Phase I study of olaparib in combination with liposomal doxorubicin in patients with advanced solid tumours

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Background: Olaparib, an oral PARP inhibitor, has shown antitumour activity as monotherapy in patients with germline BRCA1/2 (gBRCA)-mutated breast and ovarian cancer. This study evaluated olaparib capsules in combination with liposomal doxorubicin (PLD) in patients with advanced solid tumours (NCT00819221).

Methods: Patients received 28-day cycles of olaparib, continuously (days 1–28) or intermittently (days 1–7), plus PLD (40 mg m⁻², day 1); seven olaparib dose cohorts (50–400 mg bid) were explored to determine the recommended dose. Assessments included safety, pharmacokinetics, pharmacodynamics and preliminary efficacy (objective response rate (ORR)).

Results: Of 44 patients treated (ovarian, n = 28; breast, n = 13; other/unknown, n = 3), two experienced dose-limiting toxicities (grade 3 stomatitis and fatal pneumonia/pneumonitis (200 mg per 28-day cycle); grade 4 thrombocytopenia (400 mg per 7-day cycle)). The maximum tolerated dose was not reached using continuous olaparib 400 mg bid plus PLD. Grade X3 and serious AEs were reported for 27 (61%) and 12 (27%) patients, respectively. No major pharmacokinetic interference was observed between olaparib and PLD. The ORR was 33% (n = 14 out of 42; complete response, n = 3). A total of 13 responders had ovarian cancer: 10 were platinum-sensitive, 11 had a gBRCA mutation.

Conclusions: Continuous/intermittent olaparib (up to 400 mg bid) combined with PLD (40 mg m⁻²) was generally tolerated and showed evidence of antitumour activity in ovarian cancer.

Poly(ADP-ribose) polymerases (PARPs), which repair single-strand DNA breaks through the base-excision repair (BER) pathway, have emerged as important targets for cancer therapies in patients with an homologous recombination repair deficiency (HRD), because PARP inhibition leads to the formation of double-stranded DNA breaks that cannot be accurately repaired in tumours with an HRD, such as a BRCA1/2 mutation; this concept is known as synthetic lethality. In preclinical studies, PARP inhibitors have demonstrated efficacy in tumours with BRCA1/2 mutations (Moynahan et al, 1999, 2001; Bryant et al, 2005; Farmer et al, 2005).

Olaparib is a potent oral PARP inhibitor that has demonstrated efficacy as monotherapy in trials involving ovarian and breast cancer patients with germline BRCA1/2 (gBRCA) mutations and/or sensitivity to platinum-based therapies (Fong et al, 2009; Audeh et al, 2010; Fong et al, 2010; Tutt et al, 2010; Gelmon et al, 2011;
Monotherapy studies have shown that olaparib is relatively well tolerated; the most common adverse events (AEs) being nausea, fatigue, vomiting and anaemia (Fong et al, 2009; Audeh et al, 2010; Fong et al, 2010; Tutt et al, 2010; Gelmom et al, 2011; Kaye et al, 2012; Ledermann et al, 2012). Combination studies with standard chemotherapeutic agents in patients with advanced solid tumours (ASTs) have resulted in sub-therapeutic recommended doses (RD) because of haematologic toxicities (Giaccone et al, 2010; Khan et al, 2011; Samol et al, 2012). Pegylated liposomal doxorubicin (PLD) is an approved treatment for ovarian cancer patients failing platinum and taxane chemotherapies (Gordon et al, 2001; Rose, 2005), and has shown efficacy in a Phase II trial of ovarian cancer patients with gBRCA mutations (Kaye et al, 2012) and a Phase III trial in recurrent ovarian cancer patients (Gordon et al, 2001). In practice, the placement of PLD in the treatment algorithm varies between countries, with use in the second-line setting for patients with platinum-sensitive epithelial ovarian cancer in combination with carboplatin or trabectedin according to the duration of the platinum-free interval since last chemotherapy cycle. However, other second-line options exist and other regimens, such as carboplatin, gemcitabine and bevacizumab, may be applied (Aghajanian et al, 2012). The combination of PARP inhibition with PLD may provide a synergistic effect in patients with advanced ovarian cancer, especially those with HRDs, because of the decreased ability to repair chemotherapy-induced DNA damage. Preclinical studies with PARP inhibitors have shown potentiation of the cytotoxic effects of chemotherapeutic agents (Drew and Plummer, 2009). In particular, PARP inhibition has been shown to sensitise human hepatocellular carcinoma cell lines to doxorubicin treatment in a dose-dependent manner (Muñoz-Gámez et al, 2011). In another study, performed in HeLa cells, the combination of a PARP inhibitor with doxorubicin treatment led to a 50% increase in doxorubicin-mediated cell death compared with doxorubicin treatment alone (Magan et al, 2012). The toxicity profile of PLD appears to be distinct from that of olaparib, with the most common AEs associated with PLD being palmar–plantar erythrodysaesthesia syndrome (PPES), stomatitis and nausea (Kaye et al, 2012). PARP inhibition should be sustained throughout the DNA damage and repair processes but, when combining PARP inhibitors with chemotherapy, prolonged inhibition may be unnecessary provided that a critical inhibitory level is maintained during DNA repair. Consequently, intermittent olaparib treatment schedules may show comparable activity, but better tolerability, vs continuous regimens and represent an interesting option for combination studies.

