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Identifying the need to refine the potential patient risk factors for niraparib-induced thrombocytopenia

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HIGHLIGHTS

- Higher niraparib plasma concentrations/exposure due to decrease clearance would increase risk of thrombocytopenia.
- Niraparib is an inhibitor of MATE1/2 renal transporters resulting in decrease in CrCl and potentially its own clearance.
- Weight and platelet count do not identify all patients at risk for developing thrombocytopenia in clinical practice.
- Additional analysis is needed to consider patient factors that lead to overall increase niraparib drug exposure.

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ABSTRACT

Objective. Niraparib is a poly (ADP-ribose) polymerase inhibitor (PARP) approved for use in maintenance therapy for ovarian cancer that is associated with the unpredictable grade 3/4 thrombocytopenia. This study was conducted to refine patient dosing recommendations for niraparib based upon clinical practice observations of grade 3/4 thrombocytopenia.

Methods and materials. Six patient cases were reviewed to identify similarities in patient factors. An *in vitro* study was conducted using healthy volunteer blood spiked with Niraparib concentrations ranging from 0 ng/mL to 5000 ng/mL. Manual platelet counts were evaluated at different time intervals for each concentration and compared to untreated controls. Data was then analyzed based on percent change in platelet count *versus* untreated control for each concentration/time point.

Results. In three patients with body weight > 80 kg and platelet count >200 × 10^9/L, decreased creatinine clearance (CrCl) <60 mL/min was identified as potential signal. An additional three patients with weights below 77 kg and/or baseline platelet counts <150 × 10^9/L were re-evaluated, and it was observed that all had decreased CrCl of <60 mL/min. Albumin <3.5 g/dL was also observed in some patients with thrombocytopenia. The *in vitro* study, observed a direct concentration-dependent relationship between niraparib and thrombocytopenia.

Conclusion. The data suggests that renal insufficiency and hypoalbuminemia may be associated with the development of niraparib-induced thrombocytopenia. Moreover, the preliminary *in vitro* studies also demonstrated a concentration-dependent relationship between niraparib and direct toxicity to platelets.

1. Introduction

Niraparib was the first poly (ADP-ribose) polymerase inhibitor (PARP) to be approved by the U.S. Food and Drug Administration (FDA) for the maintenance treatment of ovarian cancer without a germline or somatic BRCA mutation requirement [1]. The mechanism of action of PARP inhibitors involves interference with DNA repair through inhibition of the base excision repair pathway as well as trapping of PARP-1 and PARP-2 enzymes at sites of DNA damage, which may cause more cytotoxicity than unrepaired single-stranded breaks alone [1]. This PARP trapping mechanism is more predominant in niraparib than in either olaparib or rucaparib. Niraparib's clinical efficacy was demonstrated in the NOVA study, a randomized, double-blind phase 3 trial that found progression-free survival significantly longer in the niraparib group compared to the placebo group regardless of

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the presence or absence of a germline BRCA mutation [2]. Secondary endpoints have revealed increased chemotherapy-free interval and no detrimental effect on response to subsequent therapies compared to placebo [3].

In another phase one dose escalation study with 100 participants by Sandhu and colleagues, thrombocytopenia was reported in 35% all of patients with 15% of all patients experiencing grade 3 or greater thrombocytopenia [4]. In both the phase one study and in the NOVA study, hematological toxicity was most common toxicity requiring dose modification or discontinuation, appearing to be proportional to dose and increasing in instances of cumulative doses [3,4]. In the NOVA study, overall risk of grade 3/4 thrombocytopenia in patients on 300 mg/day niraparib, was 33.8%, tending to occur early in treatment. However, by cycle three the risk decreased to only 1.2% [4]. Empirically, dose modifications to 200 mg/day and 100 mg/day during cycles one and two tended to achieve resolution of thrombocytopenia, lowering the risk to 2.2 to 3.9% [4]. Moore and colleagues proposed two predictors of increased risk for grade 3/4 thrombocytopenia: baseline platelet count $<150 \times 10^9$/L or baseline body weight <77 kg [5]. The incidence of grade 3/4 thrombocytopenia was increased in patients with at least one risk factor compared to no risk factor (39.3% *vs* 16.1%). Based on these criteria, Moore and colleagues recommended that patients who meet these criteria start at a dose of 200 mg by mouth once daily [5].

