OPIS PRZYPADKU / CASE REPORT
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Use of C_{trough} for the Therapeutic Drug Monitoring of Olaparib in the Patient with anaemia as a side effect during the treatment

Terapia monitorowana olaparybu w oparciu o stężenie minimalne w stanie stacjonarnym (C_{trough}) u pacjentki z anemią jako działanie niepożądane w trakcie leczenia

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Summary

Background. A 63-year-old patient was admitted to the Gynecological Oncology Department in March 2020 due to ovarian cancer FIGO (fr. *Fédération internationale de gynécologie et d'obstétrique*) stage IV (meta to the liver). The patient was qualified for neoadjuvant chemotherapy: paclitaxel (175 mg/m^2), carboplatin (AUC 6.0) and bevacizumab (7.5 mg/kg) administered intravenously every three weeks. The *BRCA1* gene mutation was diagnosed. The aim was assessing the correlation between anaemia and the dose of olaparib and C_{trough}. ***Material and methods.*** The concentrations of olaparib in plasma were assayed using the high-performance liquid chromatography (HPLC) method with ultraviolet (UV) detection. The severity of olaparib's adverse effects was assessed by CTCAE v.5.0 scale. ***Results.*** Anaemia in the presented patient is not correlated with the dose of olaparib and $C_{trough.}$ There is no tendency in lower C_{trough} of olaparib depending on the grade of anaemia due to CTCAE. The presented results indicate that reducing the dose of the olaparib causes an initial reduction in C_{trough} however, the observed upward trend in drug concentration and the number of tested samples performed is too small to establish a safe drug concentration and to determine the concentration of the drug requiring a reduction of the cytostatic before the occurrence of side effects. ***Conclusion.*** For this moment, C_{trough} of olaparib should not be used as a practical benefit in patients with anaemia during PARP-inhibitor treatment. In the presented case, the severity of olaparib-induced anaemia was not correlated with C_{trough}. *(Farm Współ 2022; 15: 160-166) doi: 10.53139/FW.20221518*

Keywords: olaparib, C_{trough}, anaemia

Streszczenie

Wstęp. 63-letnia pacjentka została przyjęta na oddział ginekologii onkologicznej w marcu 2020 roku z powodu raka jajnika FIGO (fr. *Fédération internationale de gynécologie et d'obstétrique*) IV – (przerzuty do wątroby). Pacjentka została zakwalifikowana do chemioterapii: paklitaksel (175 mg/m^2), karboplatyna (AUC 6.0) i bewacyzumab (7,5 mg/kg) podawanych dożylnie co 3 tygodnie. Zdiagnozowano mutację w obrębie genu *BRCA1.* Celem było sprawdzenie korelacji pomiędzy anemią a dawką olaparybu i C_{trough}. ***Materiał i metody.*** Stężenie olaparybu w osoczu pobranym od pacjentki oznaczono przy pomocy metody wysokosprawnej chromatografii cieczowej (HPLC, ang. *High Performance Liquid Chromatography*) z detekcją ultrafioletu (UV). Stopień natężenia anemii został oceniony zgodnie ze skalą CTCAE v.5.0. ***Wyniki.*** Poziom anemii u przedstawionej pacjentki nie koreluje z dawką olaparybu oraz $C_{trough.}$ Nie stwierdzono spadkowej tendencji w C_{trough} olaparybu w zależności od nasilenia anemii wg CTCAE. Przedstawione wyniki wskazują, że zredukowanie dawki olaparybu powoduje początkowy spadek C_{trough}, natomiast następowy wzrost jego stężenia oraz zbyt mała ilość testowanych próbek powoduje, że nie ma możliwości ustalenia bezpiecznego stężenia leku, przy którym warto zredukować dawkę olaparybu, zanim wystąpią działania niepożądane. ***Wnioski.*** Na podstawie uzyskanych danych określono, iż C_{trough} nie jest skorelowane z nasileniem anemii, co uniemożliwia wykorzystanie tego parametru w predykcji wystąpienia tego działania niepożądanego. *(Farm Współ 2022; 15: 160-166) doi: 10.53139/FW.20221518*

Słowa kluczowe: olaparyb, C_{trough}, anemia

A case report

A 63-year-old patient was admitted to the Gynecological Oncology Department in March 2020 due to suspicion of ovarian cancer FIGO (fr. *Fédération internationale de gynécologie et d'obstétrique*) stage IV (meta to the liver). She suffered from abdominal pain for one month. Her familiar history was positive: her sister was diagnosed with breast cancer in her 36th year, and her brother was diagnosed with Hodgkin's disease.