The aim of this study was to determine the optimal treatment schedule and RD of oral olaparib capsules when administered bid for either 1 week (intermittent) or 4 weeks (continuous), in combination with PLD, in patients with ASTs.
considered, by the investigator, to be related to combination treatment: grade 4 neutropenia lasting >5 days, grade 4 thrombocytopenia, grade ≥3 febrile neutropenia, grade ≥3 nausea and/or vomiting (despite maximal anti-emetic therapy) or any other CTCAE grade ≥3 non-haematologic toxicity. Preliminary efficacy was determined by assessing objective responses based on Response Evaluation Criteria In Solid Tumors (RECIST; v1.0) (Therasse et al, 2000) as determined by the study site investigators. Efficacy was analysed by tumour type and gBRCA mutation status. Additional analyses of ovarian cancer patients were performed by subdividing patients into platinum-sensitive (patients who experienced a progression-free interval of ≥6 months following discontinuation of the last platinum-containing chemotherapy) and platinum-resistant subgroups. Analyses by gBRCA mutation status and platinum sensitivity were not pre-specified in the study design and were performed retrospectively.

Blood samples (4 ml) were collected according to limited (escalation phase) or full (expansion phase) sampling schedules and analysed to determine plasma concentrations of olaparib and PLD (Supplementary Figure 1). Plasma concentrations were used to derive PK parameters following intermittent and continuous dosing. Olaparib concentrations were determined by solid-phase extraction and LC–MS/MS chromatography. Total doxorubicin was measured by a high-performance liquid chromatography (HPLC)/fluorescence method following liposome dispersion with Triton-X and on-line plasma extraction. PK data were analysed by non-compartmental methods.

For the determination of γH2AX, PBMCs were isolated from venous blood samples (8 ml) obtained on days 1, 8, 15 and 28 of cycle 1 (Supplementary Figure 1); fixed, and stained for intracellular γH2AX. Cytofluorimetric detection was performed with an anti-phospho-H2AX (Ser139) antibody (Cell Signaling Technology, Beverly, MA, USA). Further analyses were performed to correlate γH2AX data with preliminary evidence of autitumour activity.

Statistical analyses. No formal statistical analysis of safety/tolerability was planned. A Student’s t-test for paired data (two tailed) was applied to PK data and a Wilcoxon signed-rank test was performed for PD data to determine statistically significant differences.

RESULTS

Patient disposition. All 44 patients enrolled from January 2009–December 2010 were treated and evaluable for safety (Table 1). Two patients receiving continuous olaparib 400 mg bid are ongoing. Two patients receiving continuous olaparib (100 and 200 mg, respectively) did not complete a 28-day treatment cycle, owing to tumour-related intestinal obstruction and tumour-related ileus, so were not evaluable for DLT or efficacy evaluations. Pre-existing gBRCA mutation status data only were collected following limited radiotherapy, medical history of chronic interstitial lung disease, thromboses, infection, suppurating bronchopneumonia; the other had mediastinal radiotherapy, medical history of chronic interstitial lung disease, thromboses, infection) that potentially contributed to pneumonitis.

