Tumour cell retention of rucaparib, sustained PARP inhibition and efficacy of weekly as well as daily schedules

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Background: Poly(ADP-ribose) polymerase-1 (PARP) inhibitors (PARPi) exploit tumour-specific defects in homologous recombination DNA repair and continuous dosing is most efficacious. Early clinical trial data with rucaparib suggested that it caused sustained PARP inhibition. Here we investigate the mechanism of this durable inhibition and potential exploitation.

Methods: Uptake and retention of rucaparib and persistence of PARP inhibition were determined by radiochemical and immunological assays in human cancer cell lines. The pharmacokinetics and pharmacodynamics of rucaparib were determined in tumour-bearing mice and the efficacy of different schedules of rucaparib was determined in mice bearing homologous recombination DNA repair-defective tumours.

Results: Rucaparib accumulation is carrier mediated (Km = 8.4 ± 1.2 µM, Vmax = 469 ± 22 pmol per 10^6 cells per 10 min), reaching steady-state levels > 10 times higher than the extracellular concentration within 30 min. Rucaparib is retained in cells and inhibits PARP for > 72 h days after a 30-min pulse of 400 nM. In Capan-1 tumour-bearing mice rucaparib accumulated and was retained in the tumours, and PARP was inhibited for 7 days following a single dose of 10 mg kg^-1 i.p. or 150 mg kg^-1 p.o. by 70% and 90%, respectively. Weekly dosing of 150 mg kg^-1 p.o once a week was as effective as 10 mg kg^-1 i.p daily for five days every week for 6 weeks in delaying Capan-1 tumour growth.

Conclusions: Rucaparib accumulates and is retained in tumour cells and inhibits PARP for long periods such that weekly schedules have equivalent anticancer activity to daily dosing in a pre-clinical model, suggesting that clinical evaluation of alternative schedules of rucaparib should be considered.

Poly(ADP-ribose) polymerase-1 (PARP) is activated by DNA breaks and has a crucial role in the recruitment of repair proteins to the breaks (El-Khamisy et al., 2003). Poly(ADP-ribose) polymerase-1 is the most abundant of a family of PARP enzymes, and together with PARP-2 (and to a limited extent PARP-3) is responsible for facilitating DNA repair (De Vos et al., 2012). Poly(ADP-ribose) polymerase-1 inhibitors (PARPi) were first developed to inhibit repair and increase the antitumour activity of DNA-damaging chemotherapy and radiotherapy. Where tested, they inhibit PARP-1 and PARP-2 (Wahlberg et al., 2012). It is well established that PARPi increase the persistence of DNA breaks and the cytotoxicity of ionising radiation, DNA methylating agents and topoisomerase I poisons in cultured cells and the antitumour activity of these agents in xenograft models (Javle and Curtin, 2011). The first clinical trial of a PARPi, AG014699 (now called rucaparib) in cancer patients in 2003 was in combination with temozolomide (Plummer et al., 2008).

