
	1–2	3	4
Diarrhoea	21 (75%) [3–3–3–5–3–4] ^a	1 (4%) DL3a	—
Nausea	18 (64%) [1–2–4–6–3–2]	1 (4%) DL0	—
Anaemia	13 (46%) [2–3–2–4–1–1]	5 (18%) 2 DL3, 3 DL3a	—
Thrombocytopenia/platelet count decrease	10 (36%) [0–3–1–3–0–3]	2 (7%) DL3, DL3a	—
Fatigue	12 (43%) [1–3–3–2–1–2]	—	—
Vomiting	8 (29%) [1–1–3–2–1–0]	1 (4%) DL0	—
Abdominal pain	4 (14%) [1–1–0–0–2–0]	1 (4%) DL2	—
Anorexia	5 (18%) [1–1–1–0–1–1]	—	—
Neutrophil count decreased	3 (11%) [0–0–1–1–0–1]	—	1 (4%) DL3a
Lymphocyte count decreased	3 (11%) [0–0–0–1–1–1]	1 (4%) DL3a	—
Floater	4 (14%) [0–1–0–1–0–2]	—	—
Dysgeusia	4 (14%) [0–1–0–1–0–2]	—	—

^aCorresponding to DL0-DL1-DL2-DL3-DL2a-DL3a, respectively.

respectively. At each dose level evaluated, there were no significant differences between the mean values of the olaparib pharmacokinetic parameters determined when it was given alone on cycle 0 day 7 as compared to days 1 and 15 of cycle 1, when olaparib was taken immediately after completing the IV infusion of onalespib. In addition, there was no evidence of a trend in the mean apparent oral clearance of olaparib with respect to the onalespib dose. There were no significant differences in the mean onalespib pharmacokinetic parameters on cycle 1 day 15 as compared to cycle 1 day 1. Mean values of the olaparib steady-state pharmacokinetic parameters determined in this study were in very good agreement with data reported for the drug when given alone [27]. In addition, values of the mean CL of onalespib at each dose level were within the range previously reported in the initial Phase I clinical trial of onalespib given as a single agent to solid tumour patients, which was 40.9 to 69.1 L/h/m² [20]. Accordingly, the coadministration of olaparib and onalespib does not appear to affect the steady-state pharmacokinetics of either agent.

Clinical efficacy and targeted next-generation sequencing

Among the 22 patients who had at least one restaging scan in the study, there were no objective responses by RECIST 1.1 (Fig. 2). One patient with ovarian carcinosarcoma experienced shrinkage of her tumour by 28%; this patient decided to withdraw after three

cycles of protocol therapy. Fifteen patients (68%) had stable disease as a best radiologic response, while seven (32%) had progressive disease (PD) (Fig. 2). Seven (32%) patients derived clinical benefits from this regimen with stable disease for at least 24 weeks (Fig. 3).

We performed targeted next-generation sequencing (Oncopanel) in archival, formalin-fixed tumour specimens from the seven patients who derived clinical benefit and the one patient who experienced tumour shrinkage by 28% (Table 6). Two patients had BRCA-mutated high-grade serous ovarian cancer (HGSOC) and had both progressed through prior PARP inhibitor therapy twice. The first patient with BRCA1-mutated HGSOC had progressed through olaparib and niraparib on two separate previous occasions and completed six cycles of olaparib/onalespib before progression. The second patient with BRCA2-mutated ovarian cancer had progressed through olaparib/alpelisib (PI3K inhibitor) and olaparib/prexasertib (CHK1 inhibitor) previously and completed ten cycles before withdrawing after learning that development of onalespib was discontinued. Two other patients among those with disease stabilisation ≥24 weeks exhibited alterations involving the RB pathway; one patient with uterine serous cancer had biallelic RB1 loss and completed eight cycles and one patient with HGSOC exhibited CCNE1 gain and completed six cycles. Finally, CCNE1 amplification was observed in the patient who experienced tumour shrinkage by 28%.