Clinically significant haematological abnormalities reported as AEs included alterations in neutrophil count (n = 13) and haemoglobin (n = 4). Grade ≥3 haematologic alterations were observed in neutrophils (n = 9; 20%), platelets (n = 3; 7%), haemoglobin (n = 2; 5%) and white blood cells (n = 2; 5%).

Pharmacokinetics. The maximum plasma concentration (Cmax) and area under the plasma concentration–time curve (AUC0–10 h) of olaparib increased with dose when given alone (day 1) and in the presence of PLD; olaparib exposure tended to be higher in the presence of PLD (Table 3; Supplementary Figure 2). Following a single dose, olaparib was absorbed rapidly with a mean time to maximum observed concentration (Tmax) of 2.1 h. The minimum plasma concentrations (Cmin) of olaparib were maintained during 28 days of treatment (400 mg bid: day 8, 3.6 ± 2.2 μg ml⁻¹; day 28, 3.9 ± 2.6 μg ml⁻¹) indicating that PLD did not interfere with steady-state olaparib plasma concentrations. PLD parameters were generally similar when olaparib was administered for 7 or 28 days; a statistically significant increase in AUC0–inf and a corresponding decrease in total body clearance (CLT/ρ) were observed in patients receiving continuous olaparib 400 mg bid (day 1–28) compared with short-term administration (day 1–7).

Efficacy. The ORR in the overall population was 33% (14 out of 42). Overall, three evaluable patients (7%) achieved a complete response (CR) and 11 (26%) achieved a partial response (PR) (Table 4). Thirteen responders had ovarian cancer; the ORR in this subgroup was 50% (13 out of 26). In the ovarian subgroup, the response rate in platinum-resistant and platinum-sensitive
patients was 25% and 71%, respectively (Table 5). Eleven (61%) gBRCA-mutated patients in the ovarian subgroup achieved a response. Of the two additional patients in the ovarian subgroup who experienced a response, one patient with no gBRCA mutation had a CR and one patient with unknown gBRCA status had a PR; both were platinum-sensitive. The remaining response (PR) was in a gBRCA-mutated patient with breast cancer.

**Pharmacodynamics.** Cytofluorimetric determination of γH2AX phosphorylation level was performed in 41 out of 44 (93%) patients receiving intermittent or continuous olaparib. For both regimens, downregulation of phospho-γH2AX was particularly evident in ovarian patients on days 8 and 15 during the first treatment cycle. Decreases were statistically significant for patients with platinum-resistant ovarian cancer on day 8 ($P = 0.046$), and for patients receiving intermittent olaparib treatment, and was independent of the olaparib dose. However, in platinum-sensitive ovarian patients, the phospho-γH2AX level was stable throughout 28 days of treatment (Figure 1). A rebound of phospho-γH2AX levels occurred between days 15 and 28 in the platinum-resistant ovarian subgroup; this effect was most noticeable in patients with PR or stable disease (Supplementary Figure 3) and in those receiving intermittent dosing (data not shown). A trend towards higher basal phospho-γH2AX levels was observed in the platinum-resistant subgroup compared with the platinum-sensitive subgroup (Supplementary Figure 4). This study only measured phospho-H2AX in surrogate tissue. PBMCs, as tumour samples were not available. The measured levels of phospho-H2AX may therefore not reflect any DNA damage in the tumour target lesions induced by the combination of olaparib and doxorubicin treatment.

