Trabectedin and olaparib in patients with advanced and non-resectable bone and soft-tissue sarcomas (TOMAS): an open-label, phase 1b study from the Italian Sarcoma Group

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Brief title: Trabectedin and olaparib in advanced bone and soft tissue sarcomas

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ABSTRACT

Background. Trabectedin is an alkylating agent with a unique mechanism of action causing single- and double-strand DNA breaks that activate DNA damage response pathways. Based on our preclinical data, we hypothesized that PARP1 inhibitors might be an ideal partner of trabectedin. We report the results of olaparib and trabectedin combination in advanced bone and soft tissue sarcoma (B-STS) patients.

Methods. We ran an open-label, multicenter, phase 1b study recruiting patients aged ≥18 years with histologically-confirmed B-STS progressing after standard treatments with ECOG performance status ≤1. In a classic 3+3 design, patients received a 24-hour trabectedin infusion on day 1 and olaparib orally twice daily in 21-day cycles across six dose-levels (trabectedin 0·675 to 1·3 mg/m^2/3 weeks and olaparib 100 to 300 mg bid day 1 to 21). Intermediate dose-levels were permitted to improve safety and tolerability. The primary study endpoint was determination of the recommended phase 2 dose (RP2D). Safety and antitumor activity were assessed in all patients who received at least one dose of study drugs. Secondary endpoints were pharmacokinetics, pharmacodynamics, preliminary signs of activity, and related biomarkers. Here we report the results of the dose-escalation and dose-expansion cohorts. Trial is still active, but closed to enrollment, and follow-up for patients who completed treatment is ongoing. This trial is registered with ClinicalTrials.gov, number NCT02398058.

Findings. Between November 2014 and January 2017, we enrolled 50 patients: 28 in the escalation and 22 in the dose-expansion cohorts. Patients received a median of 4 cycles (IQR: 2-6; range 1-17+) with a median follow-up of 10 months (IQR: 5-23). Considering all dose levels, the most common grade 3/4 AEs were lymphopenia (32 [64%] of 50 patients), neutropenia (31 [62%]), thrombocytopenia (14 [28%]), anemia (13 [26%]), hypophosphatemia (20 [40%]), and alanine aminotransferase increase (9 [18%]). No treatment-related life-threatening AEs or deaths occurred. One (2%) patient interrupted treatment without progression. Observed dose-limiting toxicities were

thrombocytopenia, neutropenia >7 days, and febrile neutropenia. We selected intermediate dose-level 4b (trabectedin 1·1 mg/m^2 plus olaparib 150 mg bid) as RP2D. On 50 patients, overall response rate was 14% with 13 (26%) patients progression-free at six months. Low- *vs*. high-PARP1 expression correlated with six-month progression-free survival (p=0·01; OR 17·33; 95%CI=1·90-158·0).

Interpretation. Trabectedin and olaparib combination is feasible at active dose-levels for both drugs. Preliminary data on antitumor activity are encouraging. Therefore, two dedicated phase 2 studies will assess activity of this combination both in ovarian cancer (EudraCT2018-000230-35) and soft tissue sarcomas.

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Introduction

Patients with bone and soft tissue sarcomas (B-STS) ineligible for complete surgery have dismal prognoses and anecdotal long-term survivals.[1,2] In the last decade, new drugs have been shown to be effective in the treatment of advanced STS: trabectedin, pazopanib, eribulin and lastly olaratumab in combination with doxorubicin.[3–6] Nonetheless poor prognosis endures, demanding innovative strategies.[1]

Cancer medicine has recently witnessed many breakthroughs among which immunotherapy and inhibition of poly (ADP-ribose) polymerase-1 and -2 (PARP1/2) enzymes.[7,8] The latter takes advantage of impairing recognition and repair of DNA damage especially in tumor cells bearing defects in specific DNA-damage-response (DDR) pathways. Olaparib is a potent oral PARP1/2 inhibitor (PARP1-I) that blocks the DNA repair function of these enzymes and traps them at the DNA damage site increasing cytotoxicity.[9] So far, PARP1-Is have mainly been explored in BRCA1/2-deficient patients affected by ovarian, breast, prostate, and pancreatic cancers.[10] The rationale for their use in patients with these genetic defects stems from the synthetic lethality concept.[7] Briefly, DNA double-strand breaks are promoted by PARP1-I that blocks base-excision repair system from fixing single-strand DNA breaks. BRCA1/2 deficiency makes repair of double-strand DNA breaks possible only through quite inaccurate pathways, eventually leading to cell death.[7,11] One strategy to expand the patients benefitting from this drug class was to combine PARP1-I with agents causing DNA damage in order to make it irreparable.[7,12,13] However, this appealing idea collided with clinical findings showing a substantial increase of myelotoxicity ultimately forcing dose/exposure reductions that weaken combination antitumor activity.[12,13] Thus, PARP1-Is are used as monotherapy predominantly for BRCA1/2-deficient patients.[11]

This background spurred the hypothesis that trabectedin might be an ideal PARP1-I partner for two reasons: its favorable hematopoietic toxicity profile[14] and its unique mechanisms of action. Specifically, trabectedin binds to the minor groove of the DNA bending it towards the major one. The transcription-coupled nucleotide excision repair system in the attempt to remove trabectedin

adducts causes single and double-strand breaks.[15] In robust B-STS preclinical models, we demonstrated that trabectedin-induced PARP1 activation can be blocked by PARP1-Is that significantly boost the combined antitumor activity compared to either treatment alone.[16]

On these bases, we conducted a phase 1b trial in B-STS patients who progressed after standard treatments to assess the safety, identify the recommended phase-2 dose (RP2D), and explore preliminary signs of activity of trabectedin and olaparib combination.

Methods

Study design and participants

This open-label multicenter phase 1 trial included a conventional 3+3 dose-escalation part followed by an expansion-phase in advanced B-STS patients. Diagnosis review by an expert pathologist and these parameters characterized trial-eligible patients: ≥18 years; ECOG performance status ≤1; estimated life expectancy of at least 4 months; adequate bone marrow (hemoglobin >10·0 g/dL; absolute neutrophil count (ANC) >1,500/mm^3; platelet count >100,000/mm^3), liver (total bilirubin <1·5 times the upper limit of normal [ULN]; alanine and aspartate aminotransferase <2·5 x ULN [<5 x ULN for patients with liver metastases]; alkaline phosphatase <2·5 x ULN [if alkaline phosphatase >2·5 x ULN, hepatic isoenzymes 5-nucleotidase or gamma-glutamyltransferase [GGT] were considered to rule out bone origin]; prothrombin time international normalized ratio <1·5 x ULN; albumin >25 g/L), kidney (serum creatinine <1·5 x ULN or creatinine clearance ≥50 mL/min), lung, and heart functions (left ventricular ejection fraction ≥50%, and/or above the lower institutional limit of normality), creatine phosphokinase (CPK) <2·5 x ULN, absence of major comorbidities (*e.g.*, myocardial infarction in the last six months), tumor board and computed tomography (CT) proving inoperable progression (at least one measurable lesion per Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1) after first or further-line treatment for advanced disease. Previous exposure to either trabectedin or any PARP1-I was not allowed. A 28-day washout period from any previous treatment (surgery, chemotherapy, or radiotherapy) and AEs

recovery to G1 or less were requested before enrollment. See appendix p1-2 for further details on inclusion and exclusion criteria.

The Institutional Ethic Committees at each study Center approved the protocol (https://www.fpoircc.it/content/tomas-trabectedin-plus-olaparib-metastatic-or-advanced-sarcomas). Each patient provided written informed consent before any study-related procedure.