Table 4. Mean steady-state pharmacokinetic parameters for olaparib given alone (cycle 0) and combined with onalespib (cycle 1).^a

Dose level	Dose (mg)	Visit	No. of patients	C _{min} (ng/mL)	C _{max} (ng/mL)	AUC _t (ng h/mL)	CL/F (L/h)
0	200	C0D7	3	1134 (35.8)	5793 (12.2)	34,481 (28.7)	5.80 (28.7)
0	200	C1D1	3	783 (97.2)	5173 (74.9)	30,525 (77.6)	6.55 (77.6)
0	200	C1D15	3	1350 (195)	6218 (37.5)	37,072 (75.1)	5.39 (75.1)
1	200	C0D7	4	955 (27.4)	7058 (11.0)	36,972 (12.4)	5.41 (12.4)
1	200	C1D1	4	1069 (38.6)	6155 (18.2)	36,004 (19.3)	5.55 (19.3)
1	200	C1D15	3	895 (56.3)	6135 (8.8)	31,830 (22.1)	6.28 (22.1)
2	300	C0D7	7 ^b	2189 (51.8)	9598 (24.6)	60,713 (28.8)	4.94 (28.8)
2	300	C1D1	7 ^b	2230 (50.5)	9920 (20.7)	62,622 (22.7)	4.79 (22.7)
2	300	C1D15	4	1891 (56.4)	8232 (44.7)	54,057 (46.6)	5.55 (46.6)
3	300	C0D7	5	2408 (111)	9092 (50.5)	50,493 (56.6)	5.94 (56.6)
3	300	C1D1	5	1229 (29.9)	8095 (48.8)	47,415 (35.0)	6.33 (35.0)
3	300	C1D15	3	1326 (34.3)	6734 (9.3)	44,184 (12.6)	6.79 (12.6)
2a	200	C0D7	3	1837 (1.6)	7755 (9.3)	46,710 (14.0)	4.28 (14.0)
2a	200	C1D1	3	1579 (41.8)	7516 (29.0)	47,319 (25.2)	4.23 (25.2)
2a	200	C1D15	3	1567 (49.7)	8267 (20.3)	49,677 (21.3)	4.03 (21.3)
3a	200	C0D7	5	1380 (106)	7670 (24.3)	45,266 (25.8)	4.42 (25.8)
3a	200	C1D1	5	1507 (25.1)	8644 (13.5)	50,651 (28.2)	3.95 (28.2)
3a	200	C1D15	2	1327 (5.0)	7085 (2.1)	42,602 (0.7)	4.69 (0.7)

^aData are reported as the geometric mean (geometric %CV).^bOne patient assigned to DL3 was erroneously treated with DL2 and included in the DL2 for the PK studies.**Table 5.** Mean steady-state pharmacokinetic parameters for onalespib^a.

Dose level	Dose (mg/m ²)	Visit	No. of patients	C _{max} (ng/mL)	AUC ₂₄ (ng h/mL)	CL (L/h/m ²)	V _z (L/m ²)
0	20	C1D1	3	51.2 (62.9)	312.8 (32.8)	52.3 (34.3)	777 (28.9)
0	20	C1D15	2	46.7 (8.2)	305.8 (12.3)	49.7 (21.6)	899 (1.6)
1	40	C1D1	4	128.2 (61.3)	672.8 (10.9)	49.4 (13.4)	1,290 (179)
1	40	C1D15	3	116.8 (78.4)	657.5 (6.8)	51.1 (5.4)	732 (3.2)
2	40	C1D1	4	140.6 (188.5)	602.4 (48.9)	57.0 (48.2)	786 (34.8)
2	40	C1D15	3	196.7 (115)	782.7 (53.4)	44.7 (52.8)	561 (53.6)
3	80	C1D1	5	238.4 (94.4)	1207.0 (17.3)	58.3 (14.7)	718 (15.4)
3	80	C1D15	3	278.2 (64.1)	1073.2 (23.6)	69.2 (25.2)	662 (28.4)
2a	80	C1D1	3	271.6 (127)	1461.7 (59.0)	47.1 (57.7)	624 (50.2)
2a	80	C1D15	3	267.0 (175)	1241.7 (52.6)	57.0 (46.9)	684 (57.1)
3a	120	C1D1	5	560.3 (99.1)	2082.8 (34.2)	49.4 (33.4)	614 (59.4)
3a	120	C1D15	2	390.3 (38.5)	2003.1 (11.6)	53.7 (8.0)	597 (16.6)