### Table 1. Patient characteristics

| Characteristic              | 50 mg bid 7 day (n = 3) | 100 mg bid 7 day (n = 3) | 100 mg bid 28 day (n = 4) | 200 mg bid 7 day (n = 3) | 200 mg bid 28 day (n = 7) | 400 mg bid 7 day (n = 12) | 400 mg bid 28 day (n = 12) | Total (n = 44) |
|-----------------------------|-------------------------|--------------------------|---------------------------|--------------------------|----------------------------|---------------------------|---------------------------|-----------------|
| **Median age (range), years** | 48.0 (46–54)            | 63.0 (53–71)             | 62.5 (49–74)              | 66.0 (59–68)             | 55.0 (32–63)               | 55.0 (37–71)              | 52.0 (31–64)              | 55.5 (31–74)    |
| **Sex**                     |                         |                          |                           |                          |                            |                           |                           |                 |
| Female                      | 3 (100)                 | 3 (100)                  | 4 (100)                   | 2 (67)                   | 7 (100)                    | 11 (92)                   | 12 (100)                  | 42 (95)         |
| **ECOG status**             |                         |                          |                           |                          |                            |                           |                           |                 |
| 0                           | 3 (100)                 | 3 (100)                  | 3 (75)                    | 2 (67)                   | 3 (43)                     | 8 (67)                    | 12 (100)                  | 34 (77)         |
| 1                           | –                       | –                        | 1 (25)                    | 1 (33)                   | 4 (57)                     | 4 (33)                    | –                         | 10 (23)         |
| **Prior chemotherapy**      |                         |                          |                           |                          |                            |                           |                           |                 |
| Yes                         | 3 (100)                 | 3 (100)                  | 3 (75)                    | 3 (100)                  | 2 (29)                     | 11 (92)                   | 10 (83)                   | 35 (80)         |
| No                          | –                       | –                        | 1 (25)                    | –                        | –                          | 1 (8)                     | 2 (17)                    | 9 (20)          |
| **Primary tumour site**     |                         |                          |                           |                          |                            |                           |                           |                 |
| Ovarian                     | 3 (100)                 | 2 (67)                   | 2 (50)                    | 2 (67)                   | 3 (43)                     | 8 (67)                    | 8 (67)                    | 28 (64)         |
| Breast                      | –                       | 1 (33)                   | 1 (25)                    | –                        | 4 (57)                     | 3 (25)                    | 4 (33)                    | 13 (30)         |
| SCLC                        | –                       | –                        | 1 (25)                    | –                        | –                          | –                         | –                         | 1 (2)           |
| Prostate/Colon              | –                       | –                        | –                         | –                        | –                          | 1 (8)                     | –                         | 1 (2)           |
| Unknown                     | –                       | –                        | –                         | 1 (33)                   | –                          | –                         | –                         | 1 (2)           |
| **Evaluable patients**      |                         |                          |                           |                          |                            |                           |                           |                 |
| DLT                         | 3 (100)                 | 3 (100)                  | 3 (75)                    | 3 (100)                  | 6 (86)                     | 12 (100)                  | 12 (100)                  | 42 (95)         |
| Safety                      | 3 (100)                 | 3 (100)                  | 4 (100)                   | 3 (100)                  | 7 (100)                    | 12 (100)                  | 12 (100)                  | 44 (100)        |
| Efficacy                    | 3 (100)                 | 3 (100)                  | 3 (75)                    | 3 (100)                  | 6 (86)                     | 12 (100)                  | 12 (100)                  | 42 (95)         |
| **gBRCA mutation status**   |                         |                          |                           |                          |                            |                           |                           |                 |
| BRCA1 and/or BRCA2 positive | 3 (100)                 | 2 (67)                   | 1 (25)                    | 1 (33)                   | 2 (29)                     | 5 (42)                    | 9 (75)                    | 23 (52)         |
| Negative                    | –                       | –                        | –                         | –                        | –                          | 1 (8)                     | –                         | 1 (2)           |
| Unknown                     | –                       | 1 (33)                   | 3 (75)                    | 2 (67)                   | 5 (71)                     | 6 (50)                    | 3 (25)                    | 20 (45)         |
| **Platinum sensitivity status** |                      |                          |                           |                          |                            |                           |                           |                 |
| Sensitive                   | 3 (100)                 | –                        | –                         | –                        | 3 (100)                    | 5 (63)                    | 4 (50)                    | 15 (54)         |
| Resistant                   | –                       | 2 (100)                  | 2 (100)                   | 2 (100)                  | –                          | 3 (38)                    | 4 (50)                    | 13 (46)         |

Abbreviations: DLT = dose-limiting toxicity; ECOG = Eastern Cooperative Oncology Group; gBRCA = germline BRCA; SCLC = small-cell lung cancer.

For advanced disease.

Ovarian patients only.

