PARP INHIBITORS AS P-GLYOPROTEIN SUBSTRATES

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Abstract

The cytotoxicity of PARP inhibitors olaparib, veliparib and CEP-8983 were investigated in two P-glycoprotein overexpressing drug-resistant cell models (IGROVCDDP and KB-8-5-11). IGROVCDDP and KB-8-5-11 were both resistant to olaparib and resistance was reversible with the P-glycoprotein inhibitors elacridar, zosuquidar and valspodar. In contrast, the P-glycoprotein overexpressing models were not resistant to veliparib or CEP-8983. Olaparib and veliparib did not induce protein expression of P-glycoprotein in IGROVCDDP or KB-8-5-11 at doses which successfully inhibit PARP. Olaparib therefore appears to be a P-glycoprotein substrate. Veliparib and CEP-8983 do not appear to be substrates. Veliparib and CEP-8983 may therefore be more useful in combined chemotherapy regimens with P-glycoprotein substrates and may be active in platinum and taxane-resistant ovarian cancer.

Introduction

Parp inhibitors are a new class of chemotherapy agents which target the cell's DNA damage repair pathways. PARP inhibitors are potentially very useful for treating BRCA1/2-dysfunctional cancers, as in these cancers the DNA repair machinery is already impaired. The results of proof of concept clinical trials of the PARP inhibitor olaparib in breast and ovarian cancer patients with germline BRCA1/2 mutations have been encouraging (1, 2).

For any new chemotherapy agents it is important to establish if they are substrates of the classical ABC transporters, such as P-glycoprotein (P-gp). Agents that are not P-gp substrates may be more useful clinically, as if transporter driven drug resistance develops the cells are unlikely to be resistant to the wide range of chemotherapy drugs that are also P-gp substrates. P-gp mRNA has been detected in primary ovarian tumours (3), and its expression has been associated with poor overall survival (3). Between 16-25% of primary ovarian tumours are highly positive for P-gp by immunohistochemistry (IHC) (4-6). There is limited clinical data to support the induction of P-gp in the clinic, unlike in cancer cell lines treated with chemotherapy. Despite this, some studies have shown P-gp staining to increase in ovarian tumors after chemotherapy (6). P-gp has been shown to be an independent prognostic factor in some ovarian cancer studies (4) but not in others (5, 6). Similarly, between 44-66% (7, 8) of breast cancers stain positive for P-gp by IHC, some studies found it to be an independent prognostic factor (7) and others did not (8). Induction of P-gp in response to doxorubicin and epirubicin treatment was found to be predictive of survival in one breast cancer study (9). The role of P-gp in BRCA1 mutated clinical breast or ovarian cancer has not been studied in detail. However, a study which examined the gene expression profiles of BRCA1/2 tumours (n=34) vs sporadic ovarian cancer (n=27) in an Ashkenazi Jewish population did not find P-gp to be significantly differentially expressed (10).

There is currently limited data on the P-gp substrate status of PARP inhibitors. Olaparib has been shown to induce P-gp gene expression in an animal tumor model (11). Veliparib has been described as a weak P-gp substrate in a study using a P-gp transfected cell line (12). In contrast, the novel PARP inhibitor CEP-8983 has not been examined for its P-gp substrate status. There has also been no work to date examining PARP inhibitors using cell models of acquired drug resistance overexpressing P-gp. This study will examine the PARP inhibitors olaparib, veliparib and CEP-8983 in two cell models of acquired drug resistance where the major mechanism of drug resistance is overexpression of P-gp:- IGROVCDDP ovarian cells (13) and KB-8-5-11 cervical cells (14, 15).

Materials and Methods

2.1 Cell Culture and cytotoxicity assays

IGROV-1 and IGROVCDDP ovarian cancer cells (16, 17) were obtained from Prof. Jan Schellens (Netherlands Cancer Institute) and grown as previously described (13). KB-3-1 and KB-8-5-11 cervical cancer cells (14, 15) were obtained from Prof. Michael Gottesman (National Cancer Institute) and grown in DMEM (Sigma), 1% Pen strep, 2% L-glutamine and 1% Na Pyruvate with 10% FCS (Lonza). KB-8-5-11 cells were routinely grown in with colchicine; the drug was removed 3 days before the start of all experiments. All cell lines were maintained in a humidified atmosphere with 5% CO_2 at 37°C. All cultures were tested routinely and were mycoplasma-free. All cell lines were STR fingerprinted to confirm identity.