Procedures

Trabectedin and olaparib were started from the dose of 0·675 mg/m^2/3 weeks intravenously and 100 mg bid orally at 12-h interval two hours after meals, respectively. Starting doses were based on previous studies, expected single-drug toxicity and investigator brochure information provided by the two pharmaceutical companies. With standard corticosteroids pre- and post-medication, trabectedin was infused continuously over 24-h at 3-week intervals (one cycle). To assess expected olaparib concentration without trabectedin, a 5-day olaparib run-in period (at its cohort dose) followed by four days for wash-out preceded cycle 1. In this same cycle to study drug-drug interactions, olaparib was held for four days post infusion start, then started at its cohort dose. Six dose levels were initially planned: dose level 1, trabectedin 0·675 mg/m^2/3 weeks + olaparib 100 mg bid; dose level 2, trabectedin 0·675 mg/m^2/3 weeks + olaparib 200 mg bid; dose level 3, trabectedin 0·92 mg/m^2/3 weeks + olaparib 200 mg bid; dose level 4, trabectedin 1·1 mg/m^2/3 weeks + olaparib 200 mg bid; dose level 5, trabectedin 1·3 mg/m^2/3 weeks + olaparib 200 mg bid; dose level 6, trabectedin 1·3 mg/m^2/3 weeks + olaparib 300 mg bid (appendix p5). Treatment continued until progression, unacceptable toxicity, or patient refusal. Other predefined reasons for patients removal were investigator's choice, non-compliance with study requirements, pregnancy, use of prohibited substances, development of concurrent illness which could jeopardize clinical status and/or trial endpoints evaluation (*i.e.*, second cancer), delay in study drugs administration for more than two weeks, and death. After completion of sixth cycle, patient continued to receive additional cycles if, in the Principal Investigator's judgment, the patient was benefitting from the

treatment. Adverse events (AEs) above grade 2 (G2) according to Common Terminology Criteria for Adverse Events v4.03 (CTCAE v4.03) caused study drugs delay (trabectedin) or interruption (olaparib) until recovery or AE ≤G1. G-CSF use was allowed to treat febrile neutropenia, but not as primary prophylaxis. Dose-reductions followed pre-specified rules (appendix p3-4). In case a patient experienced a dose-limiting toxicity (DLT), he/she was allowed to continue treatment at dose level -1. A single dose reduction was permitted and re-escalation was not allowed.

Blood samples were collected for safety, pharmacokinetic, and pharmacodynamic analyses during run-in period and during cycles 1 and 2 at pre-defined timepoints (appendix p11,14). Toxic effects were monitored at least twice weekly by means of both blood tests and clinical examination during the first two cycles, and at least once weekly from the third cycle. The maximum-tolerated dose (MTD) was identified during the first two cycles. After we had defined the MTD we started the expansion cohort enrolling up to a maximum of 50 patients in the whole trial to strengthen information on combination safety and gather preliminary signs of activity. Tumors were assessed by CT scans performed at baseline and every two cycles (approximately every 6 +/- 1 weeks).

The plasma pharmacokinetic profiles of trabectedin and olaparib were determined for each drug when administered both alone (first cycle) and in combination (second cycle). Pharmacokinetics was further investigated during the expansion cohort at the RP2D. Trabectedin and olaparib plasma concentrations were determined by highly specific and sensitive validated HPLC-MS/MS methods (appendix p11-12). Patient-specific plasma concentration *vs*. time data were collected and elaborated by non-compartmental analyses to obtain pharmacokinetic profiles using NCPKA.V3, an algorithm implemented in MATLAB software (appendix p11-12).

In analogy with what reported by Fong and coworkers,[17] we considered PARP1 activity (PARylation) and DNA damage (assessed by P-H2AX expression) on peripheral blood mononuclear cells (PBMCs) as surrogate pharmacodynamic biomarkers. We employed immunocytochemistry to evaluate PARylation and DNA damage at baseline and at several

timepoints in each patient. When both pre- and post-treatment samples were available, PARylation and P-H2AX expressions were evaluated on tumor tissues as well (appendix p14-16).

On archival tumor samples collected before the first dosing date, an expert sarcoma pathologist identified tumor areas wherein to assess PARP1 expression and extract DNA after microdissection. BRCA1/2 and BRCAness alterations were evaluated searching for homozygous deletions or deleterious mutations of selected genes via targeted or whole exome sequencing (WES) (appendix p14-15).

Outcomes

Our primary objective was the safety and feasibility of trabectedin and olaparib in combination to identify the MTD and define the RP2D. Investigators assessed AEs according to CTCAE v4.03. DLTs were defined as follows: any non-hematological AE ≥G3 (with the exception of diarrhea, fatigue, nausea, vomiting, short-lasting AST and/or ALT elevation); any relevant hematological AE such as G4 neutropenia ≥7 days, febrile neutropenia, G4 thrombocytopenia; finally any other toxicity attributed to study drugs and considered severe enough to qualify for DLT at judgment of local or principal investigator.

Secondary objectives were: pharmacokinetics, pharmacodynamics, best tumor response (as per RECIST 1.1), overall response rate [ORR, the proportion of patients who achieved RECIST 1.1 confirmed complete (CR) or partial response (PR)]; disease control rate (DCR, the proportion of patients who reached an objective response or stable disease state ≥12 weeks, defined in the study protocol as clinical benefit rate); duration of response (DOR, the day-count between the date of RECIST 1.1 response and progression or death); progression-free survival (PFS, the day-count between therapy start and either disease progression or death); overall survival (OS, the day-count between first dose and death from any cause); growth modulation index (GMI, the ratio between time to progression with the experimental treatment and the time to progression with the last previous line of therapy; $GMI=TTP_n/TTP_{n-1}$).[18] Furthermore in an exploratory *post hoc* evaluation,

we analyzed six-month PFS rate (6m-PFS) defined as the estimated proportion of patients without progression at six-month. Exploratory endpoints studied the correlations between secondary objectives (ORR, DCR, PFS, and 6m-PFS) and selected pre-specified biomarkers: tumor PARP1/2 basal expression, BRCA1/2, DNA Damage Response (DDR) pathway alterations (ERCC 1-2-5, XRCC 1-2-3, RAD51, and 53BP1), and markers of DNA damage (P-H2AX, PARylation). As exploratory *post hoc* biomarkers, we also evaluated BRCAness as defined by Lord and Ashworth (appendix p 18). Exploratory endpoints were studied in patients treated at or above the third dose-level (deemed potentially active). This dose-level threshold was defined *post hoc*.

Statistical analyses

This trial was designed as a conventional 3+3 dose-escalation study and six dose-levels were initially planned (appendix p5), but intermediate dose-levels were foreseen by the protocol to better determine the safety profile and improve the MTD definition of the combination. MTD mandated DLTs in fewer than 33% of patients. The sample size of this trial could not be determined in advance because the safety profile of trabectedin and olaparib combination was unknown. We started from logistical considerations taking into account the 3+3 design dynamicity, the possible use of intermediate dose levels, and the potential need to replace some patients. With these limitations, the sample size was estimated on the worst scenario for a 3+3 phase 1 trial with the need of expanding each of the six preplanned dose levels up to six patients (for a total 36 patients). We hypothesized the need to explore at least one further intermediate dose level (three to six patients). We planned an expansion cohort of at least 12 patients eventually reaching the maximum total of 50 patients. Patients who were withdrawn for reasons other than toxicity (*e.g.*, progression), prior to completing the six-week observation period following the first olaparib and trabectedin administration, were included in the safety analysis, but were not considered evaluable for MTD assessment. In that case, an additional patient was enrolled. Safety and efficacy assessments were determined for all patients who received at least one dose of the studied drugs (intention-to-treat

population). We summarized patient demographics and AE frequencies with descriptive statistics. Qualitative variables were compared using the χ^2 and Fisher's exact tests and/or the Mantel-Haenszel odds ratio (OR) estimates when indicated. Pharmacokinetic continuous variables were compared by Mann-Whitney test. To compare baseline *vs*. post-treatment PARylation and P-H2AX expression, positive nuclei were counted and reported as mean percentage ± standard deviation and Student's t test for paired-samples was applied to calculate the p-value. Estimates of objective response and disease control rates are reported with the corresponding two-sided exact binomial 95% confidence intervals (95%CI) calculated by means of the Clopper-Pearson method. The Kaplan-Meier method estimated DOR, PFS, and OS with their respective 95%CI or inter-quartile ranges (IQR) and compared by means of the two-sided log-rank test when indicated. All statistical analyses were performed with IBM SPSS statistics v20.0 software. This study is registered with ClinicalTrials.gov, number NCT02398058.