In March 2020, the inoperable state of ovarian cancer was diagnosed in CT (computer tomography) scan. There was a biopsy of liver mass which confirmed carcinoma serosum high grade (CA125 – 508.9 U/ml)

The patient was qualified for neoadjuvant chemotherapy: paclitaxel (175 mg/m^2), carboplatin (AUC 6.0; AUC – area under the curve), and bevacizumab (7.5 mg/kg) administered intravenously every three weeks.

The patient underwent consultation and genetic tests, based on which the mutation c.5266 dup C (p. Gln 1756 Profs) in exon 19 of the *BRCA1* gene was diagnosed – this is a class 5 pathogenic change. The mutation in the patient was germinal. The characteristic of the patient is presented in table I.

Table I. Characteristics of the patient
Tabela I. Charakterystyka pacjentki

Parameter	S ± SD (%CV)
age [years]	63
body weight [kg]	66
BMI [kg/m^2]	25
WBC [10^9/L]	5.16 ± 1.37 (27)
NEUT [10^9/L]	2.75 ± 1.09 (39)
RBC [1012/L]	3.05 ± 0.55 (18)
HGB [mmol/L]	6.42 ± 1.04 (16)
HCT [L/L]	0.31 ± 0.05 (15)
PLT [10^9/L]	158.47 ± 18.04 (11)
ALT [U/L]	21,50 ± 7.57 (35)
AST [U/L]	21.39 ± 6.18 (29)
creatinine [mmol/L]	61.22 ± 14.95 (24)
glucose (mmol/l)	6.20 ± 0.39 (6)
eGFR [mL/min]	81.22 ± 14.95 (24)

In June 2020, after three courses of chemotherapy (CA125-141.5 U/ml) and in August 2020, after six courses of chemotherapy (CA125-89.5 U/ml)), the patient underwent CT examination, which revealed partial response (PR) to treatment according to RECIST 1.1 criteria [1], however, due to infiltration of the mesentery branch, cytoreductive surgery was not possible. It was decided to continue the bevacizumab immunotherapy as the maintenance therapy at the current dose. The patient noted increased blood pressure, which required the permanent introduction of one drug for hypertension. This complication is one of the most common side effects of bevacizumab treatment. The result of the genetic test allowed the initiation of maintenance treatment with olaparib. Treatment was started eight weeks after the last administration of chemotherapy at a dose of 2x300 mg/24 h in tablets, according to the PAOLA study. PAOLA study was a randomised, double-blind, phase III Trial of Olaparib *vs Placebo* in Patients with Advanced FIGO Stage IIIB – IV High-Grade Serous or Endometrioid Ovarian, Fallopian Tube, or Peritoneal Cancer treated with standard First-Line Treatment, Combining Platinum-Taxane Chemotherapy and Bevacizumab Concurrent with Chemotherapy and in Maintenance.

The patient tolerated the initial treatment with bevacizumab and olaparib well, but after the first course, there was symptomatic CTCAE (Common Terminology Criteria for Adverse Events) [2] grade 2 anaemia. The patient required transfusion of 2 RBC (Red Blood Cells) concentrate transfusion, a week break in the administration of tablets and a reduction of the olaparib dose to 2×250 mg/24h from the following treatment course. The patient tolerated the second course of olaparib treatment at a reduced dose and the maintenance treatment with bevacizumab well and did not report any side effects. After the third course, the patient was diagnosed with asymptomatic anaemia Grade 2 – the one-week break was proposed. The bevacizumab treatment was finished after the 18th course (due to Polish rules of National Program).

On the day of administration of the 16th course of bevacizumab, the patient was diagnosed with anaemia grade 3 – again, transfusion of RBC and reduction of olaparib to 2×200 mg/24h in tablets was necessary. Nowadays, she continues olaparib maintenance therapy 2×200 mg/24h in tablets. There is still an effective antineoplastic treatment which is confirmed in a CT scan done in January 2022. The haemoglobin concentration depending on the patient's treatment time is presented in figure 1.