The convenience of once daily dosing and FDA approval regardless of BRCA mutation status or platinum sensitivity status, makes niraparib a favorable option for PARP inhibitor maintenance treatment in clinical practice. However, as it moved into clinical practice, the frequent, unpredictable grade 3/4 thrombocytopenia has led to some hesitancy with the use and has highlighted a need to gain a better understanding of niraparib pharmacology to refine patient dosing recommendations. After observing three patient cases of significant grade 3/4 thrombocytopenia that had neither of the predictive risk factors from the NOVA study radar analysis by Moore and colleagues, patient parameters were reviewed to consider if there were any other potential similarities in these patients that may have contributed to risk of significant toxicity. An additional three patient cases from another institution were also evaluated that had either baseline weight <77 kg and/or baseline platelet count $<150 \times 10^9$/L to see if there were any clinical similarities with the first three cases. A hypothesis was generated that the presence of decreased creatinine clearance and/or low albumin will lead to higher niraparib plasma concentrations and exposure that results in significant thrombocytopenia. To explore this hypothesis, an *in vitro* study was conducted to determine if there is a concentration-dependent cytotoxic effect of niraparib on platelets.

2. Methods and materials

2.1. Patient risk factor assessment

First, a list of patient-related factors which may influence the susceptibility to drug toxicity was created. This list included: age, BRCA mutation status, platinum sensitivity, number of prior chemotherapy regimens, creatinine clearance, albumin, and the prior risk factors identified by Moore and colleagues: baseline weight and baseline platelet counts. Patient data that was evaluated included the dose and duration of niraparib therapy to onset of thrombocytopenia (platelet count $<100 \times 10^9$/L) or nausea through the time period that the adverse effects resolved. Additional data was collected for one case that patient was re-challenged. There was neither direct patient contact nor patient identifiers collected. This data was collected to identify better patient risk factors to predict toxicity in patients on niraparib therapy. For decades there has been controversy and debate on how to estimate creatinine clearance for drug dosing. As per the FDA Center for Drug Evaluation and Research (CDER) guidance, for this analysis creatinine clearance was calculated by the Cockcroft and Gault equation using actual body weight (ABW) unless this value was greater than ideal body weight (IBW) by 30% or more, in which case adjusted body weight (AdjBW) was calculated and used [6,7]. The formula used for adjusted body weight was: IBW + [0.4(ABW-IBW)]. Laboratory-specified normal ranges of albumin and platelets were 3.5 to 5.0 g/dL and 133 to 450 $\times 10^9$/L.

2.2. In vitro study

Healthy volunteer blood (165 mL) was obtained and aliquoted into 6 mL samples then spiked with selected concentration of niraparib and incubated at 37 °C on a rocker. The following nine niraparib concentrations selected included: 0 ng/mL, 39.06 ng/mL, 78.12 ng/mL, 156.25 ng/mL, 312.5 ng/mL, 625 ng/mL, 1250 ng/mL, 2500 ng/mL, 5000 ng/mL and each evaluated in triplicate. Samples were collected at the following time points: baseline (0), 1, 4, 8, 24, 48, 72 and 96 h. The niraparib concentrations and sampling time points were selected based upon niraparib therapeutic concentrations and pharmacokinetic profile [8]. Each niraparib concentration was assayed in triplicate to determine the mean percent change in platelet count based on untreated control for each respective timepoint to account for inherent *in vitro* platelet degradation as well. Platelet counts were estimated in 500 μL samples by unstained peripheral blood smears, a method detailed by Umashankar and colleagues [9]. Briefly, thin blood smears were prepared in triplicate for each sample on clean glass slides and were allowed to air dry. The unstained smears were examined under light microscopy immediately after preparation initially under 40× objective to select an ideal area for platelet counting where RBCs are uniformly spread without any overlapping, then examined under 100× objective without adding immersion oil. Number of platelets was counted per 1000 RBCs and the average number of platelets from three smears was calculated for each sample.