Role of the funding sources

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Results

Between November 17, 2014, and January 30, 2017, a total of 54 patients were screened for trial inclusion. Four patients were excluded for not meeting eligibility criteria (one for brain metastases

at baseline CT scan, two for inadequate bone marrow function, and one for performance status deterioration during screening period). Thus, we enrolled 50 patients whose characteristics are summarized in Table 1. Twenty-eight (56%) of 50 patients were enrolled in the dose-escalation phase and 22 (44%) in the dose-expansion phase. At data cutoff (December 31, 2017), the median duration of follow-up across all patients was 10 months (IQR 5-23). Three (6%) of 50 patients remained on active treatment and 17 (34%) were in follow-up (Figure 1). Overall, the full study population received a median of 4 cycles (IQR 2-6; range 1-17+), which corresponds to 237 cycles having been administered in total.

No DLTs were observed during dose escalation between levels 1 and 4. At level 5 (trabectedin 1·3 mg/m^2/3 weeks + olaparib 200 mg bid), we observed three DLTs (two G4 neutropenia lasting ≥7 days and one G4 thrombocytopenia) in six patients. Consequently, dose escalation was interrupted. In light of the observed hematological toxicity and according to protocol rules, we deemed it safer to reduce olaparib at the intermediate dose of 150 mg bid. At dose-level 5b (trabectedin 1·3 mg/m^2/3 weeks + olaparib 150 mg bid), we enrolled three patients and observed two DLTs (one G4 thrombocytopenia and one short-lasting G4 febrile neutropenia without seriousness criteria). These results suggested us to explore another intermediate dose-level 4b (trabectedin 1·1 mg/m^2/3 weeks + olaparib 150 mg bid). At this level, we reported two DLTs (one G4 thrombocytopenia and one G4 neutropenia ≥7 days duration) in six patients, and concluded level 4b to be both the MTD and RP2D (appendix p6). Thereafter, we enrolled 22 more patients into the dose-expansion cohort at RP2D for a total of 28 patients who received a median of 4 cycles (range 1-17+; IQR 2-7). Three (11%) of these 28 patients were still on treatment at the last follow-up.

At RP2D during the first two cycles, in five (18%) out of 28 patients we observed seven DLTs: G4 neutropenia lasting ≥7 days (three), G3 febrile neutropenia (one), and G4 thrombocytopenia (three). All were reversible with either temporary interruption of the study drugs, or antibiotics plus a short course (5 days maximum) of G-CSF in selected cases. During the first two cycles across all dose levels, 10 (20%) of 50 patients experienced DLTs. Of these patients, three (30%) withdrew from

treatment due to disease progression after experiencing the DLT(s), and seven (70%) remained on trial reducing both drugs of one dose level (details on patients treated during dose-escalation phase are reported in appendix p6). No drug-related deaths or life-threatening AEs were recorded in the safety population (N=50).

Overall, with a median safety follow-up of 4 months (IQR 3-10), most common and/or clinically relevant AEs were anemia (50 [100%] patients, associated with macrocytosis in about one third of the cases), lymphopenia (47 [94%]), neutropenia (37 [74%]), thrombocytopenia (30 [60%]), fatigue (42 [84%]), alanine or aspartate aminotransferase increase (38 [76%] and 29 [58%], respectively), nausea and vomiting (35 [70%] and 14 [28%], respectively], constipation or diarrhea (23 [46%] and 12 [24%], respectively), creatinine increase (15 [30%]), and dysgeusia or anorexia (15 [30%] and 18 [36%], respectively) (Table 2 and appendix p7-10). Considering all cycles at RP2D (28 patients), the most frequent (≥20%) G3/4 drug-related AEs were neutropenia (19 [68%]), lymphopenia (17 [61%]), hypophosphatemia (11 [39%]), anemia (7 [25%]), thrombocytopenia (7 [25%]), and alanine aminotransferase increase (6 [21%]). Other relevant G3/4 toxicities included febrile neutropenia (4%) and hyperglycemia (7%). Toxicity was manageable and all but one patient discontinued experimental treatment due to progression. This patient withdrew from the trial on his request without reporting any specific toxicity.

Overall, patients treated for more than four cycles reported short and temporary olaparib interruptions (average 6 days; range 4-10). Thirteen (26%) of 50 patients were treated for six cycles or more (range 6-17+). After the sixth cycle, trabectedin was administered every 4 instead of 3 weeks in 7 of 13 (54%) patients; those treated for more than 8 cycles received trabectedin nearly every 4 weeks to improve compliance and to attenuate chronic toxicity (*e.g.*, fatigue and asymptomatic neutropenia).

All 50 patients were evaluated for pharmacokinetics. Figure 2A-B shows the mean trabectedin plasma concentration *vs*. time profiles from patients treated at the RP2D obtained during cycle 1 (trabectedin alone), and cycle 2 (trabectedin + olaparib). Visual inspection of the curves shows that

olaparib does not affect the levels of trabectedin. During the 24-h infusion period, trabectedin achieved a C_{max} of 1·4±1·1 ng/mL when administered alone and 1·2±0·5 ng/mL when administered in combination with olaparib (p=0·67). Thereafter, trabectedin concentration declined rapidly (as much as 10-fold) within one hour, and was followed by a protracted elimination phase characterized by a long half-life of about 6 days. Key pharmacokinetic parameters, calculated for both expansion and dose-escalation patient cohorts, are listed in appendix p12-13. There was high inter-patient variability of drug exposure, but similar values of plasma clearance at all investigated doses confirmed that trabectedin pharmacokinetics is not dose dependent (appendix p13).

Figure 2C and appendix p13 report the pharmacokinetics of olaparib. The T_{max} for olaparib absorption was 1-3 h; the apparent half-life was about 4-6 h. The mean plasma concentration *vs*. time profiles exhibited non-significant trend toward reduced olaparib concentrations with concomitant trabectedin administration, both in the escalation and RP2D patient cohorts. Indeed, differences seen in the profiles did not reach statistical significance (p=0·22) when translated into the pharmacokinetic parameters (appendix p13).

Forty-four (88%) of 50 patients underwent pharmacodynamic assessment; 38 (86%) of 44 patients were fully evaluable at all timepoints. PARP1 enzymatic activity was significantly reduced post run-in period (p<0·0001 *vs*. baseline), significantly increased after trabectedin infusion (p<0·0001 pre- *vs*. post-dose), and significantly reduced at day 8 during olaparib administration (p<0·0001 day 8 *vs*. day 2; Figure 3).

We also explored pharmacodynamic biomarkers in post-treatment tumor specimens available from two patients. Tumor samples showed strong baseline PARP1 activity that was reduced in post-treatment specimens (appendix p16). Moreover, the post-treatment tumor samples displayed a significant increase in P-H2AX positive cells (pre-treatment mean ± standard deviation 4·9% ± 2·0% *vs*. post-treatment 43·8% ± 7·3%; p<0·0001; appendix p16).

All 50 patients were assessable for activity with a median follow-up of 4 months (IQR 3-10): seven (14%, 95%CI 6-27%) achieved a PR, and 16 (32%, 95%CI 20-47) a stable disease ≥12 weeks for a

DCR of 46% (95%CI 32-61%). The median DOR was 7 months (95%CI 7-8). Two (4%) of 50 patients (retroperitoneal leiomyosarcoma and malignant phyllodes tumor with lung metastases), underwent surgery and achieved complete remission (ongoing) (Figure 4). The median PFS were 1·5 (95%CI 1-3) and 3 months (95%CI 1-4) for the entire population and for the cohort of patient treated above the third dose-level, respectively. GMI was evaluable in 37 (74%) of 50 patients. We observed a median GMI of 0·77. In particular, 23 (62%) of 37 patients had a GMI <1, five (14%) a GMI between 1 and 1·33, and nine (24%) a GMI >1·33. Thirteen (26%) of 50 patients experienced a prolonged benefit ≥6 months (five leiomyosarcomas, three dedifferentiated liposarcomas, one myxoid liposarcoma, two synovial sarcomas, one solitary fibrous tumor, and one malignant phyllodes tumor). Within the 11 (22%) of 50 patients affected by bone sarcomas, none showed either an objective or clinical response. In the 39 (78%) patients affected by STS, we observed the following outcomes: ORR 18% (95%CI 8-34), DCR 56% (95%CI 40-72), 6m-PFS 38% (95%CI 22-54%). The 6m-PFS was evaluated as an exploratory *post hoc* analysis. Of the 50 enrolled patients, 33 (66%) died of disease progression. With a median follow-up of 10 months (IQR 5-23), the median OS of the 50 evaluable patients was 11 months (95%CI 5-18).