However, the discovery that cells and tumour xenografts defective in homologous recombination DNA repair, including BRCA1 and BRCA2 mutations, were selectively killed by PARPi...
The tumour after clearance from the plasma (Calabrese et al, 2005) distributed well to the tumour and was retained in the brain. In the study reported here we show that uptake of rucaparib results in PARP inhibition for several days in the extracellular space. From this value the amount of non-transported contaminating [14C]rucaparib in the extracellular fluid was calculated and, subtracted from the total. For time course of uptake experiments exponentially growing SW620 cells were incubated with 400 nm [14C]rucaparib in medium containing 7.5 μl ml⁻¹ [14C]sucrose/inulin (Amersham International, GE Healthcare, Amersham, UK) and 100 μl layered onto 100 μl silicone oil (9:11 Dow Corning 556 (Sp. Gr. 0.98): Dow Corning 550 (Sp. Gr. 1.068), final Sp. Gr. 1.028; BDS, Poole, UK) overlaying RPMI-1640 plus 0.5 ml silicone tubes at intervals for up to 2 or 1 h in the presence of ouabain (30 or 100 μM), cytochalasin B (0.1 or 1 μM), dipyridamole (10 or 100 μM) or BCH (1 or 2 μM), or in transport buffer (130 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 5 mM Na₂HPO₄, 10 mM glucose and 10 mM HEPES, pH 7.4) where the NaCl was replaced with choline chloride. The tubes were immediately centrifuged at 12 000 g for 2 min at room temperature causing the cells to pass through the oil and into the KOH. After centrifugation, the tubes were capped and cut in the oil layer such that the bottom portion (cells solubilised in KOH) fell into a 20–ml scintillation vial. To disperse and neutralise the KOH 1 ml of 0.25 M acetic acid was injected into the tube and, following the addition of 10 ml of Optiphase HiSafe scintillant (Fischer Chemicals, Loughborough, UK), the radioactivity was determined by a dual-label assay using an LKB-Wallac 1410 β-counter (Wallac, Croydon, UK). Cell volume was calculated in a similar manner using [3H]sucrose/inulin. To determine the kinetics of uptake, SW620 cells were exposed to increasing concentrations of [14C]rucaparib (0–80 μM) plus [3H]sucrose for 30 min and the intracellular concentration was determined as above. To measure the retention of drug, cells were incubated with [14C]rucaparib for 30 min then washed and transferred to fresh medium, and the intracellular [14C]rucaparib concentration was determined as above. To determine the retention over extended periods, cells (5 × 10⁶ cells for incubations of up to 24 h and 2 × 10⁶ cells for longer incubations) were seeded into six-well plates and allowed to attach overnight. They were then exposed to 400 nm [14C]rucaparib + 7.5 μl ml⁻¹ [3H]sucrose/inulin for 30 min prior washing three times with PBS and solubilising immediately with 0.2 M KOH or incubated with fresh medium for up to 72 h before solubilising with 0.2 M KOH. Samples were neutralised with acetic acid, mixed with scintillant and counted as above. In parallel with these studies, SW620 cells were exposed to 400 nm rucaparib for 30 min then either immediately washed three times with PBS, harvested by trypsinisation, resuspended in 1 ml medium + 10% DMSO and frozen at −80°C, or washed and re-incubated with fresh medium for up to 72 h before harvesting and cryopreserving, such that all samples could be assayed for PARP activity together.

**Cytotoxicity assays.** Exponentially growing Capan-1 cells and MX-1 cells were seeded at low density (500–2000 per well
for control and up to 10 000 per well for rucaparib treatment) in six-well dishes. After 18–24 h, they were exposed to increasing concentrations of rucaparib (0–50 μM) for 24 h before replacement with drug-free medium. After 10–14 days, colonies were stained with 0.4% crystal violet, were counted and survival relative to control was calculated.

Establishment of Capan-1 and MX-1 xenografts. All experiments involving mice were reviewed and approved by the relevant institutional animal welfare committee and performed according to the UK Coordinating Committee on Cancer Research Guidelines for the Welfare of Animals in Experimental Neoplasia and UK law. Female CD-1 nude mice aged 10–12 weeks (Charles River laboratories, Wilmington, MA, USA) maintained and handled in isolators under specific pathogen-free conditions, with 5 mice per cage and 20 cages per isolator) were used in all xenograft experiments. 100 exponentially growing Capan-1 cells in 50 μl of phosphate-buffered saline were implanted into one site on the right flank of each mouse. Exponentially growing MX-1 cells (2 x 10⁶ cells per mouse) were implanted in a 1:1 mix of growth factor reduced, basement membrane Matrigel matrix (BD Biosciences, Oxford, UK), and medium.

Rucaparib plasma pharmacokinetics and tissue distribution. Rucaparib was dissolved in sterile deionised water to give a final concentration such that mice would receive the appropriate dose in 250–300 μl by the i.p. route or 375–450 μl by the oral route. A single dose of rucaparib (10 mg kg⁻¹ i.p. or 50, 100 or 150 mg kg⁻¹ p.o.) was administered to CD-1 nude mice bearing established (≥5 x 5 mm) Capan-1 xenografts (18 mice per group), and at 0.5, 4, 24, 48, 72 and 168 h three mice per group were bled by cardiac puncture under general anaesthesia and then killed. The brains and tumours were removed, were snap frozen in liquid nitrogen and were stored at −80 °C before analysis. Plasma was derived from the blood samples using standard methods and was stored at −80 °C. Three untreated control animals were processed equivalently. Similarly, mice bearing MX-1 xenografts were given a single dose of rucaparib i.p. (50 mg kg⁻¹) or orally (150 mg kg⁻¹), and tumours and livers were harvested and snap frozen 24 h later. In a satellite study, murine PBMCs were harvested as previously described (Plummer et al., 2008) in parallel with collection of plasma 30 min, 6 and 24 h after a single dose of rucaparib (10 mg kg⁻¹ i.p.). To obtain sufficient volume for lymphoprepation, blood was pooled from three mice per sample.