3. Results

Unexpected significant thrombocytopenia was observed within the first month of niraparib therapy in three patients. In all three patient cases, had a body weight >80 kg and platelet count $>200 \times 10^9$/L, however, a decreased creatinine clearance of <60 mL/min was identified as potential similarity. (Table 1) There were no consistent similarities with the other patient-related factors that may have influenced susceptibility to drug toxicity which included: age, BRCA mutation status, platinum sensitivity, number of prior chemotherapy regimens. Across the duration of niraparib treatment, the time of platelet count recovery after stopping niraparib therapy was consistently eight to 11 days which is approximately five to seven half-lives for niraparib. Even when one patient received 12 units of platelets over 3 days in hospital, the platelet counts did not improve, suggesting a direct systemic cytotoxic effect on platelets until the niraparib had significantly cleared the body. The additional three patient cases from another independent institution with body weight below 77 kg and/or baseline platelet counts $<150 \times 10^9$/L were evaluated and it was observed that all these patients also had decreased creatinine clearance <60 mL/min. (Table 1) In addition, lower albumin <3.5 g/dL was also observed in two of the six cases with thrombocytopenia that were reviewed, another three patients albumin levels at the lower end of normal limits.

Patient case 3 experienced niraparib-induced thrombocytopenia as the team's clinical observations suspecting impaired renal clearance was associated with thrombocytopenia had emerged. Since she had had an impressive decrease in CA-125 suggesting response to niraparib, the clinical team decided after her counts had recovered to re-challenge her with an empiric dose reduction to niraparib 100 mg once daily based on current creatinine clearance of 65 mL/min and albumin of 2.9 g/dL. She tolerated this dose for an additional 4 months then presented with both thrombocytopenia and anemia; her creatinine clearance had declined to 42 mL/min with albumin of 2.2 g/dL. Despite holding the niraparib dose and multiple blood transfusions over

Table 1
Summary of patient characteristics and platelet concentration trends.
Creatinine clearance was calculated by the Cockcroft and Gault equation using actual body weight unless this value was greater than ideal body weight by 30% or more, in which case adjusted body weight was calculated and used. Days until platelet recovery refer to the number of days between identification of thrombocytopenia and when platelets began to trend upwards.

	Patient 1	Patient 2	Patient 3	Patient 4[b]	Patient 5[a,b]	Patient 6[a]
Age	73	51	61	71	70	72
BRCA status	BRCA negative	BRCA negative	BRCA positive	BRCA negative	BRCA negative	BRCA negative
Platinum sensitivity	Platinum resistant	Platinum sensitive	Platinum resistant	Platinum sensitive	Platinum sensitive	Platinum sensitive
# Prior regimens	3	5	6	2	2	3
Actual body weight (kg)	91	108	80	91	76	68
Ideal body weight (kg)	59	78	46	52	55	46
Adjusted body weight (kg)	72	78	60	67	63	54
Creatinine clearance (mL/min)	51	47	32	51	57	39
Baseline albumin (g/dL)	3.7	3.7	2.4	3.8	4.2	3.5
Baseline platelets ($\times 10^9$/L)	234	262	400	140	101	747
Platelet nadir ($\times 10^9$/L)	6	112	29	32	32	8
Days until platelet recovery	8	11	9	7	7	11

[a] Denotes patient had baseline weight <77 kg.
[b] Denotes patient had baseline platelet count $<150 \times 10^9$/L.

1 month, the patient's nutritional status continued to decline with progression of disease and she ultimately was switched to an alternative chemotherapy regimen.

3.1. In vitro study

The *in vitro* study revealed an inverse dose-dependent relationship between niraparib concentration and mean percent change in platelet count over a 96-hour period (Fig. 1). This dose-dependent relationship was largely consistent throughout eight time points over nine concentrations of niraparib ranging from 0 to 5000 ng/mL. After 96 h, the maximum tested niraparib concentration of 5000 ng/mL averaged a 90% reduction in platelets. In comparison, after 96 h the niraparib concentrations of 39.06 ng/mL and 78.12 ng/mL, correlating to the steady state concentration range of niraparib, averaged only a 30% and 40% reduction compared to the untreated control in platelets, respectively.