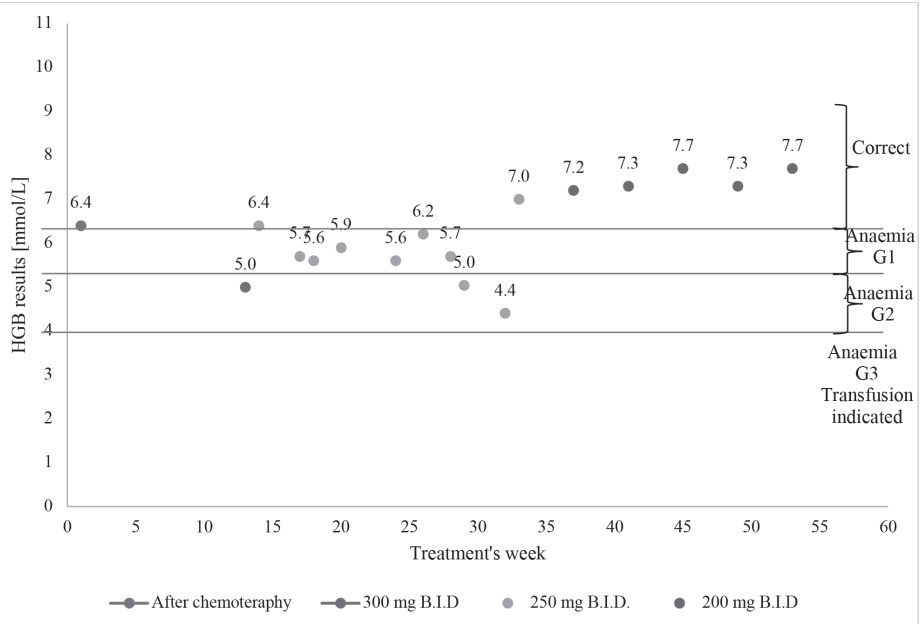

Figure 1. Dependence of the haemoglobin concentration value on the treatment week, taking into account the dose of olaparib

Rycina 1. Zależność stężenia hemoglobiny od dawki olaparybu w trakcie tygodni leczenia

During the whole treatment, she took enoxaparinum (varicose veins) and ferrum during the first three months of treatment (low iron level). There are no drug-drug interactions for those drugs and olaparib as they were analysed to find inhibitors/inducers of CYP3A4/5, P-gp.

Aim of the study

The study aimed to assess plasma trough concentrations of olaparib at steady state (C_{trough}) in a patient with ovarian cancer to determine if the severity of olaparib-induced anaemia is correlated with minimum concentration of olaparib at steady state.

Materials and methods

Reagents

Olaparib was purchased from LGC Standards (Łomianki, Poland) and Internal Standard – acetaminophen from Sigma Aldrich. HPLC (high-performance liquid chromatography) mobile phase methanol and glacial acetic acid were from Merck. Water used in the mobile phase was deionised, distilled and filtered through a Millipore system Direct Q3 before use. The extractive mixture consists of ethyl acetate and chloroform from Merck.

The patients received Lynparza® (AstraZeneca Pharma Poland Sp. z o.o.) in tablets (batch: 70818, RN192).

Table II. C_{trough} of olaparib depending on the dose

Table II. Stężenie C_{trough} olaparybu w zależności od przyjmowanej dawki

Parameter	Tablets		
dose	300 mg bid	250 mg bid	200 mg bid
C_{trough} [ng/mL]	1235.54	1042.28	854.25
		807.36	504.60
		1087.17	1148.98
		576.37	1426.85
		2181.25	1753.49
		1032.91	
S ± SD (%CV)	1235.54	1,121.22 ± 553.93 (49)	1,137,63 ± 485.81 (92)

Assays

The analytical method was developed at the Clinical Pharmacy and Biopharmacy Department of the Poznan University of Medical Sciences.

A blood sample taken from the patient before taking the dose of Lynparza® was developed by centrifugation and plasma separation. Into a clean test tube, 475 µL of the patient's plasma was measured. 25 µL of the internal standard (40 µg/mL paracetamol solution) was dosed into the plasma. After that, 100 µL of an alkaline bicarbonate buffer (pH~9) was added. In the end, 2 mL of tert-butyl methyl ether was added to the sample. The tube with a stopper was mixed with an electric shaker and centrifuged. From the test tube, 1.6 mL of the organic layer was taken into the next clean test tube, and the solvent was evaporated. The dry residue was dissolved in 100 µL of a phase (9.9 mL of ultrapure water and 100 µL of 80% acetic acid). The entire content of the tube was transferred to the autosampler insert.