PARP inhibitors olaparib and veliparib and zosuquidar were obtained from Selleck chemicals. CEP-8983 was obtained from Cephalon Inc. Elacridar was obtained from Santa Cruz Biotechnology. Valspodar was obtained from Sigma. To determine the cytotoxicity of the chemotherapy drugs, cells were plated into flat-bottomed, 96-well plates at a cell density of 2 x 10^4 cells/well and allowed to attach overnight. Twenty-four hours later wells were treated in triplicate with serial dilutions of drug in a final volume of 200 μL. The concentration ranges of chemotherapy drugs and P-gp inhibitors used for the cytotoxicity assays used on each cell line is given in Table S1. Drug-free controls were included in each assay. Plates were incubated for a further 5 days at 37°C in a humidified atmosphere with 5% CO_2 and cell viability was determined using an acid phosphatase assay for IGROV-1, IGROVCDDP and an MTT assay for KB-3-1 and KB-8-5-11 (18). The MTT assay was used for KB-3-1 and KB-8-5-11 as these cell lines have a low-level of acid phosphatase yielding a low absorbance with confluent cells. Similarly, the acid phosphatase assay was used for IGROV-1 and IGROVCDDP as low absorbances were obtained on confluent cells with the MTT assay.

2.2 Western blots

The western blots were performed as previously described (13). Primary and secondary antibodies used are listed in Table S2.

2.3 Taqman Low Density Arrays (TLDA)

The TLDAs were performed as previously described (13).

2.4 Statistical Analysis

All experiments were performed at minimum in biological triplicate. Two-sample, two-tailed student's t-tests were used to determine significant differences using $p \leq 0.05$ as a cut off.

Results

IGROVCDDP and KB-8-5-11 are resistant to known P-gp substrates

IGROVCDDP and KB-8-5-11 cells were resistant to known P-gp substrates doxorubicin and vincristine (Tables 1 and 2) (19). The resistance to these agents was reversed in both cell lines by treatment with P-gp inhibitors elacridar (20), zosuquidar and valspodar (21) ($p < 0.05$). The dose of 0.25μM elacridar has been previously shown to prevent P-gp transport activity in IGROVCDDP (13) and KB-8-5-11 cells (22) and has a minimal growth inhibitory effect. The doses of zosuquidar (1.5 μM) and valspodar (0.25 μM IGROV-1 and IGROVCDDP; 31.25 nM KB-3-1 and KB-8-5-11) were optimised to have a minimal growth inhibitory effect on the cell lines while reversing the known P-gp substrate doxorubicin. Zosuquidar used at 1 – 3 μM has been previously shown in the literature to specifically reverse P-gp transport activity in a variety of cell models (23, 24). Similarly, valspodar has been shown to reverse P-gp activity in the dose range of 2 nM – 4 μM. (25-27).

Olaparib appears to be a P-glycoprotein substrate

IGROVCDDP cells were more resistant to olaparib than parental IGROV-1 cells (8.96-fold resistant, $p = 6.88 \times 10^{-9}$, Figure 1A, Table 1). Elacridar, zosuquidar and valspodar all partially reversed the olaparib resistance in IGROVCDDP (3.99-fold, 2.43-fold and 5.56 fold respectively, Figure 1A, Table 1). This reversal of resistance in IGROVCDDP in response to the inhibitors were all significant ($p = 2.4 \times 10^{-6}$, $p = 7.81 \times 10^{-6}$, $p = 2.14 \times 10^{-5}$ respectively).

KB-8-5-11 cells were more resistant to olaparib than parental KB-3-1 cells (2.59-fold resistant, $p = 1.38 \times 10^{4}$, Figure 1B). Elacridar, zosuquidar and valspodar all completely reversed the olaparib resistance in KB-8-5-11 (0.33-fold, 0.61-fold and 0.28 fold respectively, Figure 1B, Table 2). This reversal of resistance in KB-8-5-11 in response to the inhibitors were all significant ($p = 4.73 \times 10^{-5}$, $p = 1.14 \times 10^{-3}$, $p = 9.08 \times 10^{-9}$ respectively).