The correlation with activity of pre-specified biomarkers (BRCA1/2 or BRCAness status and PARP1 basal expression) was evaluated in tumor samples from 42 (98%) of 43 patients treated at or above the third dose-level (a *post hoc* dose-level threshold). After having eliminated cases of degraded DNA or poor immunohistochemistry quality (*e.g.*, decalcified bone lesions), DDR alterations and PARP1 protein expression were evaluated in 33 (79%) and 35 (83%) of 42 samples, respectively. Among the 33 patients evaluable for BRCA1/2 status, only patient #29 harbored a BRCA1 mutation (T231M) classified as variant of unknown significance (VUS), while other 4 (12%) of 33 showed BRCAness (appendix p17). We assessed ORR, DCR, median PFS, and 6m-PFS; not one significantly differed according to BRCAness status. According to basal PARP1 status (high in 22 [63%] *vs*. low in 13 [37%] of 35 samples), we observed the following results: overall response rate 27% *vs*. 8% (p=0·22; OR 4·50, 95%CI 0·48-42·50); DCR 73% *vs*. 31% (p=0·02; OR

6·00, 95%CI 1·33-27·05); median PFS 8 (95%CI 3-15) *vs*. 2 months (95%CI 1-3) (p=0·01); 6m-PFS 59% (95%CI 37-81) *vs*. 8% (95%CI 0-24) (p=0·01; OR 17·33, 95%CI 1·90-158·00) (Figure 5).

Discussion

In this multicentric investigator-initiated trial, we identified the recommended phase 2 dose of trabectedin and olaparib combination demonstrating its feasibility in terms of toxicity and interesting preliminary activity. Indeed, no death or life-threatening AEs were observed, and the combination as a second/further-line treatment showed a 14% ORR. Furthermore, tumor basal PARP1 expression revealed a subset of patients who exhibited a higher activity from the combination in terms of both disease control rate and progression-free survival. This suggests that PARP1 assessment might be a useful biomarker in patient selection.

To impede tumor cell DNA repair to enhance cytotoxicity induced by other therapeutics is an exciting hypothesis. In fact, the first PARP1 inhibitor iniparib was studied in combination with chemotherapy, but due to an initial pharmacological misinterpretation, drug development was abandoned.[19] Thereafter, work with the potent inhibitor olaparib made identification of combination chemotherapeutics challenging due to myelotoxicity, a common Achilles' heel of this drug class.[12,13,20] In the carboplatin and paclitaxel study, olaparib was administered half-time at half-dose (200 mg bid for 10 days per cycle) with a reduced dose of carboplatin (AUC4). Notwithstanding, 43% of patients experienced neutropenia ≥G3, while olaparib was either reduced or interrupted in 37% of patients. Finally, antitumor activity showed no differences compared to chemotherapy alone in the combination part of the study.[13] Also veliparib in combination with carboplatin and paclitaxel failed to improve pathological response compared to the same cytotoxics.[12] Therefore, olaparib and other PARP1-Is were mainly developed as maintenance monotherapies.[8,21,22] These results prompted us to consider a different combination aimed at exploiting the ability of trabectedin to cause single-strand DNA breaks at an inferior myelotoxicity.[14,15]

With the purpose to curb hematological toxicity, we selected low initial doses of both drugs and paid attention not simply to MTD, but also to the type of toxicity observed. Thus, we explored intermediate lower doses of olaparib aiming to attenuate as much as possible myelotoxicity. Indeed, our pharmacodynamic data demonstrated that the potent PARP1 activation induced by trabectedin was suppressed by 150 mg bid of olaparib tablets (that have a higher bioavailability compared to capsules).[23] Moreover, the RP2D pharmacokinetic analyses of olaparib (average AUC_{ss} in the range of 30-40 μg*h/mL) showed drug concentrations known to inhibit PARP1.[17] Overall, these doses allowed us to minimize the need for drug reductions and continue treatment for as long as tumor control was maintained. However, number and type of previous lines of treatment may jeopardize study drugs activity and toxicity. Indeed, patients who had received more than two lines of therapy had a higher risk to develop DLTs. After six/eight cycles to keep non-progressing patients on treatment, short interruptions/delays were required due to myelotoxicity and fatigue. This strategy exploits the potential of long-term tumor control of these two drugs.[8,14] Of course, only a randomized trial comparing trabectedin *vs*. its combination with olaparib will answer whether the reported myelotoxicity and reduced trabectedin dose are justified by an increased antitumor activity. In STS there is a general consensus to select later treatment lines on histotype.[3–5] This study was not designed to identify specific histotype sensitivities and our data should be considered preliminary. Indeed, STS heterogeneity is a general constraint to most trials in mesenchymal tumors because it may be difficult or simply inappropriate to make general assumptions based on data related to a specific histotype. With the above-mentioned limitation, our results do not encourage any further development of this combination in bone sarcomas. We did not observe any disease stabilization lasting longer than three months or even transitory improvement of patient symptoms. This is particularly disappointing for Ewing's sarcoma wherein PARP1 inhibitors have been studied on strong rationale,[24,25] without clinically meaningful results.[26] Of course, it can be speculated that this might be due to the limited activity of trabectedin in bone sarcomas.[27]

Advanced sarcomas are still an unmet medical need. A standard second-line treatment as trabectedin is expected to reach an ORR around 10% in selected histotypes.[4] Our drug combination resulted in a 14% ORR (18% excluding bone sarcomas) in several different sarcomas. Furthermore, PR in two cases converted inoperable patients to surgery-eligible with ongoing prolonged tumor control. Despite the fact that this was a phase 1 study, the observed PFS rates are encouraging. Indeed, in the STS subgroup the 6-month PFS of 38% is above the 14% threshold to consider active an experimental drug in pretreated patients according to EORTC.[28] Notwithstanding, only a randomized trial will clarify the additional role, if any, played by olaparib in this combination activity.

The Holy Grail in oncology is identification of a biomarker to improve patient selection. In this series, we validated our preclinical finding on the central role of tumor PARP1 basal expression to determine trabectedin and olaparib antitumor activity.[16] In fact, this biomarker identified patients with the highest probability to benefit from the combination in terms of ORR, DCR, and PFS. These analyses were performed in the selected cohort of patients treated at or above the third dose-level and therefore, are to be considered *post hoc* in nature. Of course, achievable dose-levels were unknown and any assumption beforehand was simply impossible. We considered more informative to study prespecified explorative biomarkers at drug doses deemed active. Correlation between PARP1 expression and PARP1-I activity was shown also in preclinical lung cancer models; however, the authors suggested that other DDR components may drive the observed activity. Overall, PARP1-I mechanisms of action are far from having been completely elucidated. Hence, also the impact of tumor PARP1 basal expression requires further investigation in a randomized fashion to assess its definite role.

As of today, PARP1 inhibitors development has been predominantly bound to BRCA1/2 deficiency,[8,21,22] and later to the concept of BRCAness.[7,10,29] In STS, the expected rate of BRCA1/2 deficiency and BRCAness is approximately 2% and 5%, respectively.[30] Our data are consistent with these results but are inconclusive as to specific differential activity in patients bearing these genetic

alterations. Indeed, our study was underpowered to assess the predictive role of both BRCA1/2 deficiency and BRCAness. Furthermore, paraffin-embedded tumor samples do not guarantee adequate DNA quality to assess genome alterations in all cases, as in decalcified tumor specimens. This conclusion applies also to other genetic alterations of DNA repair mechanisms such as ERCC and XRCC families of enzymes. Theoretically, patients with a DDR abnormality should be more sensitive to our study drugs,[10,29,31] but clinical validation of this assumption requires a larger patient cohort. In fact, the precise role of DDR status to predict trabectedin activity in monotherapy is not yet completely elucidated. Several authors have addressed this issue suggesting a positive correlation.[31,32] On the contrary, the only prospective evaluation of BRCA1 alteration was not correlated with trabectedin activity.[32] That said, it is an attractive idea to move PARP1 inhibition from BRCA1/2-deficient and BRCAness tumors to a broader patient population.[13,20–22]

Unfortunately, patients who experienced clinical and objective responses later progressed, highlighting the need to delve into resistance mechanisms. A better understanding would improve not only patient selection but also exploitation of other compounds in patients with primary or secondary resistance. Trabectedin is less active in tumors bearing deficient transcription-coupled nucleotide excision repair mechanism.[15] In the case of PARP1-Is, resistance comprehension is quite elusive.[11,33] Indeed, several proteins involved in DNA repair, such as 53BP1 and RAD7 have been identified *in vitro*, but only revertant mutations in BRCA1/2 restoring homologous recombination repair have been demonstrated in the clinical setting.[11,34]

In summary, this combination exploits the potential of two different first-in-class drugs and demonstrates feasibility and activity in homologous repair-proficient tumors. The activity in soft tissue sarcomas will be assessed in a randomized phase 2 study comparing trabectedin *vs*. the combination of trabectedin and olaparib stratifying patients according to PARP1 expression. Moreover, given the activity of both drugs in ovarian cancer,[8] we are opening a phase 2 after-platinum-failure study (EudraCT2018-000230-35) regardless of patients BRCA1/2 and BRCAness status.