^aData are reported as the geometric mean (geometric %CV).

DISCUSSION

This Phase 1 dose-escalation study showed that combining the HSP90i onalespib and the PARPi olaparib is feasible, with no unexpected toxicities or safety signals identified. Although the majority of patients enrolled were heavily pretreated, the olaparib and onalespib combination was well tolerated and myelosuppression was not an issue. Adverse events that defined DLTs included anaemia in two patients and thrombocytopenia in one patient. Dose levels up to olaparib 300 mg/onalespib 40 mg (DL1) and olaparib 200 mg/onalespib 80 mg (DL2a) were deemed safe without any DLTs, but dose level DL3a (olaparib 200 mg/onalespib 120 mg) did not complete evaluation (one DLT in four evaluable patients) as enrolment was suspended due to discontinuation of further development of onalespib. PK analysis indicated that the

coadministration of olaparib and onalespib does not appear to affect the steady-state pharmacokinetics of either agent.

Although there were no objective responses by RECIST, 7 (32%) patients exhibited disease stabilisation for ≥ 24 weeks and one additional patient experienced shrinkage of her tumour by 28%; this patient decided to withdraw from the study after three cycles of protocol therapy. This activity is clinically meaningful considering that most of these patients were heavily pretreated (four patients with ≥ 7 prior lines and three with ≥ 3 prior lines) and had no alternative treatment options. Among the seven patients with disease stabilisation, two patients had BRCA-mutated HGSOc (1 BRCA1-mutated and 1 BRCA2-mutated) that had progressed twice through prior PARPi therapy. Activity in that setting is consistent with our preclinical findings from the PDX models whereby

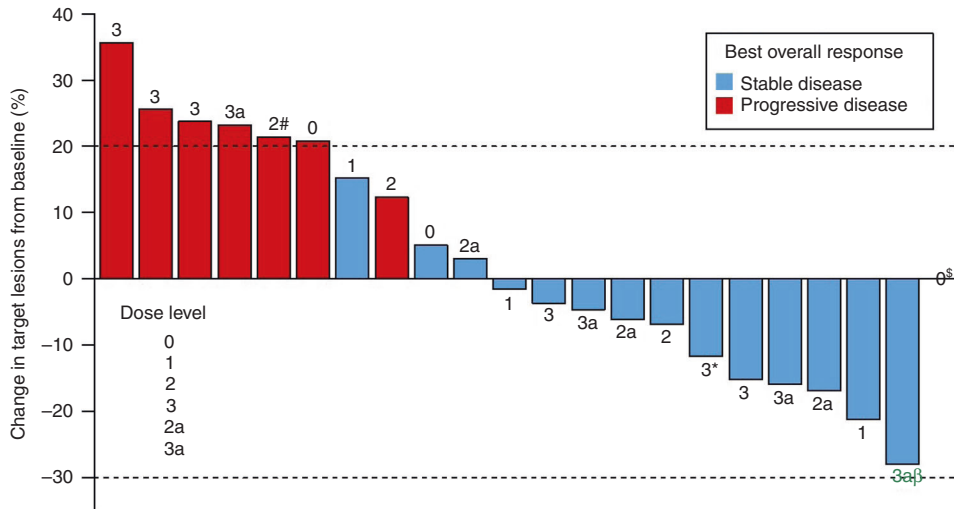


Fig. 2 Response to study treatment (n = 22). Summary of the maximum reduction in the sum of target lesion sizes. Dose level is indicated for each patient. ^sPatient in DL0 in the far right had no measurable disease. *This patient was replaced due to being enrolled under DL3 but treated at DL2. #This patient was replaced due to not having received 75% of Cycle 1 dosing. ^βThis patient had tumour shrinkage by 28%.