Veliparib and CEP-8983 do not appear to be P-glycoprotein substrates

IGROVCDDP and KB-8-5-11 were not resistant to veliparib (Tables 1 and 2). In general, treatment with the P-gp inhibitors had a mild sensitising effect on the cell lines. IGROVCDDP became resistant to veliparib at a very low-level (1.1 – 1.23 fold) when treated in combination with zosuquidar or valspodar. While statistically significant, this low-level resistance is the product of a drop in IC_{50} of the parental IGROV-1 cell line, rather than a gain of resistance by the resistant cell line.

IGROVCDDP was not resistant to CEP-8983 (Table 1). KB-8-5-11 were significantly resistant to CEP-8983 but at a very low-level (1.31 fold, $p = 0.031$, Table 2). This 1.31-fold resistance to CEP-8983 was not reversed in KB-8-5-11 by valspodar treatment. The fold resistance to CEP-8983 was reduced to 0.90 on treatment with zosuquidar. However, this was due to an increase in the IC_{50} of the parental KB-3-1 cells rather than a drop in IC_{50} of KB-8-5-11. The fold resistance to CEP-8983 was reduced to 1.06 on treatment with elacridar.

Treatment with doses of olaparib and veliparib that inhibit PARP does not induce the expression of P-gp

Olaparib and veliparib were chosen for further investigation. IGROV-1, IGROVCDDP, KB-3-1 and KB-8-5-11 cells were treated with their IC_{50} doses of olaparib or veliparib for 72 hours. IGROVCDDP and KB-8-5-11 cells both express significantly more P-gp than their parental cells. IGROVCDDP express 3-fold more P-gp than IGROV-1 ($p = 0.005$, Figures 2A and B). P-gp was not detected in KB-3-1 cells by western blot so calculation of a fold increase in KB-8-5-11 was not possible. IC_{50} doses of olaparib and veliparib did not increase the expression of P-gp in any of the cell lines (Figures 2A and B).

To confirm the inhibition of PARP at this dose and exposure time, a western blot of Poly(ADP-ribose) (PAR) was performed. Formation of PAR by PARP results in the releasing of PARP from damaged DNA (28). Therefore, PAR is a marker to indicate if PARP inhibitors have successfully inhibited PARP's activity. PAR has been used for this purpose in several clinical trials (29, 30). PAR expression was significantly decreased in IGROV-1 and KB-3-1 in response to both olaparib and veliparib. Reductions in the range of 14-29 fold were observed ($p < 1.0 \times 10^{-5}$, Figure 2C and D). Olaparib and veliparib decreased PAR expression in IGROVCDDP and KB-8-5-11 but these changes were only significant in response to veliparib in both cases. This indicates that the doses of parp inhibitors chosen for treatment were successful at inhibiting PARP.

Discussion

Olaparib appears to be a P-gp substrate

IGROVCDDP and KB-8-5-11 are suitable cell models for studying P-gp transport, as they both overexpress P-gp and no other ABC transporters such as MRP1-6 and BCRP (Table S3). P-gp has been previously shown to be functionally active by accumulation assays in both IGROVCDDP and KB-8-5-11 cells (13, 31).

Olaparib was the only examined PARP inhibitor to which IGROVCDDP and KB-8-5-11 were both resistant (8.96 and 2.59 fold respectively). This resistance was also significantly reversed by elacridar, zosuquidar and valspodar in both cell lines (Figure 1A and B). Very low-level resistance to CEP-8983 was observed in KB-8-5-11 (1.3-fold, $p = 0.031$). However, this resistance was not reversed by zosuquidar or valspodar treatment. Drug resistance when it occurs in the clinical treatment of cancer is typically in the range of 2-12 fold (32-38). Therefore, we are regarding the statistically significant 1.3 fold resistance to CEP-8983 in KB-8-5-11 as below the level of biological significance. Therefore, olaparib appears to be a P-gp substrate whereas veliparib and CEP-8983 appear not to be substrates (Tables 1 and 2).