Research in context

Evidence before this study

We searched PubMed through April 30, 2018 for clinical studies exploring combinations between PARP1 inhibitors and chemotherapy in patients with metastatic/advanced cancer using the following search terms "combination" AND "PARP inhibitor OR olaparib OR veliparib OR niraparib OR rucaparib OR talazoparib" (refining research for clinical trials). Several cytotoxic compounds and target therapies have been tested, both in ovarian cancer and other solid tumors. In general, these trials demonstrated that chemotherapy and PARP1 inhibitors combinations were feasible, but required reductions in both dosing and treatment duration, potentially diluting the synergistic effects. In this context, we hypothesized that trabectedin might be an ideal drug to be combined with PARP1 inhibitors due to its mechanism of action and a relatively low incidence of overlapping toxicities. With this aim, we studied and demonstrated in robust sarcoma preclinical models that olaparib potentiated trabectedin activity. Indeed, single- and double-strand DNA breaks caused by trabectedin were made irreparable by olaparib blocking and trapping PARP1 at damaged DNA sites. Thus, we focused on advanced sarcomas where trabectedin has been most extensively studied and the need for innovative options continues. To date, no clinical trial has studied this combination.

Added value of this study

To our knowledge, this hypothesis-driven study is the first to investigate the safety and antitumor activity of two first-in-class drugs: trabectedin, a minor groove-binding cytotoxic, and olaparib, a PARP1 inhibitor. The combination was feasible and we observed an acceptable toxicity profile at drug concentrations deemed active. Furthermore, though preliminary, overall response rate and duration of response were both encouraging. Finally, we propose basal PARP1 tumor expression as a biomarker to improve patient selection based on the differential signs of antitumor activity we registered.

Implications of all the available evidence

The combination of trabectedin and olaparib showed interesting activity regardless of BRCA status, thus widening the possibility to exploit PARP1 potential. In particular, it warrants studies in two areas: ovarian cancer and sarcomas. First, a phase 2 study will explore the activity of this combination in the unfavorable subset of patients affected by ovarian cancer after failing platinum-based chemotherapy (EudraCT2018-000230-35). The second area requires a randomized phase 2 trial to compare the activity of trabectedin *vs*. trabectedin + olaparib in second- or further-line treatments in advanced and unresectable STS.

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Contributors

GG, LD, SA, SS, RB, DS, MD, PP, SF, and MA contributed to study conception and design.

GG, LD, EP, PB, SA, SS, RB, RP, SM, FT, EM, SF, and MA contributed to patient recruitment.

AP and APDT reviewed the diagnoses.

MZ and MD did the pharmacokinetic analyses.

YP, GC, DS, AP, APDT, LN, AB, and AB did the pharmacodynamic and biomarker evaluation.

LD, YP, PB, SA, SM, FT, GC, DS, LN, AB, EM collected and assembled the data.

GG, LD, YP, EP, MZ, RP, DS, AB, PP, MA analyzed and interpreted the data.

All authors participated in writing the paper and approved the final version of the paper.

Declaration of interests

GG has received fees for consulting and advisory board roles from PharmaMar, Lilly, Novartis, Bayer, Eisai. LD, SA received travel expenses from PharmaMar. SS has received fees for consulting or advisory board roles from Amgen, Lilly, PharmaMar, Daiichi Sankyo, Karyopharm Therapeutics, Lilly, PharmaMar, Plexxikon; SS Institution received funding from Amgen, ARIAD, Bayer, Daiichi Sankyo, GlaxoSmithKline, Lilly, Novartis, Pfizer, PharmaMar. APDT received

honoraria and grants from PharmaMar. MD has received consultancy fees for consulting and advisory board roles from PharmaMar. PP has received fees for consulting or advisory board roles from Takeda. SF received grants from PharmaMar. MA has received fees for consulting or advisory board roles from Bristol-Myers Squibb, Merck, Roche. The other authors declare no competing interests.

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Figures and Tables.

Table 1. Demographics and baseline characteristics. IQR, interquartile range; ECOG PS, Eastern Cooperative Oncology Group Performance Status; UPS, undifferentiated pleomorphic sarcoma; MPNST, malignant peripheral nerve sheath tumor; SFT, solitary fibrous tumor; STS, soft tissue sarcoma. *Patients with ECOG PS 2 for orthopedic problems solely were eligible. **Ewing sarcoma, synovial sarcoma, and undifferentiated pleomorphic sarcoma were accounted as high grade tumors.

Figure 1. Trial profile. DLTs, dose-limiting toxicities.

Table 2. Adverse events at recommended phase 2 dose (all cycles). WBC, white blood cells; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CPK, creatine phosphokinase; GGT, gamma-glutamyltransferase.

Figure 2. Pharmacokinetics. Comparison of the mean trabectedin (panel A) and olaparib (panel C) concentrations obtained during the first 2 cycles during the expansion phase. Panel B shows a focus on the first 48h of trabectedin kinetic.

Figure 3. Immunostaining of PARP1 enzymatic activity (PARylation) in peripheral blood mononuclear cells during run-in period, cycle 1 and 2. A staining intensity score was attributed to PARylation positive cells at each timepoint for all patients (appendix p 14-15). Average PARylation score (bars) and the relative standard error mean (lines) are reported for each timepoint.

Figure 4. Time on treatment and observed responses. Graph shows all patients treated at or above the third dose level (trabectedin 0·920 mg/m^2/3 weeks + olaparib 200 mg bid).

Figure 5. Progression-free survival according to tumor PARP1 expression. Green line high-PARP1 expression, blue line low-PARP1 expression.

All patients	N = 50
Sex	
-Male	29 (58%)
-Female	21 (42%)
Age	
-Median	53
-Range	19-80
-IQR	39-63
ECOG PS	
-0	22 (44%)
-1	25 (50%)
-2*	3 (6%)
Time from diagnosis	
-Median (months)	32
-IQR	15-53
Disease extension at study start	
-Locally advanced	3 (6%)
-Metastatic	47 (94%)
Previous lines	
-Mean	2
-Median	2
-Range	1-8
-IQR	1-3
Type of chemotherapy	
-anthracyclines	50 (100%)
-ifosfamide	31 (62%)
-gemcitabine	19 (38%)
-docetaxel/paclitaxel	16 (32%)
-dacarbazine	15 (30%)
-etoposide	10 (20%)
-pazopanib	9 (18%)
-cisplatin/carboplatin	9 (18%)
-methotrexate	6 (12%)
-vincristine	6 (12%)
-cyclophosphamide	6 (12%)
-temozolomide+irinotecan	4 (8%)
-busulfan/melphalan	3 (6%)
-other	9 (18%)
Grade**	
-Intermediate	3 (6%)
-High	47 (94%)
Histotype	
-Leiomyosarcoma	15 (30%)
Uterine	8 (16%)
Non-uterine	7 (14%)
-Osteosarcoma	7 (14%)
-Liposarcoma	6 (12%)
Dedifferentiated	4 (8%)
Myxoid	1 (2%)
Pleomorphic	1 (2%)
-Synovial sarcoma	6 (12%)
-Ewing's sarcoma	4 (8%)
-UPS	3 (6%)
-MPNST	2 (4%)
-Malignant Myoepithelioma	2 (4%)
-SFT	1 (2%)
-Angiosarcoma	1 (2%)
-Other STS/bone	3 (6%)