Number of patients.
This study evaluated olaparib (intermittent and continuous dosing up to 400 mg bid) in combination with the optimal dose of PLD (40 mg m\(^{-2}\) every 28 days) in patients with ASTs. Although the MTD was not reached, our results suggest that continuous dosing with olaparib capsules, at the recommended mono-therapy dose of 400 mg bid, combined with PLD (40 mg m\(^{-2}\)) could be considered for Phase II trials of longer duration.

The AEs reported were consistent with known events associated with olaparib and PLD when given as monotherapy (Gordon et al., 2001; Fong et al., 2009; Audeh et al., 2010; Fong et al., 2010; Tutt et al., 2010; Gelmon et al., 2011; Kaye et al., 2012; Ledermann et al., 2012), and the combination was generally tolerated up to the highest doses administered. Two DLTs were reported in patients from separate cohorts (continuous 200 mg: intermittent 400 mg); the MTD was not reached, as the protocol did not permit exploration of olaparib doses above continuous 400 mg bid. The maximum dose permitted in this trial was olaparib 400 mg bid because this dose was determined as the MTD in a previous trial (Fong et al., 2009). The PLD 40 mg m\(^{-2}\) dose investigated in this trial is a commonly used single-agent dose in clinical practice (Julius et al., 2013), despite being lower than the FDA-approved dose for patients with breast and ovarian cancer (50 mg m\(^{-2}\)). In addition, this study used the capsule formulation of olaparib, whereas ongoing Phase III studies in ovarian and breast cancer use the tablet formulation of olaparib. It has been shown that exposure with tablet doses $\geq$300 mg bid matched or exceeded that of the 400 mg bid capsule, and olaparib 300 mg bid is the recommended tablet dose for Phase III studies (Mateo et al., 2013).

The tolerability profile observed in this study compares favourably to that seen in studies of PLD monotherapy and in combination with carboplatin. The Phase III CALYPSO study compared PLD (30 mg m\(^{-2}\)) plus carboplatin (AUC5) every 4 weeks with paclitaxel plus carboplatin in 976 patients with platinum-sensitive ovarian cancer. PLD plus carboplatin treatment was associated with severe non-haematological toxicity in 28.4% of patients and Grade 3 – 4 neutropenia in 35.2% of patients. Grade $\geq$2 fatigue, nausea and hand-foot syndrome occurred in 36.9%, 35.2% and 12% of patients, respectively (Pujade-Lauraine et al., 2010). Treatment with single-agent PLD (mainly 50 mg m\(^{-2}\) every 4 weeks) is associated with fewer events of neutropenia, anaemia, thrombocytopenia, and gastrointestinal toxicity, but increased cutaneous toxicity compared with other monotherapies (Gibson et al., 2013). Compared with other second-line regimens seen in the clinic, olaparib and PLD combination therapy was associated with fewer grade $\geq$3 AEs than carboplatin, gemcitabine and bevacizumab as determined in the OCEANS study (Aghajanian et al., 2012), and carboplatin plus paclitaxel regimens (Pignata et al., 2014). In the current trial, three patients experienced serious AEs of pneumonitis, resulting in death in two patients. The three events occurred in different patient cohorts (100 mg 28-day cohort, 200 mg 28-day cohort and 400 mg 7-day cohort) and were all considered to be related to study treatment by the investigator. Of the two patients who died because of lung toxicities, both had a history of medical conditions that may have contributed to the observed pneumonitis. The third patient had no known risk factors associated with lung toxicity, but developed grade 3 pneumonitis after receiving five cycles of therapy and, following withdrawal of treatment, made a full recovery. Although previous lung conditions may have contributed to both fatal cases of pneumonitis, we cannot exclude the role of olaparib in the observed events.