4. Discussion

In this small, observational case series it was observed that baseline weight <77 kg and baseline platelet counts $<150 \times 10^9$/L were consistently not predictive of grade 3/4 thrombocytopenia in these patients, contrary to the recommendations by Moore and colleagues [4]. However, in all six patients with significant thrombocytopenia, a decreased creatinine clearance was observed. A lower albumin level was also associated with thrombocytopenia. From a pharmacology perspective, it is important to note that, both decreased drug clearance and decreased albumin levels lead to increased free fraction (active) of drug concentration and ultimately an overall increased drug exposure that would increase the potential risk for toxicity. Niraparib, as well as the other PARP inhibitors, all have high plasma protein binding >80%. Hence when plasma proteins such as albumin are low it results in higher free-fraction/unbound drug concentration [11,12]. The follow up *in vitro* study demonstrated a clear concentration-dependent relationship between niraparib concentrations and cytotoxicity to platelets.

The class of PARP inhibitors have been identified to be both substrates and inhibitors of the renal drug transporters, multidrug and toxic compound extrusion (MATE) proteins MATE1 and MATE2 located in the proximal renal tubule [10,11]. Specifically niraparib is an inhibitor of MATE1 and MATE2 transport proteins involved in proximal tubular drug secretion, the niraparib concentration to achieve 50% inhibition (IC_{50}) is 0.18 μM for the MATE1 transport and less than or equal to 0.14 μM for MATE2 transport [11]. After basolateral uptake of drugs/compounds, MATE proteins facilitate tubular section or clearance [10]. In addition to the PARP inhibitors, some of the most common MATE substrates are creatinine, corticosteroids and the platinum analogs. Drug/drug interactions involving inhibition of renal transporters are often overlooked but can alter the efficacy and toxicity of drugs that

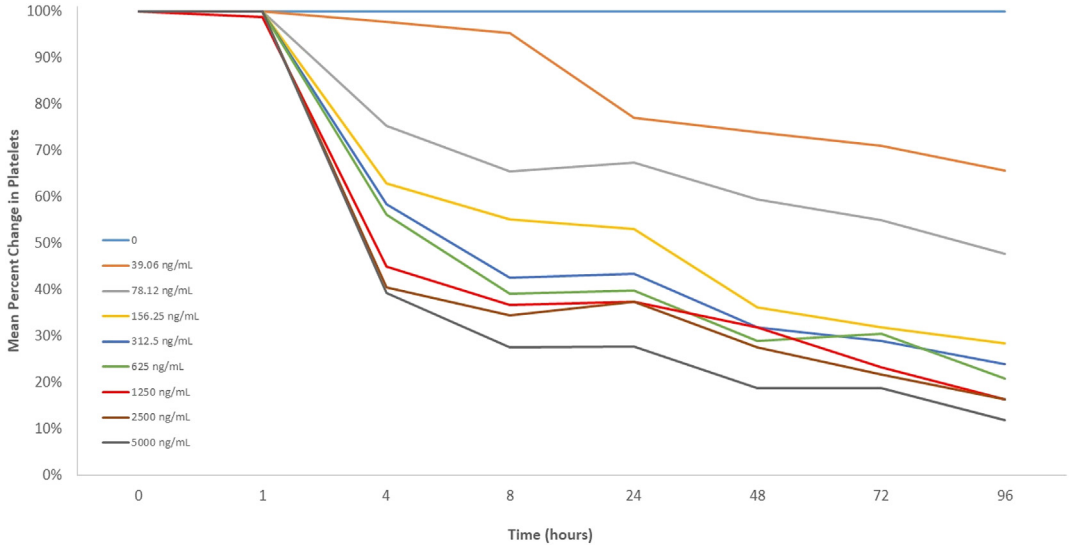

Fig. 1. Summary of niraparib concentration-dependent effect on platelet concentration *in vitro*. Mean concentration of remaining platelets as percentage of baseline value over time for each concentration of niraparib. Each concentration of niraparib was assayed in triplicate.