Table 1. Demographics and baseline characteristics. IQR, interquartile range; ECOG PS, Eastern Cooperative Oncology Group Performance Status; UPS, undifferentiated pleomorphic sarcoma; MPNST, malignant peripheral nerve sheath tumor; SFT, solitary fibrous tumor; STS, soft tissue sarcoma. *Patients with EOCG PS 2 for orthopedic problems solely were eligible. **Ewing sarcoma, synovial sarcoma, and undifferentiated pleomorphic sarcoma were accounted as high grade tumors.

	trabectedin 1·1 mg/m^2 olaparib 150 mg bid n =28							
	Grade 1-2		Grade 3		Grade 4		Grade 5	
Adverse Event	n	%	n	%	n	%	n	%
Hematologic toxicities								
Anemia	21	75%	7	25%	0	0%	0	0%
Lymphocyte count decreased	8	29%	14	50%	3	11%	0	0%
Macrocytosis	10	36%	0	0%	0	0%	0	0%
Neutrophil count decreased	1	4%	7	25%	12	43%	0	0%
Platelet count decreased	9	32%	3	11%	4	14%	0	0%
WBC decreased	8	29%	9	32%	8	29%	0	0%
Febrile neutropenia	0	0%	1	4%	0	0%	0	0%
Gastrointestinal toxicities								
Abdominal Pain	9	32%	0	0%	0	0%	0	0%
Anorexia	7	25%	0	0%	0	0%	0	0%
Constipation	15	54%	0	0%	0	0%	0	0%
Diarrhea	8	29%	0	0%	0	0%	0	0%
Nausea	16	57%	1	4%	0	0%	0	0%
Stomatitis	5	18%	0	0%	0	0%	0	0%
Vomiting	7	25%	0	0%	0	0%	0	0%
General disorders								
Edema limbs	6	21%	0	0%	0	0%	0	0%
Fatigue	24	86%	1	4%	0	0%	0	0%
Fever	12	43%	0	0%	0	0%	0	0%
Musculoskeletal and connective tissue disorders								
Arthralgia/myalgia	9	32%	0	0%	0	0%	0	0%
Cardiovascular								
Hypertension	1	4%	2	7%	0	0%	0	0%
Infections and infestations								
Infection	15	54%	2	7%	0	0%	0	0%
Investigations								
ALT increased	15	54%	6	21%	0	0%	0	0%
Alkaline phosphatase increased	9	32%	0	0%	0	0%	0	0%
AST increased	13	46%	3	11%	0	0%	0	0%
Bilirubin increase	3	11%	0	0%	0	0%	0	0%
CPK increased	6	21%	5	18%	0	0%	0	0%
creatinine increased	6	21%	0	0%	0	0%	0	0%
GGT increased	16	57%	3	11%	0	0%	0	0%
Hyperglycemia	24	86%	2	7%	0	0%	0	0%
Hyperkalemia	7	25%	0	0%	0	0%	0	0%
Hyperuricemia	4	14%	0	0%	0	0%	0	0%
Hypoalbuminemia	12	43%	0	0%	0	0%	0	0%
Hypokalemia	10	36%	3	11%	0	0%	0	0%
Hyponatremia	5	18%	4	14%	0	0%	0	0%
Hypomagnesemia	8	29%	0	0%	0	0%	0	0%
Hypophosphatemia	10	36%	10	36%	1	4%	0	0%
Lipase increased	9	32%	0	0%	0	0%	0	0%
Serum amylase increased	11	39%	0	0%	0	0%	0	0%
Endocrine								
Hyperthyroidism	6	21%	0	0%	0	0%	0	0%
Nervous system disorders								
Dysgeusia	5	18%	0	0%	0	0%	0	0%
Pain								
Non-cardiac chest pain	5	18%	0	0%	0	0%	0	0%
Psychiatric disorders								
Insomnia	4	14%	0	0%	0	0%	0	0%
Respiratory								

Dyspnea	3	11%	0	0%	0	0%	0	0%
Ear and labyrinth disorders								
Vertigo	3	11%	0	0%	0	0%	0	0%

Table 2. Adverse events at recommended phase 2 dose (all cycles). WBC, white blood cells; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CPK, creatine phosphokinase; GGT, gamma-glutamyltransferase.

Figure 1.

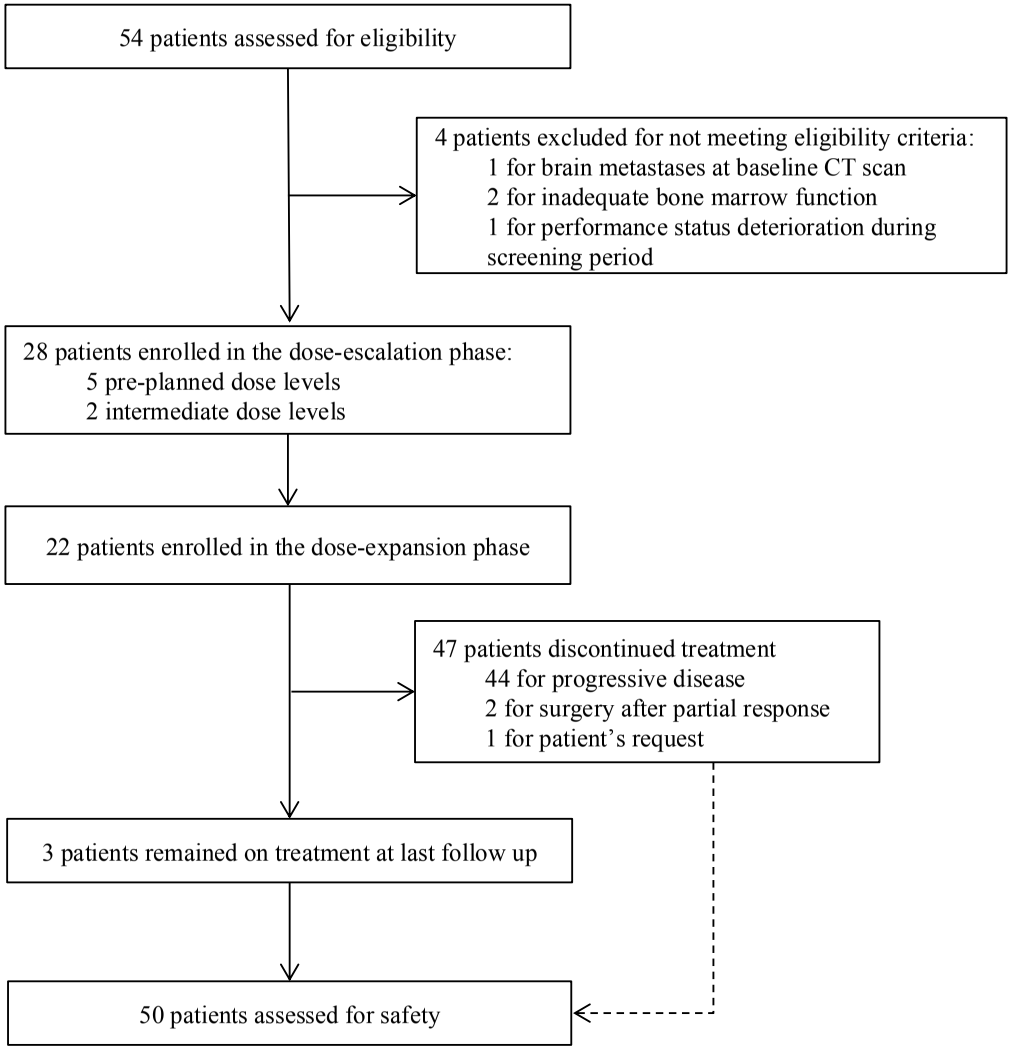
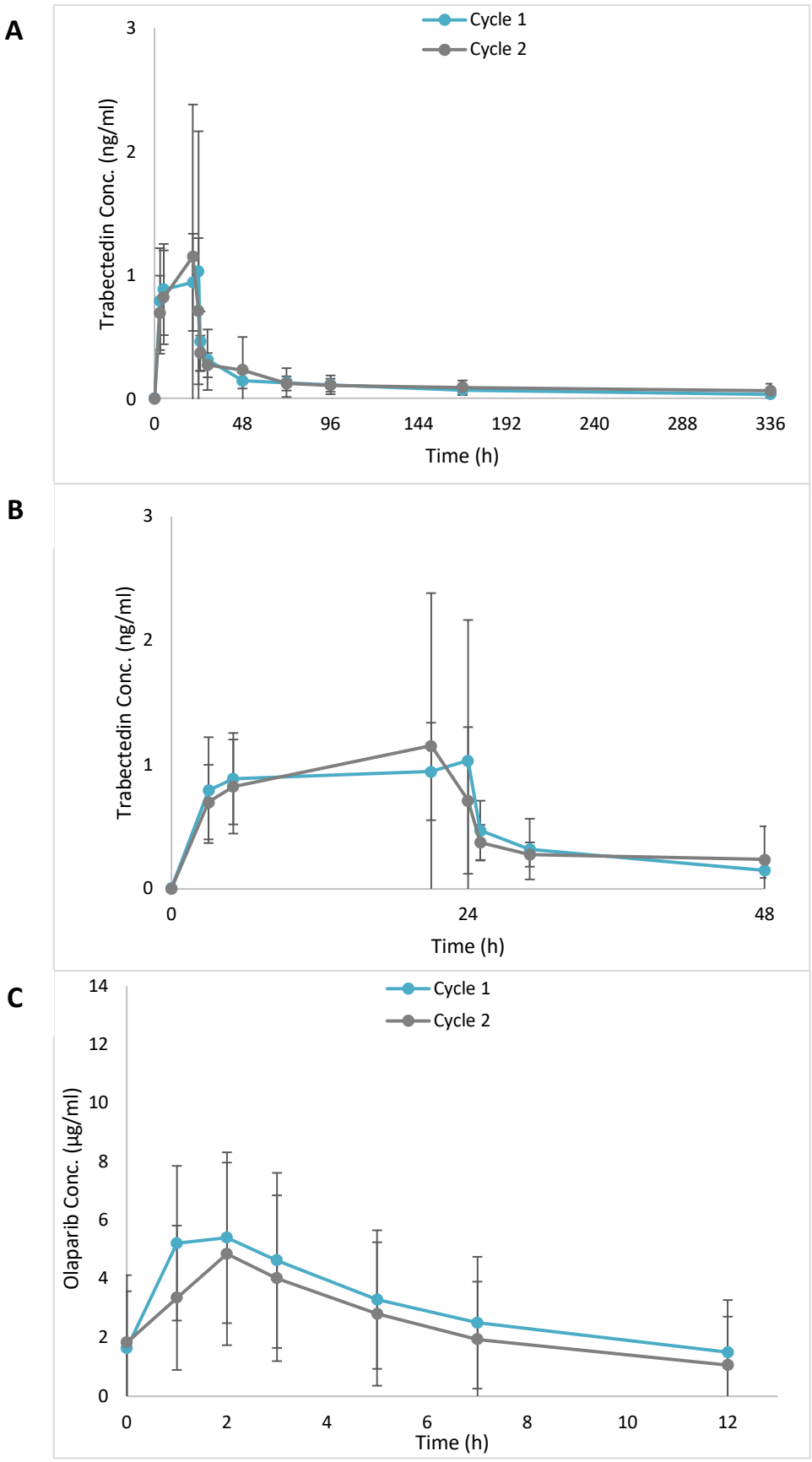