Brain, liver and tumour samples were homogenised 1 in 4 (w/w) in isotonic buffer (7 mM HEPES, 26 mM KCl, 0.1 mM dextran, 0.4 mM EGTA, 0.5 mM MgCl₂ and 45 mM sucrose (pH 7.8)) using a PRO Scientific 2000 hand held homogeniser (Pro Scientific, Inc., Monroe, CT, USA). Samples were analysed using a Waters Alliance 2690 (Waters Corporation, Milford, MA, USA) HPLC, coupled with a Waters 996 Photo Diode Array detector and Agilent (Wokingham, UK) 1200 fluorescent detector. Waters Empower 2 software (Waters Corporation) was used to control the instrument. Samples and standards were precipitated with acetonitrile (HPLC grade; Fischer Scientific, Loughborough, UK), the supernatant evaporated to dryness under nitrogen then reconstituted in mobile phase and 50 μl was analysed using a Supelcosil LC-CN 5 μm 25 cm × 4.6 column (Sigma-Aldrich, Supelco, Gillingham, UK) with a Security guard C18 ODS 4 mm × 3 mm precolumn (Phenomenex, Cheshire, UK). A mobile phase of 0.02 M Na formate, pH3.5, in acetonitrile (1:1 v/v) was used with a flow rate of 1 ml min⁻¹. Plasma concentrations of rucaparib and the carboxyl metabolite were determined by reference to standards prepared in human plasma with fluorescence detection (λex 238/λem 470 nm).

RESULTS

Rucaparib accumulation and PARP inhibition in cells. Following exposure of SW620 cells to 400 nm [³¹C]rucaparib in exponentially growing SW620 cells and also in Capan-1 and MX-1 cells after a 30-min pulse exposure to 400 nm rucaparib. Poly(ADP-ribose) polymerase-1 activity was also measured in mouse PBMCs, and brain, liver and tumour homogenates at various times after administration of rucaparib at various doses and routes using a validated assay as previously described (Plummer et al., 2005, Daniel et al., 2010). Tissues were homogenised in 3 volumes of isotonic buffer then further diluted in isotonic buffer (1:5000 final dilution for brain and 1:20 000 final dilution for tumour) immediately before determining the PARP activity. In brief, PARP activity in 500 permeabilised cells per PBMCs or 50 μl of tissue homogenate was maximally stimulated with a double-stranded oligonucleotide in the presence of excess NAD (350 μM) and the amount of ADP-ribose polymer formed quantified by immunoblot using anti-PAR antibody (clone 10H, from Professor Dr Alex Burkle University of Konstanz) by reference to a PAR standard curve (Enzo Life Sciences, Exeter, UK). Data are expressed as pMol PAR per 10⁶ cells by reference to cell counts or pMol per mg protein by reference to protein assay of the homogenate (Pierce assay; Thermo Scientific, Leicestershire, UK).

Rucaparib efficacy study. Mice bearing Capan-1 or MX-1 xenografts were randomly assigned to treatment groups (described in the Results section) once they had palpable tumours (i.e., ≥5 x 5 mm). Mice were weighed and tumour volumes were determined three times per week from two-dimensional caliper measurements and the equation a² x b/2 (where a = width and b = length of the tumour). Tumour data are presented as the dimension-less parameter, relative tumour volume (RTV). For example, RTV1 is the tumour volume on the first day of treatment (day 0), RTV2, 3 and 4 are when the tumours are 2, 3 or 4 times larger than RTV1. Mice bearing tumours that had grown to 10 x 10 mm or >15 mm in any dimension, or showing signs of stress, were humanely killed.