A previous case of presumed treatment-related pneumonitis leading to treatment discontinuation was seen in a Phase I trial of combination olaparib, gemcitabine and cisplatin (Rajan et al., 2012); however, cases of pneumonia have also been seen in previous trials of PLD (Numico et al., 2002; Berenson et al., 2012). The combination of olaparib with platinum-based chemotherapies has previously been associated with increased myelosuppression;

### DISCUSSION

**Table 2. Summary of common treatment-related AEs* and CTC grade $\geq$3 AEs**

| Adverse event                  | Olapar dose cohort |
|-------------------------------|-------------------|
|                               | 50 mg bid 7 day   | 100 mg bid 7 day | 100 mg bid 28 day | 200 mg bid 7 day | 200 mg bid 28 day | 400 mg bid 7 day | 400 mg bid 28 day | Total n=44, n (%) |
|                               | (n=3)             | (n=3)            | (n=4)             | (n=3)            | (n=7)            | (n=12)           | (n=12)           |                 |
| Stomatitis                    | 3\(^a\)           | 3                | 2                 | 2                | 5                | 6                | 11               | 32 (73)         |
|                               | –                 | 2\(^e\)          | –                 | –                | 1                | 2                | 2                | 7 (16)          |
| Nausea                        | 3                 | 2                | 2                 | 3                | 8                | 8                | 28 (64)          |
|                               | –                 | 1                | –                 | –                | 4                | –                | 5 (11)           |
| Asthenia                      | 2                 | 1                | 2                 | 2                | 6                | 6                | 21 (48)          |
|                               | –                 | –                | –                 | –                | –                | 1                | –                | 2 (5)           |
| Anorexia                      | –                 | –                | –                 | –                | 3                | 4                | 4                | 12 (27)         |
|                               | –                 | –                | –                 | –                | 1                | –                | –                | 2 (5)           |
| Vomiting                      | 3\(^b\)           | 3                | 2                 | 1                | 6                | 3                | 3                | 13 (30)         |
|                               | –                 | 3                | –                 | –                | 1                | –                | 1                | 2 (5)           |
| Decreased neutrophil count    | 2                 | 3                | 2                 | 1                | 1                | 2                | 2                | 13 (30)         |
|                               | –                 | 3                | 1                 | –                | 1                | 1                | 1                | 9 (20)          |
| PPES                          | –                 | –                | –                 | –                | 1                | 2                | 4                | 4                |
|                               | –                 | –                | –                 | –                | –                | 1                | 1                | 11 (25)         |

Abbreviations: AEs = adverse events; CTC = Common Terminology Criteria; PPES = palmar-planter erythrodysesthesia syndrome.

\(^a\)AEs experienced by $\geq$25% patients overall.

\(^b\)Values in bold denote the number of patients (n, %) with AEs.

\(^c\)Values in non bold denote the number of patients (n, %) with grade $\geq$3 AEs.
in a Phase I study, 5 out of 23 (22%) patients with ASTs receiving olaparib plus cisplatin and gemcitabine experienced haematological DLTs (Giaccone et al., 2010). Although, in the present study, 30% of patients experienced alterations in neutrophil count, the events appeared not to be dose related, there were no neutropenia-associated DLTs and the overall tolerability profile of olaparib plus PLD appeared more favourable than that observed in most previous olaparib combination studies (Giaccone et al., 2010; Khan et al., 2011; Balmaña et al., 2012; Samol et al., 2012). A Phase I study of olaparib plus weekly paclitaxel showed higher-than-expected rates of neutropenia despite prophylactic administration of granulocyte colony-stimulating factor (Dent et al., 2013).

PK interference between olaparib and PLD was minor and unlikely to have clinical relevance. \( C_{\text{max}} \) and \( AUC_{0-10\text{h}} \) of olaparib in the presence of PLD increased with increasing doses, suggesting lack of acute interference on the absorption and distribution of olaparib (Supplementary Figure 2). A trend towards increased olaparib \( AUC_{0-10\text{h}} \) and \( C_{\text{max}} \) on day 2 was observed and was statistically significant with the 400 mg dose; this is probably the result of drug accumulation between the doses. The PK parameters of PLD were similar, regardless of whether olaparib was administered for 7 or 28 days (Table 3). Differences in \( AUC_{0-\text{inf}} \) of PLD were similar, regardless of whether olaparib was administered for 7 or 28 days (Table 3). Differences in \( AUC_{0-\text{inf}} \) of PLD were similar, regardless of whether olaparib was administered for 7 or 28 days (Table 3).