54 patients assessed for eligibility
4 patients excluded for not meeting eligibility criteria:
1 for brain metastases at baseline CT scan
2 for inadequate bone marrow function
1 for performance status deterioration during screening period
28 patients enrolled in the dose-escalation phase:
5 pre-planned dose levels
2 intermediate dose levels
22 patients enrolled in the dose-expansion phase
47 patients discontinued treatment
44 for progressive disease
2 for surgery after partial response
1 for patient's request
3 patients remained on treatment at last follow up
50 patients assessed for safety

Figure 2.


A
Cycle 1
Cycle 2
Trabectedin Conc. (ng/ml)
0
1
2
3
0
48
96
144
192
240
288
336
Time (h)
B
Trabectedin Conc. (ng/ml)
0
1
2
3
0
24
48
Time (h)
C
Cycle 1
Cycle 2
Olaparib Conc. (µg/ml)
0
2
4
6
8
10
12
14
0
2
4
6
8
10
12
Time (h)

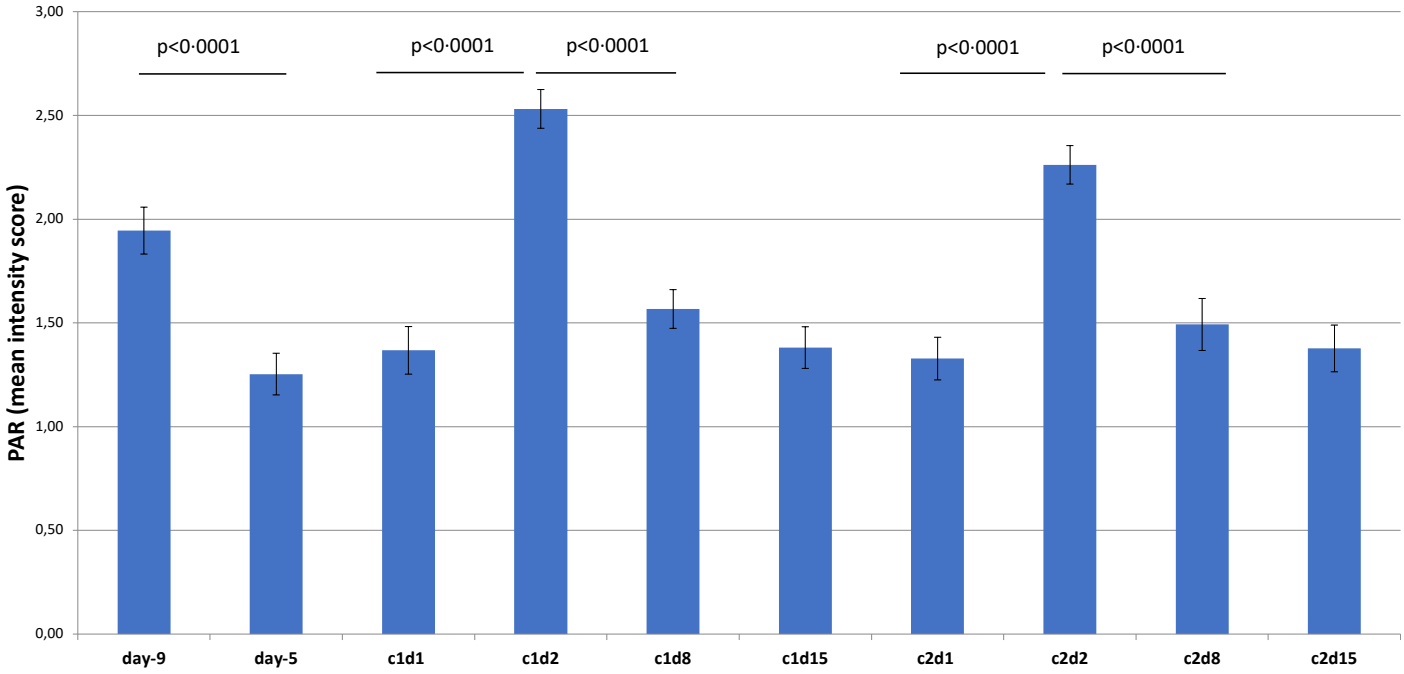

3,00
2,50
2,00
1,50
1,00
0,50
0,00
PAR (mean intensity score)
p<0·0001
p<0·0001
p<0·0001
p<0·0001
p<0·0001
day-9
day-5
c1d1
c1d2
c1d8
c1d15
c2d1
c2d2
c2d8
c2d15

Figure 4.