Poly(ADP-ribose) polymerase-1 activity. In parallel with the [³¹C]rucaparib retention studies, the PARP activity was determined following a 30-min exposure to 400 nm rucaparib in exponentially growing SW620 cells and also in Capan-1 and MX-1 cells after a 30-min pulse exposure to 400 nm rucaparib. Poly(ADP-ribose) polymerase-1 activity was also measured in mouse PBMCs, and brain, liver and tumour homogenates at various times after administration of rucaparib at various doses and routes using a validated assay as previously described (Plummer et al., 2005, Daniel et al., 2010). Tissues were homogenised in 3 volumes of isotonic buffer then further diluted in isotonic buffer (1:5000 final dilution for brain and 1:20 000 final dilution for tumour) immediately before determining the PARP activity. In brief, PARP activity in 500 permeabilised cells per PBMCs or 50 μl of tissue homogenate was maximally stimulated with a double-stranded oligonucleotide in the presence of excess NAD (350 μM) and the amount of ADP-ribose polymer formed quantified by immunoblot using anti-PAR antibody (clone 10H, from Professor Dr Alex Burkle University of Konstanz) by reference to a PAR standard curve (Enzo Life Sciences, Exeter, UK). Data are expressed as pMol PAR per 10⁶ cells by reference to cell counts or pMol per mg protein by reference to protein assay of the homogenate (Pierce assay; Thermo Scientific, Leicestershire, UK).
activity at 72 h (Figure 2A). Further studies in Capan-1 and MX-1 cells revealed that PARP inhibition persisted for 72 h after a 30-min pulse with 400 nM rucaparib in these cells too and that PARP activity was still below baseline 1 week after drug removal (Figure 2B). Rucaparib was cytotoxic in these cells with the IC50 being 5 μM in Capan-1 cells and only 100 nM in MX-1 cells (Figure 2C), which is in line with our previous studies of rucaparib in Capan-1 cells (Drew et al, 2011) and previous reports of the differential cytotoxicity of PARPi in these cells (Shen et al, 2013).

Rucaparib pharmacokinetics and tissue distribution in tumour-bearing mice. Mice bearing Capan-1 tumours were given a single administration of rucaparib either by the i.p. route at a dose of 10 mg kg\(^{-1}\), the dose previously shown to be active in repeated dosing schedule (Drew et al, 2011), or orally at 50, 100 or 150 mg kg\(^{-1}\). Parent drug was detectable in the plasma only at 30 min after 10 mg kg\(^{-1}\) i.p and up to 4 h for 50–150 mg kg\(^{-1}\) p.o. in all three mice per group (Figure 3A). At 24 h, one mouse in the 100 mg kg\(^{-1}\) group and at 48 h one mouse in the 150 mg kg\(^{-1}\) group had detectable levels of parent drug. Rucaparib reached higher concentrations in the tumour, such that levels were up to 10 × the plasma concentration at 4 h. The drug was retained in tumour for longer than in plasma, and although levels were much lower than at earlier time points, rucaparib was still detectable in most mice receiving oral rucaparib at 3 days (Figure 3B). Uptake into the brain was ≤10% of plasma concentrations (Figure 3C). A metabolite was detected, identified as 4-(8-fluoro-6-oxo-3,4,5,6-tetrahydro-1H-azepino[5,4,3-cd]indol-2-yl)benzoic acid, that reached higher concentrations than the parent drug in the plasma, but lower in the tumour (Supplementary Figure 2A and B).

Experiments to determine PARP inhibition by this compound showed that when added to permeabilised cells it inhibited PARP activity with an IC50 of ~550 nM; however, pre-incubation of intact cells with the compound did not result in PARP inhibition, suggesting that it does not easily cross the plasma membrane (Supplementary Figure 2C). There was some suggestion of a modest cumulative effect in that PARP activity was suppressed by 71 ± 3% (n = 3) in tumours 24 h after a single p.o. dose of 150 mg kg\(^{-1}\) and by 79 ± 0.6% (n = 3) 24 h after the third weekly dose, but this was not significant (P = 0.0589) (Supplementary Table 1).
Persistence of tumour PARP inhibition. In parallel with the PK study, PARP activity was determined in tumour and brain tissue. Poly(ADP-ribose) polymerase-1 activity in the tumours was reduced to \( \leq 20\% \) of baseline between 30 min and 24 h after all doses and routes of administration (Figure 4A). Poly(ADP-ribose) polymerase-1 activity showed a modest recovery to \( \approx 30\% \) of baseline after 48 h in mice receiving rucaparib at 10 mg kg\(^{-1}\) i.p., but showed no further recovery over the subsequent 4 days. Following oral dosing, there appeared to be no substantial recovery in PARP activity for the entire monitoring period following a single dose and PARP activity in mice receiving both 50 and 150 mg kg\(^{-1}\) was still \( \approx 10\% \) of control at 7 days. As expected from the much lower drug concentrations achieved in the brain, PARP inhibition in brain tissue was more modest. Following the single 10 mg kg\(^{-1}\) i.p. dose PARP was initially inhibited by about 50%, but activity recovered to baseline levels between 24 and 48 h with some indication of a stimulation of PARP activity at 3 days before return to normal at 7 days. Following oral dosage, there was a \( > 60\% \) reduction in PARP activity at 4 h followed by a recovery to \( \approx 70-80\% \) of baseline, but activity was still suppressed at 7 days (Figure 4B). In a satellite PK/PD study, plasma rucaparib and PARP in PBMCs were measured following a single injection of rucaparib (10 mg kg\(^{-1}\) i.p.). Data are mean \( \pm \) s.e.m. from three mice per time point (PBMC triplicates from pooled blood of three mice per time point).