The concentrations of olaparib in plasma were assayed using the high-performance liquid chromatography (HPLC) method with ultraviolet (UV) detection. Acetaminophen was used as the internal standard (IS). Samples were analysed on Alliance 2695 HPLC UV-Vis system with Empower Software No 1154 Waters Corporation. The separation was done using the gradient method. Phase A was 0.2% acetic acid in water, phase C was ultrapure water, and phase D was methanol. The gradient started at 100% C and decreased linearly to 11% C, 1% A and increased 88% D in 17 min, then changed to 1% D, 98% C and on 25 min it returned to starting condition for column equilibration. The flow rate was 1 mL/min. Chromatography was run on Waters Symmetry C8, 5 µm, 4.6 mm × 250 mm analytical column. The column temperature was maintained at 25°C, the UV detection wavelength was set at 254 nm, and the injection volume was 20 µL. The method was validated according to European Medicines Agency guidelines [3]. The method validation confirmed good precision (CV% <15%), accuracy (92.3-115.0%) and linearity (r=0.9994) in the range of 100-4000 ng/mL. Olaparib recovery was constant at 19.23 ± 2.27%. The average recovery result for the internal standard was 28%.

The severity of olaparib's adverse effects was assessed by CTCAE v.5.0 scale. In the CTCAE scale, the severity of adverse effects can be divided from mild (G1) to death (G5) [2]. The severity of anaemia in CTCAE v.5.0 scale depends on haemoglobin concentration (G1: Hgb <LLN – 6.2 mmol/L; G2: Hgb <6.2 – 4.9 mmol/L; G3: Hgb <4.9 mmol/L, transfusion indicated; G4: life-threatening consequences, urgent intervention indicated; G5: death).

Results

The treatment of the patient was conducted at the Gynecological Oncology Division of Poznan University of Medical Sciences, and the measurements were done in the Department of Clinical Pharmacy and Biopharmacy, University of Medical Sciences, Poznan, Poland, with the approval from the Bioethics Committee, University of Medical Sciences, Poznan, Poland (697/20). During the olaparib treatment, the patient had 12 measurements of blood $C_{trough.}$ Measurements were taken during the intake of the dose of 2x300 mg/24h (1 sample) and also in reduced doses of 2x250 mg/24h (6 samples), and then 2x200 mg/24h (5 samples). The samples were taken before each course of olaparib treatment and at the time of diagnosed anaemia. If the patient is presented with asymptomatic anaemia grade 2, then a one-week break of olaparib is introduced. If the increase in red cell colony is not enough, it is possible to make a maximal 28 days break. If there is symptomatic anaemia grade 2 or anaemia grade 3, then the red blood cells (RBC) concentration should be transfused. The C_{trough} values measured during olaparib treatment are presented in table II. Due to symptomatic anaemia that occurred after two months of treatment and Red Blood Cells transfusion, the patient underwent the first reduction of olaparib to the dose of 2x250 mg, and then C_{trough} ranged from 576.373 to 1,042.280 ng/mL.

Unfortunately, after five consecutive courses of olaparib, the patient returned to symptomatic anemia requiring transfusion of RBC, a one-week treatment break, and a second dose reduction to 2x200 mg/24h. At that time, C_{trough} ranged from 504.604 to 854.249 ng/mL during the first three months of measurements, while after this time, it began to increase again, reaching a concentration of 1,753.448 ng/mL. In figure 2, measurements from the last months are quite surprising because the patient does not take additional medications, and yet a significant increase in C_{trough} concentration has been noticed without any decreasing tendency in blood count parameters, especially in the red cell line.