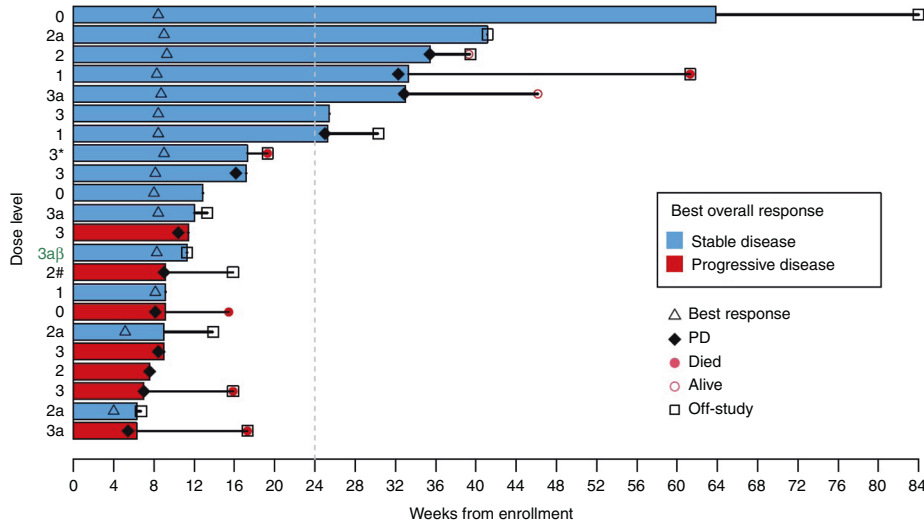


Fig. 3 Duration of treatment and time to progression (n = 22). Duration and time to progression are defined as the interval from the date of enrolment to date of either progression or date of last disease assessment. *This patient was replaced due to being enrolled under DL3 but treated at DL2. #This patient was replaced due to not having received 75% of cycle 1 dosing. ^βThis patient had tumour shrinkage by 28%.

Table 6. Genomic aberrations among patients with stable disease ≥24 weeks and in the patient with 28% tumour shrinkage.

Patient	Tumour type	Best response	Cycles	Prior lines	Prior PARPi	Relevant genomic alterations
1	Ovarian (HGSOC)	SD	14	11	NO	ARID1A, TP53 mutations
2	Uterine serous	SD	8	3	NO	KRAS, PIK3CA, TP53 mutations, RB1 mutation and copy loss
3	HGSOC	SD	6	7	Yes (x2)	gBRCA1, TP53 mutations
4	Malignant solitary fibrous tumour	SD	8	2	NO	PIK3CA mutation
5	HGSOC	SD	6	4	NO	TP53 mutation, CCNE1 gain
6	HGSOC	SD	10	7	Yes (x2)	BRCA2, TP53 mutations
7	Uterine high-grade endometrioid	SD	8	3	NO	TP53, CTNNB1, PTEN mutations
8	Ovarian carcinosarcoma	SD (-28%)	3	7	NO	TP53 mutation, CCNE1 amplification

olaparib/onalespib demonstrated efficacy against *BRCA1*-mutated models with acquired PARPi resistance due to restoration of HRR proficiency (DF101 and DF59 models, Fig. 1, top panel).