Efficacy of rucaparib on weekly and daily schedules. In view of the marked persistence of PARP inhibition by rucaparib, we investigated different schedules of administration. Mice bearing Capan-1 xenografts were randomised (10 mice per group) when tumours were \( \geq 5 \times 5 \) mm to receive vehicle control; rucaparib at 10 mg kg\(^{-1}\) i.p. daily for 5 days per week for 6 weeks (daily \( \times \) five
to the original clinical schedule, had only marginal effect on tumour growth that was not significant ($P = 0.3720$) (Figure 5 and Supplementary Figure 3A). There was no significant weight loss on any of the schedules (Supplementary Figure 3B).

As the MX-1 cells were around 50 times more sensitive to rucaparib cytotoxicity in vitro, we hypothesised that MX-1 xenografts would respond well to rucaparib administration. Pilot studies suggested that these cells only established well in nude mice if implanted in Matrigel. Fewer mice developed tumours and they were randomised to the following groups (7–8 mice per group) when tumours were $\geq 5 \times 5$ mm: vehicle control; rucaparib at $10 \text{ mg kg}^{-1}$ i.p. daily five times weekly for 6 weeks; rucaparib at $50 \text{ mg kg}^{-1}$ i.p. once weekly for 6 weeks; or rucaparib at $150 \text{ mg kg}^{-1}$ p.o. once weekly for 6 weeks. Despite the Matrigel, tumours grew slowly only doubling in size after 30 days, and tumour growth was not delayed in any of the rucaparib treatment groups (Supplementary Figure 3C). Rucaparib did not cause any significant weight loss in the mice ($\leq 4\%$ weight loss at nadir, Supplementary Figure S3D). Formal PK-PD studies were not conducted with this xenograft, but in a pilot study PARP was inhibited by $>80\%$ 24 h after a single dose of $150 \text{ mg kg}^{-1}$ p.o. and $>55\%$ after $50 \text{ mg kg}^{-1}$ i.p. in the tumours but not in the livers from these mice, indicating a tumour-specific effect (Supplementary Figure 3E). In additional satellite studies, PARP was inhibited by 87–92\% 3 days after $150 \text{ mg kg}^{-1}$ but only 44–74\% 7 days after $150 \text{ mg kg}^{-1}$. Interestingly in this study MX-1 tumours were found to have 3–4 times higher PARP activity than Capan-1 tumours, such that at 7 days after rucaparib the levels in the MX-1 tumours were comparable to those in Capan-1 tumours from untreated mice (Supplementary Figure S3F).

**DISCUSSION**

Rucaparib is a potent PARPi that has been undergoing clinical evaluation since 2003. Evidence from studies of the pharmacodynamic effect of rucaparib in patients suggested that it caused durable PARP inhibition (Plummer et al, 2005). The aim of the current study was to identify if this was the case to explore the mechanisms and to see if this was exploitable using intermittent dosing regimens. We found that rucaparib uptake into cells displays saturable, classic Michaelis–Menten kinetics, indicating that it is carrier mediated. It is possible that uptake is by a carrier that would normally transport nicotinamide, as rucaparib, like most PARPi, contains a nicotinamide pharmacophore. Transport of nicotinamide and its metabolites is poorly defined; early studies suggested that uptake of nicotinamide was via the glucose transporter (Sofue et al, 1992), but more recent data suggest that nicotinamide riboside is the extracellular NAD$^+$ precursor and that this enters via the nucleoside transporter (Nikiforov et al, 2011). Our data indicate that neither the glucose nor nucleoside transporters were responsible for the uptake of rucaparib. The amino-acid carrier was also excluded and uptake appeared to be sodium independent. Rucaparib accumulated in cells against a concentration gradient, suggesting either that uptake was energy driven or once inside the cell rucaparib was rapidly sequestered or bound to cellular protein. The accumulation of rucaparib into PARP-1 null MEFs was similar to that of their wild-type counterparts (data not shown); thus, it seems unlikely that accumulation is driven by binding to PARP-1. Associated with the cellular accumulation and retention, we found that PARP activity was inhibited by $\geq 70\%$ in all three cell lines for at least 72 h after a 30-min pulse and, where measured, PARP activity was still $\geq 30\%$ below baseline 1 week after initial exposure. We do not believe this is analogous to trapping of PARP-1 on DNA (Satoh and Lindahl, 1992; Murai et al, 2012) because we were able to measure PARP