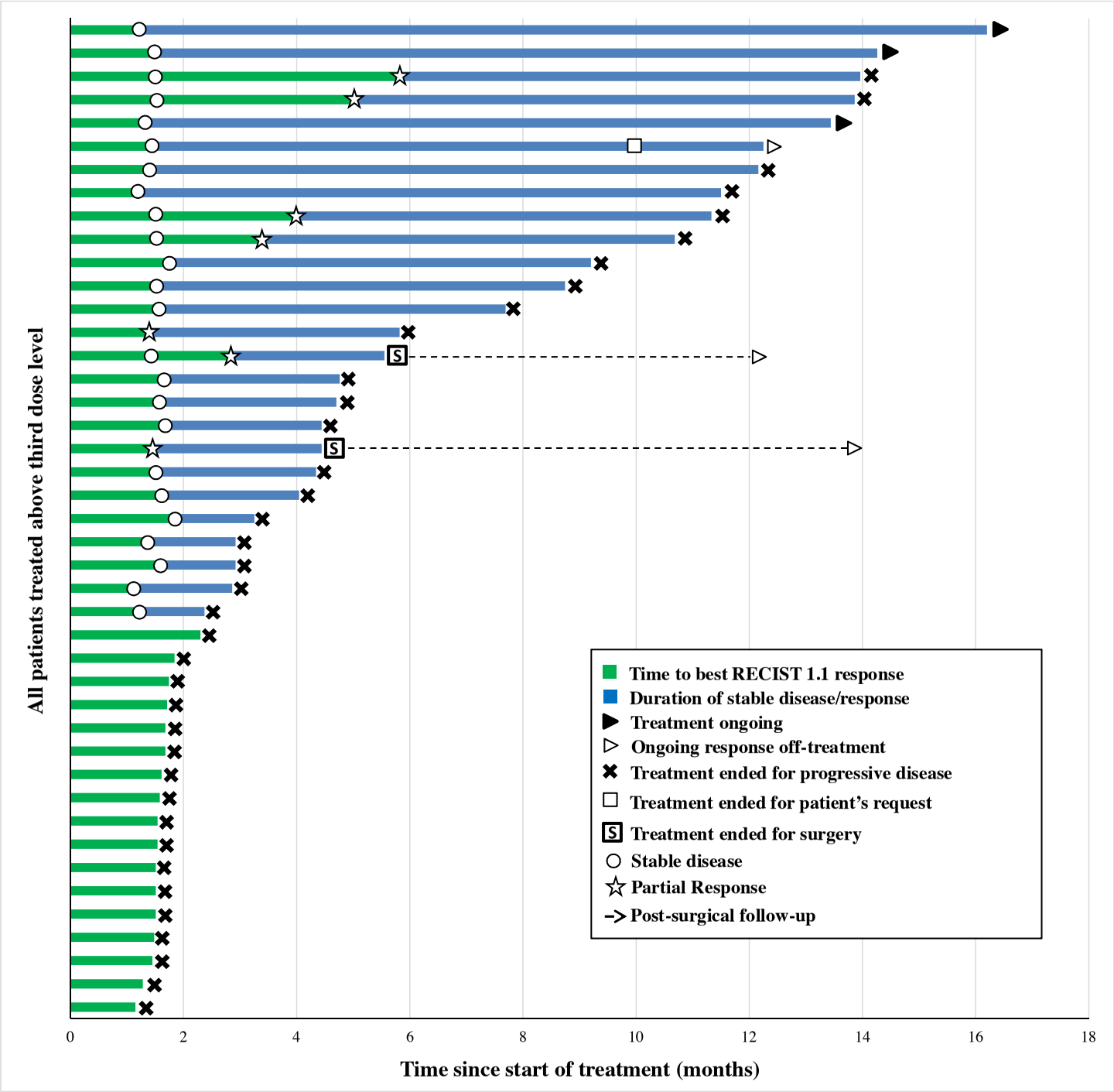
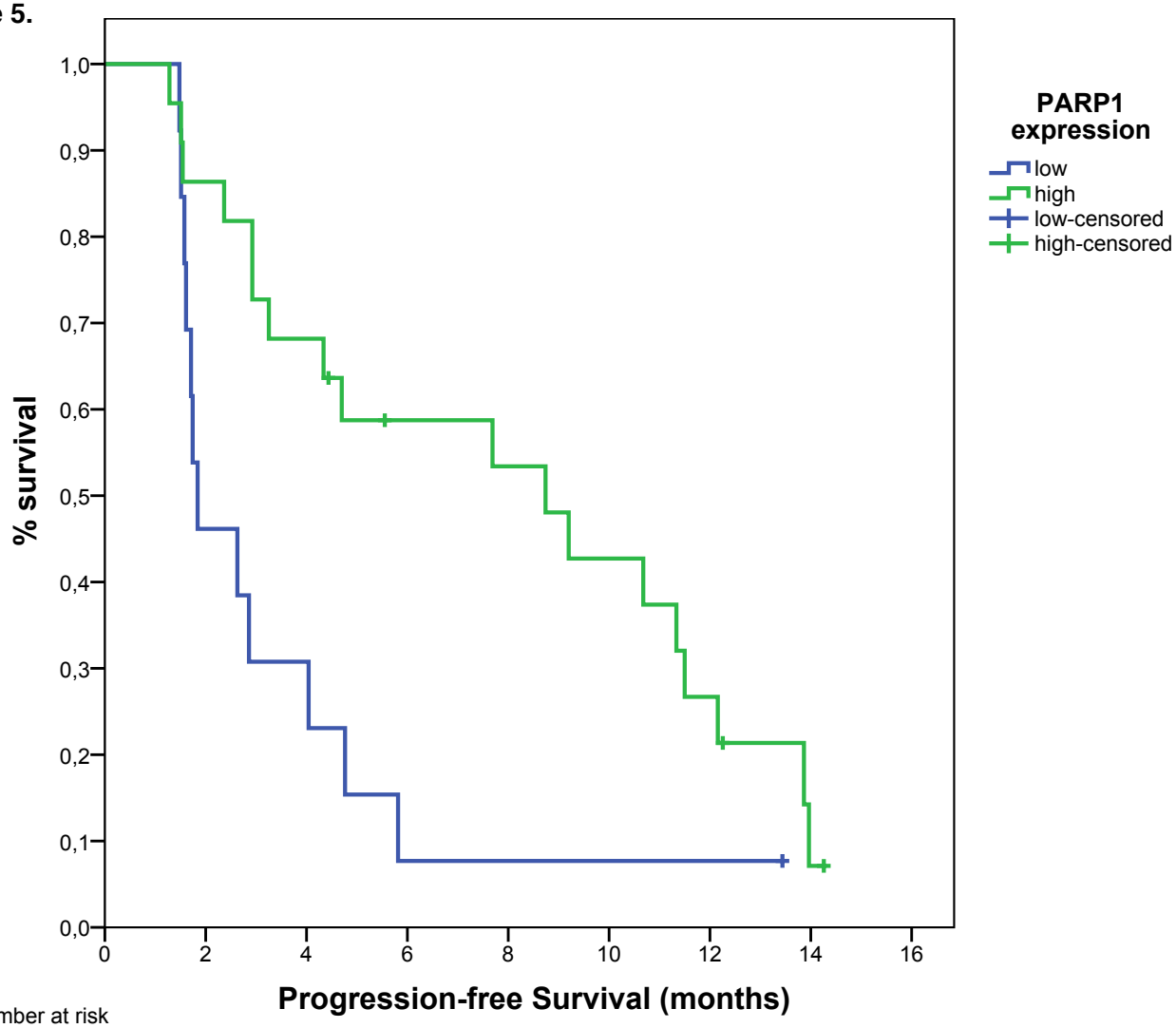

All patients treated above third dose level
Time since start of treatment (months)
0
2
4
6
8
10
12
14
16
18
Time to best RECIST 1.1 response
Duration of stable disease/response
Treatment ongoing
Ongoing response off-treatment
Treatment ended for progressive disease
Treatment ended for patient's request
Treatment ended for surgery
Stable disease
Partial Response
Post-surgical follow-up

Figure 5.



Number at risk
(number censored)

low PARP1	13 (0)	6 (0)	4 (0)	1 (2)	1 (2)	1 (2)	1 (2)	0 (3)	0 (4)
high PARP1	22 (0)	19 (0)	15 (0)	11 (0)	10 (0)	8 (0)	5 (0)	1 (1)	0 (1)