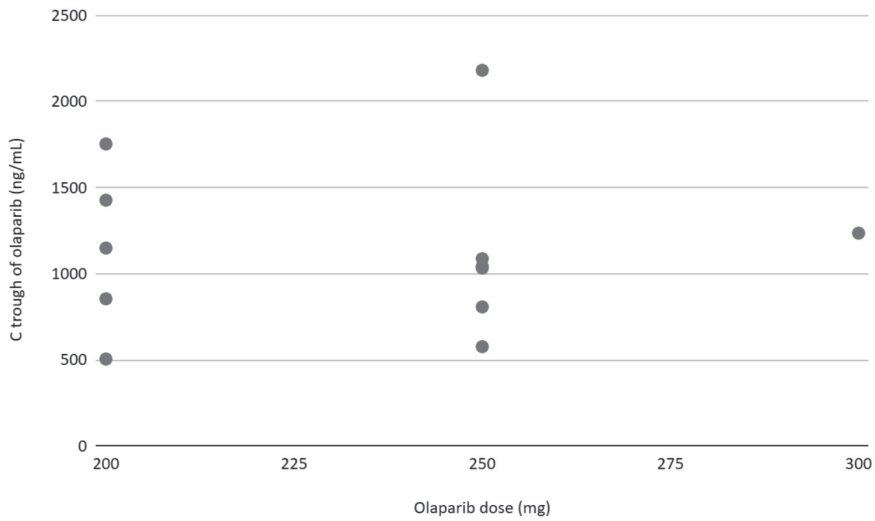

Figure 2. Analysis of C_{trough} of olaparib depending on the dose
Rycina 2. Analiza C_{trough} olaparybu w zależności od dawki

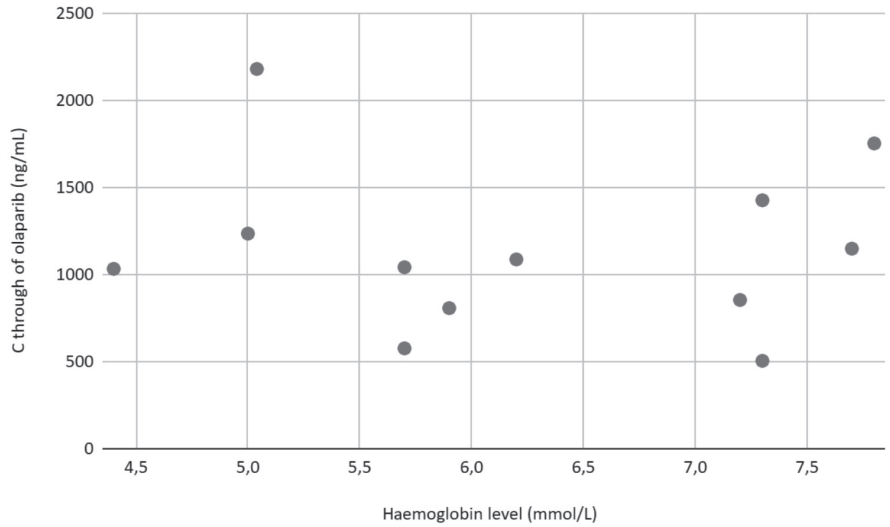

Figure 3. Analysis of C_{trough} of olaparib depending on the haemoglobin concentration
Rycina 3. Analiza C_{trough} olaparybu w zależności od stężenia hemoglobiny

It should be marked that anaemia in the presented patient is not correlated with the dose of olaparib (Pearson correlation coefficient -0,8692) and C_{trough} – (Pearson correlation coefficient -0,0612) (figure 3). There was no trend in the lower C_{trough} of olaparib depending on the grade of anaemia due to CTCAE (figure 4).

Disscussion

Olaparib is one of the better-tolerated anti-cancer drugs. The most commonly reported side effects during treatment were anaemia, neutropenia, fatigue, nausea, and vomiting. There are no analyses which would unambiguously answer the question of whether there