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**Table 1. Response and survival in Capan-1 tumour-bearing mice treated with rucaparib**

| Treatment                  | Complete regressions | Mice remaining at day 40 |
|----------------------------|----------------------|-------------------------|
| Vehicle control            | 0/10                 | 3*                     |
| 10 mg kg$^{-1}$ i.p., d x 5 w x 6 | 1/10                 | 6                      |
| 50 mg kg$^{-1}$ p.o., d x 5 w x 6 | 0/10                 | 2                      |
| 150 mg kg$^{-1}$ p.o., d x 5 w x 6 | 1/10                 | 5                      |
| 150 mg kg$^{-1}$ p.o., 1 x w x 6 | 3/10                 | 7                      |
| 150 mg kg$^{-1}$ p.o., 3 x w x 6 | 1/10                 | 5                      |
| 150 mg kg$^{-1}$ p.o., 5x every 3w | 1/10                 | 5                      |

Abbreviations: d = daily; w = weekly; x = times

*Mice killed due to tumour burden as described in Materials and Methods.

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weekly x six), as this was a schedule previously shown to be effective (Drew et al, 2011); 50 or 150 mg kg$^{-1}$ p.o. daily x five weekly x six, as a direct comparison with the i.p. schedule; 150 mg kg$^{-1}$ p.o. once per week for 6 weeks or three times per week for 6 weeks to investigate intermittent schedules, or 150 mg kg$^{-1}$ p.o. daily for five days every 3 weeks to mimic the schedule originally given to BRCA mutation carrier patients with 150 mg kg$^{-1}$ p.o. once per week for 6 weeks to investigate intermittent dosing regimens. We found that rucaparib uptake into cells displays saturable, classic Michaelis–Menten kinetics, indicating that it is carrier mediated. It is possible that uptake is by a carrier that would normally transport nicotinamide, as rucaparib, like most PARPi, contains a nicotinamide pharmacophore. Transport of nicotinamide and its metabolites is poorly defined; early studies suggested that uptake of nicotinamide was via the glucose transporter (Sofue et al, 1992), but more recent data suggest that nicotinamide riboside is the extracellular NAD$^+$ precursor and that this enters via the nucleoside transporter (Nikiforov et al, 2011). Our data indicate that neither the glucose nor nucleoside transporters were responsible for the uptake of rucaparib. The amino-acid carrier was also excluded and uptake appeared to be sodium independent. Rucaparib accumulated in cells against a concentration gradient, suggesting either that uptake was energy driven or once inside the cell rucaparib was rapidly sequestered or bound to cellular protein. The accumulation of rucaparib into PARP-1 null MEFs was similar to that of their wild-type counterparts (data not shown); thus, it seems unlikely that accumulation is driven by binding to PARP-1. Associated with the cellular accumulation and retention, we found that PARP activity was inhibited by $\geq 70\%$ in all three cell lines for at least 72 h after a 30-min pulse and, where measured, PARP activity was still $\geq 30\%$ below baseline 1 week after initial exposure. We do not believe this is analogous to trapping of PARP-1 on DNA (Satoh and Lindahl, 1992; Murai et al, 2012) because we were able to measure PARP

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inhibition in PBMCs from patients receiving rucaparib even after they had been subjected to lymphopreparation and cryopreservation prior to ex vivo stimulation of PARP activity by an oligonucleotide mimicking DNA breaks in the presence of 350 μM NAD^+. Together, these observations and the data presented here, coupled with the Ki (1.4 nM) for rucaparib being <1/50th of the Km for NAD^+ (estimated range from 75 to 200 μM), which suggest avid binding of rucaparib to the enzyme.