### Table 3. Pharmacokinetic parameters of olaparib alone (day 1), olaparib in the presence of PLD (day 2), and PLD 1-h infusion by olaparib administration schedule (mean ± s.d.)

| Olaparib (mg bid) | n | \( AUC_{0-10\text{h}} \) (\( \mu g \times h \\text{ml}^{-1} \)) | \( C_{\text{max}} \) (\( \mu g \text{ml}^{-1} \)) |
|------------------|---|------------------------------------------------|------------------|
| **50**           |    |                                                |                  |
| Day 1 alone      | 3  | 8.7 ± 5.8                                     | 1.8 ± 1.1        |
| Day 2 + PLD      |    |                                                | 1.4 ± 0.7        |
| **100**          |    |                                                |                  |
| Day 1 alone      | 3  | 6.8 ± 2.7                                      | 1.7 ± 0.8        |
| Day 2 + PLD      |    | 9.4 ± 3.0                                      | 2.2 ± 1.0        |
| **200**          |    |                                                |                  |
| Day 1 alone      | 2  | 29.5 (12.2, 46.9)                              | 5.6 (3.3, 7.9)   |
| Day 2 + PLD      | 3  | 46.2 ± 52.0                                    | 5.2 ± 2.8        |
| **400**          |    |                                                |                  |
| Day 1 alone      | 11 | 25.9 ± 9.0                                     | 5.1 ± 1.7        |
| Day 2 + PLD      |    | 35.2 ± 17.1*                                   | 6.6 ± 2.0*       |

| Olaparib (mg bid) | n | \( AUC_{0-\text{inf}} \) (\( \mu M \times h \)) | \( C_{\text{max}} \) (\( \mu M \)) | \( T_{1/2} \) (h) | \( CL_{TB} \) (l/h) | \( V_{ss} \) (l) |
|------------------|---|---------------------------------------------|-----------------|--------------|----------------|--------|
| **50**           |    |                                              |                 |              |                 |        |
| Q7               | 3  | 732 ± 42                                     | 36.3 ± 0.8      | 77 ± 7       | 17 ± 4         | 1.5 ± 0.1 |
| Q28              | 3  | 623 (574–672)                                | 38.9 ± 16.4     | 83 ± 24      | 15 ± 3         | 1.6 ± 0.6 |
| **100**          |    |                                              |                 |              |                 |        |
| Q7               | 3  | 4685 (4232–5537)                             | 30.0 ± 0.5      | 82 ± 13      | 22 ± 5         | 2.0 ± 0.02 |
| Q28              | 3  | 566 ± 50                                     | 4129            | 82 ± 13      | 22 ± 5         | 2.0 ± 0.02 |
| **200**          |    |                                              |                 |              |                 |        |
| Q7               | 2  | 4885 (4232–5537)                             | 33.9 (34.2–33.6)| 67 (69–65)  | 18 (18–18)     | 1.6 (1.7–1.5) |
| Q28              | 3  | 3846 (3823–3868)                             | 36.2 ± 3.1      | 74 ± 10      | 17 ± 1         | 1.5 ± 0.3  |
| **400**          |    |                                              |                 |              |                 |        |
| Q7               | 11 | 3968 ± 44                                    | 30.6 ± 4.3      | 72 ± 12      | 23 ± 6*        | 2.0 ± 0.3 |
| Q28              | 12 | 4209 ± 928*                                  | 33.5 ± 5.2      | 77 ± 13      | 18 ± 4*        | 1.7 ± 0.4 |

Abbreviations: \( AUC \) = area under the plasma concentration–time curve; \( C_{\text{max}} \) = maximum concentration; \( CL_{TB} \) = total body clearance; PLD = pegylated liposomal doxorubicin; Q7 = 7-day dosing of olaparib; Q28 = 28-day dosing of olaparib; \( T_{1/2} \) = half-life; \( V_{ss} \) = distribution volume at steady state.

*\( P < 0.01 \) by Student’s t-test for paired data.