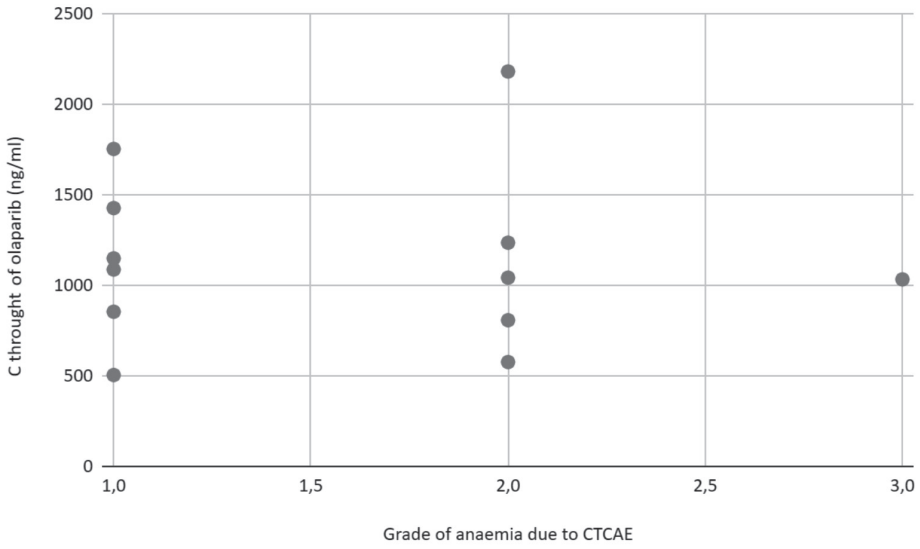

Figure 4. Analysis of C_{trough} of olaparib depending on the grade of anaemia due to CTCAE
Rycina 4. Analiza C_{trough} olaparybu w zależności od stopnia nasilenia anemii wg CTCAE

is a concentration of the olaparib C_{trough} that would recommend a drug reduction before the occurrence of side effects, without worsening the response to the anti--cancer treatment used, and yet significantly reducing the deterioration of QoL (Quality of life).

Anaemia is one of the most common complications occurring during olaparib therapy. It was noticed primarily in clinical trials (STUDY 19, SOLO-2, SOLO-1, SOLO-3) [4]. To effectively control anaemia, patients should have a complete blood count test before they undergo maintenance therapy and monthly during the olaparib therapy – before each subsequent course of the treatment and in case of clinical indications. During the treatment, the severity of anaemia is described according to the CTCAE 5.0 classification of adverse events [2].

Cytotoxic chemotherapy causes rapidly dividing cells damage, among others, bone marrow cells. During treating patients after the finished line of chemotherapy by PARP inhibitor, there is a possible synergistic effect on inhibiting myeloid repair. Inhibiting PARP enzymes is the stronger the greater the damage to the cells, whether it is a cancer cell or not. Due to this effect, there is a break between finishing the chemotherapy and starting olaparib treatment. To control, patients have regular cell blood line elements tests performed. PARP enzymes are essential factors in the modification of DNA processes, which cause a high risk of bone marrow damage and induction of anemia [5,6]. Olaparib is one of the anaemia-inducing drugs. It can be seen that the olaparib-toxicity can be managed by drug dose reduction. Many patients can still continue the PARP-inhibitor treatment after dose reduction of the drug without lower effectiveness. Initially, the patient exhibited toxicity which required not one but two olaparib-dose reductions in the first months of treatment. Nowadays, she continues maintenance therapy in minimal doses without any decrease in haemoglobin level. Therapeutic drug monitoring (TDM) is testing the amount of certain medicines in the blood. It is done to ensure the amount of medicine the patient is taking is safe and effective and to determine the best dosages for patients taking olaparib. It is important to remember to collect the blood sample at the proper time. It is worth emphasising that if G3 or G4 anaemia occurs and it is necessary to transfuse RBCs, it must be remembered to take the blood sample for the C_{trough} test should be collected before rather than after an RBC transfusion because the mixing of the donor and recipient's blood may result in a measurement error.

The presented results of patient indicate that reducing the dose of the olaparib causes an initial reduction in C_{trough} however, the observed upward trend in drug concentration and the number of tested samples performed is too small to establish a safe drug concentration and to determine the concentration of

the drug requiring a reduction of the cytostatic before the occurrence of side effects.

Summary

This case report is interesting from the approaches to patient management as well as the specific interpretation in the information of drug monitoring and eventually modifying dosing while maintaining efficacy – but always remembering about resulting in improved treatment and decisions which would be safe for patients. Some clinicians will remain unconvinced, but this real-life example suggests that for this moment, C_{trough} of olaparib should not be used as a practical benefit in patients with anaemia during PARP-inhibitor treatment. In the presented case, the severity of olaparib-induced anaemia is not correlated with minimum concentration of olaparib at steady state (C_{trough}). For the moment, C_{trough} of olaparib during the treatment cannot be used as a predictor point for the reduction of the dose. Other measurement options are needed.

Conflict of interest
None

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