Rucaparib was also retained in tumour xenografts after it had been cleared from the plasma, similar to our previous observations with the related inhibitor, AG14361 (Calabrese et al., 2004). Most remarkably, PARP activity was inhibited in tumour xenografts for 1 week after a single injection. There is some suggestion that this might be a tumour-specific effect, as PARP activity in the liver appeared to have recovered completely within 24 h and there was a >35% recovery in PBMC PARP activity at this time. Haematological toxicities have been reported clinically with PARPi, particularly when administered with cytotoxic drugs (Do and Chen, 2013). Further studies are warranted to determine whether careful scheduling may allow the cytotoxic to be administered when PARP activity has recovered in normal tissues but remains inhibited in the tumour, thereby increasing the therapeutic window. As with the in vitro uptake studies, the mechanism by which rucaparib is taken up into and retained within the tumour remains to be elucidated. There was some suggestion of a cumulative effect in the tumours with repeated dosing, but further studies following repeated daily or weekly dosing would be required to confirm whether this effect was likely to be of clinical relevance.

The oral bioavailability of rucaparib was similar to that in humans (Shapiro et al., 2013) but with a more rapid elimination from plasma. The measured concentrations varied widely between animals. However, there was apparent dose linearity, consistent with clinical data. The carboxylic acid metabolite, although it has been cleared from the plasma, similar to our previous observations (Shen et al., 2013). Further studies following repeated daily or weekly dosing would be required to confirm this effect was likely to be of clinical relevance.

The in vivo efficacy study in mice bearing Capan-1 xenografts confirmed our previous finding that 0 mg kg^−1 once a day for 5 days per week for 6 weeks significantly inhibited tumour growth with both complete and partial regressions. For the first time, we show that a weekly schedule of a PARPi has significant antitumour activity. A single administration of rucaparib once weekly was at least as effective as daily administration, with three complete regressions on this schedule, consistent with the prolonged PARP inhibition after a single dose. The use of tumour xenografts does, of course, have its limitations, and Capan-1 subcutaneous xenografts do not model closely to the pathology of pancreatic tumours in humans, where there is a large stromal component. Nevertheless, the first clinical trials of single-agent PARPi were initiated following promising xenograft studies with mutant or genetically engineered murine cells, and have been sufficiently successful to result in pre-registration trials being conducted with four different PARPi. Disappointingly, there was no antitumour activity of rucaparib in the MX-1 xenografts, this may have been because of the slow growth of the tumours. Other studies using xenotransplantation report much faster growth of MX-1 xenografts (Shen et al., 2013). Whether the method of implantation of the tumour or insufficient potency of rucaparib is responsible for the lack of effect has not been determined. It is worth noting that we found that MX-1 tumours had very high PARP activity and 7 days after receiving 150 mg kg^−1 dose it was only suppressed by ~75% compared with 90% inhibition in the Capan-1 cells. Thus, 7 days after receiving rucaparib, the PARP activity in MX-1 tumours was equivalent to untreated Capan-1 tumours and presumably sufficient to sustain repair of endogenous DNA breaks. However, the continuous schedule of rucaparib was no more effective than the weekly dosing, indicating that it was not because of inappropriate scheduling. To date, the only report of regressions of MX-1 xenografts are following once or twice daily administration of BMN 673, and in this study olaparib at 100 mg kg^−1 daily for 28 days was completely ineffective (Shen et al., 2013).

In conclusion the remarkable persistence of PARP inhibition by rucaparib in cell line and xenograft models, shown here for the first time, indicate that where the tumour is sensitive to rucaparib and PARP activity can be maintained below a certain threshold this results in equivalent efficacy of weekly vs daily schedules. These pre-clinical data suggest that clinical trials to evaluate alternative schedules of rucaparib, with appropriate pharmacodynamic monitoring, are warranted.

ACKNOWLEDGEMENTS

We gratefully acknowledge the support from CR UK (grant number C240/A7409 ) (to JM, SK, CJ and HT) and research funding from Pfizer GRD (awarded to NJC) for this work.

CONFLICT OF INTEREST

NJC is in receipt of research funding from Pfizer, GL and ZH are former employees of Pfizer. The remaining authors declare no conflict of interest.

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Supplementary Information accompanies this paper on British Journal of Cancer website (http://www.nature.com/bjc)