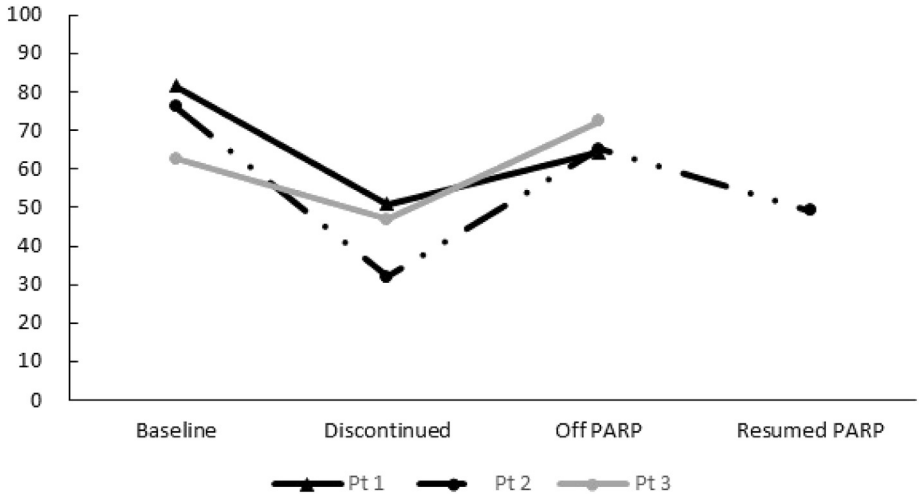

Fig. 2. Example of change in creatinine clearance in presence of MATE1/2 inhibitor, niraparib. Data was available from three patients to demonstrate impact on creatinine (known MATE 1/2 substrate) clearance (CrCL) in presence of niraparib, a known inhibitor of MATE 1/2 transporter. Thrombocytopenia also correlated with the decrease in CrCl.

are substrates of these renal transporters. While estimated creatinine clearance is most often described as an estimate of renal function, in respect to the renal transporters it can also specifically represent changes in drug clearance for MATE substrates. Fig. 2 demonstrates how creatinine clearance was inhibited while on niraparib then once stopped, resumed back to baseline. If re-challenged creatinine clearance declined again in presence of niraparib. In this case review, the Cockcroft and Gault equation was used to estimate creatinine clearance and it did correlate with thrombocytopenia [7]. However, niraparib plasma concentrations were not obtained to confirm the hypothesis that decreased creatinine clearance leads to higher niraparib plasma concentration and ultimately toxicity but it merits further prospective evaluation. As mentioned, inhibition of MATE1 and MATE2 renal transporters is a PARP inhibitor drug class effect. Olaparib, the first PARP inhibitor approved by the FDA for treatment of recurrent ovarian cancer and now also as maintenance therapy, has specific dose modification recommendations based on decreased creatinine clearance [12].

The radar analysis to evaluate the clinical data for potential patient risk factor for niraparib-induced thrombocytopenia did not identify calculated creatinine clearance as potential risk factor in development of thrombocytopenia. This may be because serum creatinine and the estimated glomerular filtration (eGFR) at baseline were evaluated as opposed to a calculated creatinine clearance over the duration of treatment. A serum creatinine level is not representative of changes in creatinine clearance, specifically in consideration of niraparib's inhibition the MATE1 and MATE2 renal transporter. Again Fig. 2 demonstrated impact on creatinine (known MATE 1/2 substrate) clearance (CrCL) in presence of niraparib, a known inhibitor of MATE 1/2 transporter. In this review, the proposed patient parameters of baseline weight and platelet counts have not been consistently predictive of thrombocytopenia in our clinical practice to date. Based on data in this case review, renal insufficiency and low albumin appeared to be potential risk factors in all six patients suggesting that plasma niraparib concentration is associated with risk for toxicity. Preliminary *in vitro* studies demonstrated a concentration-dependent relationship between niraparib and direct toxicity to platelets. This data identified there is need for refinement in the radar analysis and additional prospective pharmacology studies to determine the role of CrCl and low albumin for developing niraparib-induced thrombocytopenia.

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Data presentation

Partial data we presented at the 2018 Western Association of Gynecologic Oncology Annual Meeting, Lunch & Learn session, June 14th, 2018 and 2018 Salt Lake City, UT; Updates in Obstetrics & Gynecology Research, Houston TX June 20, 2018.

Conflict of interest

This research was unfunded and the authors have no conflicts of interest to declare.

Authors' contributions

JA Smith: Principal investigator involved in all aspects of study designs, data collection, data analysis, manuscript writing and final approval; T. Le: participated in clinical data collection & data analysis and manuscript writing/final approval; GA Martin: clinical data collection, manuscript review/revising and final approval; A. Gaikwad conducted preclinical studies, data analysis and manuscript writing/final approval; CH. Sun: data review/audit, manuscript final approval; EK. Nugent: study design, clinical data interpretation, manuscript review/final approval; JA Lucci III, M.D.: study design, clinical data interpretation, manuscript review/final approval.

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