**\( P = 0.0276 \).**

\( P = 0.0233 \) by Student’s t-test for unpaired data.
randomized trials of single-agent PLD (18–20%) (Gordon et al., 2001; Kaye et al., 2012). As a result, the combination of olaparib (400 mg bid) and PLD (40 mg m–2) may offer an advantage over either agent alone, particularly since both drugs were combined at their full recommended monotherapy dosages. The ORR in patients with ovarian cancer is within the range achieved by other potential second-line regimens seen in the clinic (Monk et al., 2010; Pujade-Lauraine et al., 2010; Aghajanian et al., 2012). Responses were achieved by 25% of platinum-resistant and 71% of platinum-sensitive ovarian patients. Consistent with previous olaparib trials (Fong et al., 2010; Gelmon et al., 2011), the ORR was higher in platinum-sensitive patients with a gBRCA mutation (67%); however, responses were also seen in platinum-sensitive ovarian patients with wild type or unknown gBRCA mutation status (100%). The ORR in platinum-resistant patients with a gBRCA mutation (50%) was in line with that observed in a recent Phase II trial (Gelmon et al., 2011). Consistent with findings by Gelmon et al. (2011), few objective responses were observed in the subgroup of evaluable patients with breast cancer (8%), although only 3 out of 13 were known to have a gBRCA mutation. Although a formal comparison of intermittent and continuous olaparib administration schedules was not performed, antitumour activity was observed with both schedules (7 out of 21 and 7 out of 23 patients, respectively), and both appeared similar in terms of tolerability.

Phosphorylation of γH2AX is associated with cytotoxic agents and has been used widely as a marker of DNA damage (Sedelnikova and Bonner, 2006; Bonner et al., 2008; Fong et al., 2009; Redon et al., 2010). We studied γH2AX in isolated, fixed PBMCs to determine the effects on DNA repair. In contrast to results reported by Fong et al. (2009), downregulation of phospho-γH2AX was observed with both continuous and intermittent olaparib regimens during the first treatment cycle. This effect was independent of olaparib dose and most noticeable in platinum-resistant ovarian patients, who presented with higher baseline levels of this marker. Although the decrease in phospho-γH2AX levels was unexpected, peak levels have previously been shown to occur within 6–7 h of treatment with PARP inhibitors (Fong et al., 2009; Kummar et al., 2011, 2012), whereas our observations were not conducted until days 8, 15 and 28. As the phosphorylation of γH2AX is a dynamic phenomenon, we studied the late phase of this event (Supplementary Figure 4). Our aim was to assess changes in γH2AX phosphorylation during chronic treatment with olaparib plus PLD combination; therefore, we selected time points from day 8 onwards so that olaparib had reached a steady-state plasma concentration. In accordance with the results reported by Fong et al. (2009), which were unavailable when our study was initiated, we cannot exclude the possibility that, in our study, peak levels of γH2AX phosphorylation may have occurred before day 8. Phosphorylation of γH2AX may be a useful marker for future studies provided that samples are collected at early time points (≤6 h post treatment).

In conclusion, our data suggest that continuous olaparib 400 mg bid (capsule formulation) in combination with PLD 40 mg m–2 would be suitable for assessment in Phase II studies in patients with ovarian cancer. However, it should be noted that, following
Figure 1. Analysis of γH2AX phosphorylation in ovarian cancer patients. Both treatment regimens are shown: continuous olaparib dosing, days 1–28 in platinum-resistant (A) and platinum-sensitive (C) patients, and intermittent olaparib dosing, days 2, 8, in platinum-resistant (B) and platinum-sensitive (D) patients. A Wilcoxon signed-rank test was performed ($P = 0.046$; statistical significance was defined as $P < 0.05$). Median values are indicated.

Recent results from a Phase I study, the recommended mono-therapy dose for the olaparib tablet formulation is 300 mg bid (continuous dosing). The encouraging efficacy results seen in ovarian cancer patients were not limited by gBRCA mutation status or sensitivity to platinum therapy, and the tolerability profiles appeared distinct, suggesting that the combination of olaparib with PLD should be explored further.

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CONFLICT OF INTEREST

Advisory/consulting roles have been held by RVM for Amgen, GlaxoSmithKline, Novartis, Merck Sharp & Dohme and Bristol-Myers Squibb; by CS and RC for AstraZeneca; and by LG for GlaxoSmithKline, Novartis, Merck Sharp & Dohme and Bristol-Myers Squibb; by CS and RC for AstraZeneca; and by LG for GlaxoSmithKline, Novartis, Merck Sharp & Dohme and Bristol-Myers Squibb. No potential conflict of interest were disclosed by the other authors.

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Olaparib plus PLD in advanced solid tumours

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