Of note, alterations involving the RB pathway were identified in two patients among those with disease stabilisation ≥ 24 weeks (one patient with HGSOc exhibited *CCNE1* gain and one patient with uterine serous cancer had biallelic *RB1* loss) and in the patient who experienced tumour shrinkage by 28% (HGSOc with *CCNE1* amplification). Loss of RB-pathway regulation is common in both uterine and ovarian high-grade serous tumours, and may occur via *CCNE1* amplification, *RB1* alterations (mutations or homozygous deletion), and *CDKN2A* loss. *CCNE1*, *RB1* and *CDKN2A* alterations are mutually exclusive, reflecting a strong selection pressure for loss of RB-pathway regulation and rapid G1- \rightarrow S phase entry in these tumours [28]. These observations are again consistent with our preclinical findings from the PDX models whereby olaparib/onalespib also demonstrated activity in tumours with RB-pathway alterations, i.e. models with *CDKN2A* loss and *CCNE1* overexpression. Mechanistically, cyclin E activates CDK2 which phosphorylates RB leading to activation of E2F transcriptional activity and G1- \rightarrow S phase progression [29, 30]. Interestingly, cyclin E is a client protein of HSP90 raising the possibility that abrogation of its activity may contribute to the activity of olaparib/onalespib against *CCNE1*-amplified or overexpressing tumours [31, 32]. This activity may also be explained by the fact that *CCNE1*-amplified tumours are dependent on HRR and sensitive to abrogation of BRCA1 [33]; therefore, downregulation of BRCA1 and other HRR proteins by onalespib, may also explain the activity against *CCNE1*-amplified/overexpressing tumours. Loss of *CDKN2A* leads to activation of CDK4 and CDK6, which similarly phosphorylate RB leading to activation of E2F transcriptional activity and accelerated G1- \rightarrow S phase transition [30, 34]. As with cyclin E, CDK4 and CDK6 are also client proteins of HSP90 suggesting that the activity against tumours with *CDKN2A* loss may be related to abrogation of CDK4 and CDK6 activity by HSP90i [32, 35–37]. Finally, activity in tumours with biallelic *RB1* loss may be explained by increased E2F1 activity with subsequent increase in *CCNE1* transcription in a feed-forward loop that would similarly facilitate susceptibility to HSP90i. Another mechanism may be relevant in HGSOc whereby there is the correlation (co-occurrence) between *RB1* loss and HRR deficiency (i.e., *RB1* loss co-occurs with HRR deficiency) [38]. Therefore, if tumours with *RB1* loss are also HRR deficient, they should respond to PARPi alone; however, if they have become HRR proficient (due to prior platinum or PARPi exposure), then the activity may be explained by HSP90i reversal of acquired HRR proficiency and sensitisation to PARPi as discussed above.

Taken together, the combination of olaparib and onalespib was feasible, and exhibited some preliminary evidence of activity manifesting as prolonged disease stabilisation, in this heavily pretreated patient population with advanced solid tumours. Data from PDX models and clinical data from patients indicate that this combination may provide disease stabilisation in patients with BRCA-mutated HGSOcs and acquired PARPi resistance as well as patients with tumours harbouring RB-pathway alterations such as *CCNE1* amplification, *CDKN2A* loss and *RB1* loss. Mechanistically, abrogation of HRR may explain the activity against BRCA-mutated HGSOcs with acquired PARPi resistance, while destabilization of cyclin E and of CDK4/CDK6 may explain the activity against tumours with *CCNE1* amplification and *CDKN2A* loss, respectively.

Moving forward, further development of onalespib has been discontinued due to its limited efficacy in monotherapy and combination studies. Therefore, we do not anticipate any further development of the olaparib/onalespib combination. However, it was recently announced that an alternative HSP90 inhibitor TAS-116 (pimitespib), which is administered orally, met its primary endpoint of prolongation of PFS in patients with previously treated gastrointestinal stromal tumours (GISTs) in a

pivotal Phase III trial (CHAPTER-GIST-301 trial). Pimitespib is a novel, selective inhibitor of cytosolic HSP90a and b that does not inhibit HSP90 paralogs such as endoplasmic reticulum GRP94 or mitochondrial TRAP1 and has a different structure from other HSP90 inhibitors resulting in less hepatic and neurologic toxicity [39]. Furthermore, oral administration allows for a more flexible dosing schedule compared with intravenous administration. In addition, there is now interest in alternative ways of inhibiting HSP90 such as targeting cell division cycle 37 (CDC37), a ubiquitous co-chaperone of HSP90 that directs client proteins into the HSP90 chaperone cycle [5]. In this regard, small-molecule inhibitors of CDC37 or its interaction with HSP90 have shown promising preclinical activity against several tumour models [40–43]. Of interest, ATP competitive CDK4/6 inhibitors can also block CDC37 binding and deprive CDK4 and CDK6 access to the Hsp90 chaperone system [35]. Therefore, although further development of olaparib/onalespib is not anticipated, it is our hope that the preclinical and clinical data presented here may support future evaluation of novel combinations of olaparib (or other PARP inhibitors) with alternative HSP90 inhibitors such as pimitespib or novel agents that inhibit CDC37 or its interaction with HSP90.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

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AUTHOR CONTRIBUTIONS

PAK conceived, supervised, coordinated, and analysed all the data and wrote the manuscript; S-CC performed the statistical analysis and was the lead statistician of the study; JGS performed the PK analyses; MP, BB, HS, PB, MH and JC contributed to data collection and coordinated the processing and distribution of the clinical trial samples; AEW, PI, NH, AAW and SMC provided clinical data and contributed to the analysis of data; EVI, CPP, SP, JFL, ADD and DC performed and contributed to the preclinical studies and to the analysis of data; UAM and GIS supported and supervised the analyses of this study. All authors contributed to the writing and editing of the manuscript.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The clinical trial was approved by the NCI Central Institutional Review Board (CIRB) and the US Food and Drug Administration (NCT02898207). All procedures involving human participants were carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from patients or guardians before enrolment in the study.

CONSENT TO PUBLISH

Not applicable.

COMPETING INTERESTS

Dr. Konstantinopoulos has participated in advisory boards for Alkermes, AstraZeneca, GSK, BMS, Repare, Artios and Kadmon, serves in the gynaecologic oncology scientific committee for AstraZeneca and has received institutional support as P.I. of clinical trials from AstraZeneca, Bayer, Eli Lilly, GSK, Merck, Merck KGaA, Pfizer and GSK. Dr. Cloud Paweletz has sponsored research agreements with Daiichi Sankyo, Bicycle Therapeutics, Transcenta, Bicara Therapeutics, AstraZeneca, Intellia Therapeutics, Janssen Pharmaceuticals, Array Biopharma, has stock and other ownership interests in XSphera Biosciences, has received honoraria from Bio-Rad and ThermoFisher and has consulting or advisory role for DropWorks and XSphera Biosciences. Dr. Liu has participated in advisory boards from AstraZeneca, Clovis, Eisai, EpsilaBio, Genentech, GSK/Tesaro, Regeneron Pharmaceuticals and has received institutional support as P.I. of clinical trials from 2X Oncology, Aravive, Arch Oncology, AstraZeneca, Bristol-Myers Squibb, Clovis Oncology, CytomX Therapeutics, GlaxoSmithKline, Regeneron, Surface Oncology, Tesaro, Vigeo therapeutics. Dr. Campos has participated in the advisory board for AstraZeneca. Dr. Wright has participated in the advisory board for GSK. Dr. Matulonis has participated in advisory boards for Novartis, Blueprint Medicines, Trillium, in data safety monitoring boards for Advaxis and Symphogen and has been a consultant for NextCure, AstraZeneca and Merck. Dr. D'Andrea is a consultant/advisory board member for Lilly Oncology, Merck-EMD-Serono, Intellia Therapeutics, Sierra Oncology, Cyteir Therapeutics, Third Rock Ventures, AstraZeneca, Ideaya Inc.,

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ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41416-021-01664-8>.

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