Resistance to olaparib has been previously shown to be associated with increased gene expression of P-gp in a mouse tumour model (11). In contrast, we do not see any induction of P-gp protein expression in response to a 72-hour olaparib treatment in any of the cell lines examined (Figure 2A and B). These same doses of drug were shown to decrease PAR, a marker of PARP inhibition (Figure 2C and D). However, it should be noted that it often takes a long-term exposure to a P-gp substrate, such as in drug-resistant cell line development to induce the expression of P-gp. We are currently developing parp-inhibitor resistant cell lines to address this issue. Veliparib was previously found to be a weak substrate for P-gp in transfected cells (12). Our results show that veliparib is not a substrate for P-gp in IGROVCDDP and KB-8-5-11 which are also consistent with these findings (Tables 1 and 2).

The reversal of olaparib resistance by elacridar in IGROVCDDP was only partial compared to that seen in KB-8-5-11 (Figure 1). IGROVCDDP was 8.96-fold resistant to olaparib while KB-8-5-11 was only 2.59-fold resistant. Therefore, complete reversal may have been easier to achieve in KB-8-5-11. The partial reversal in IGROVCDDP may also be due to other non-P-gp mechanisms of drug resistance that cause resistance to olaparib. IGROVCDDP cells are resistant to cisplatin. As platinums and olaparib both affect DNA damage and repair pathways there may be an overlap in the mechanisms of resistance between these agents.

P-glycoprotein has a very broad substrate specificity and is believed to have multiple binding sites. Most of the classic P-gp substrates are natural products that cannot be unambiguously aligned with each other due to a lack of similar orientation points or chemical domains (39). Therefore, the presence or absence of a particular chemical domain cannot predict if a compound is a P-gp substrate. However, several factors relating to the structure of a compound can suggest if it's a P-gp substrate. The chemical structures of olaparib, veliparib and CEP-8983 are given in Figure 3A-C. A molecular weight of >400 is typical of P-gp substrates (39), out of the drugs we investigated Olaparib is the only one exceeding 400 (MW 434.46), Veliparib and CEP-8983 are smaller (MW 244.29 and MW 306.31 respectively). Compounds with a combined number of oxygen and nitrogen atoms ≥8 are often P-gp substrates, and ≤4 non-substrates (39). None of the parp inhibitors we have examined are easily defined by this rule. Olaparib has a combined number of 7 (N_4O_3) and Veliparib and

CEP-8983 both have a combined number of 5 (N_4O_1 and N_2O_3 respectively). However, Olaparib is higher towards the criteria of P-gp substrate and Veliparib and CEP-8983 are lower towards the criteria of non-substrate. This is consistent with our data (Tables 1 and 2).

There are a variety of online in silico tools which can predict the P-gp substrate status of a compound based on its molecular structure. Using the tool developed by Wang et al, doxorubicin is predicted to be a P-gp substrate with a probability of 0.74 (40). Olaparib and Veliparib are both predicted to be substrates with probabilities of 0.87 and 0.77 respectively. CEP-8983 had a 0.55 probability of being a substrate. In contrast, another online tool which makes a binary substrate/non-substrate classification categorised olaparib as a substrate and veliparib as a non-substrate (41). This suggests that these tools are valuable for screening large numbers of compounds, but that there is still value in in vitro conformation of P-gp substrate status.

Clinical implications

P-gp in the intestine may become saturated with rapidly absorbed drugs due to the large concentration of drug present. Olaparib is rapidly absorbed in the intestine with peak-plasma levels occurring 1-3 hours after dosing (42, 43). This suggests that olaparib's P-gp substrate status is not having a significant impact on intestinal absorption. However, veliparib is absorbed faster than olaparib, peak-plasma levels occurring 0.5-1.5 hours after dosing (44). One factor in this faster absorption may be that veliparib is not a P-gp substrate.

P-gp has a greater impact at the individual tissue level where the concentration of xenobiotic is lower compared to the intestine (45). The role of P-gp in clinical drug resistance is controversial, as outlined in the introduction with some studies finding it a prognostic marker (4, 7) and others not (5, 6, 8). As personalised biomarker panels are developed for ovarian and breast cancer treatment, it is potentially relevant to include P-gp, and to use this to guide the choice of parp inhibitor for an individual patient.

The IGROVCDDP cisplatin-resistant ovarian cell line is an unusual model, as it is also cross-resistant to paclitaxel which is mediated by P-gp (13). IGROVCDDP models the resistance phenotype of ovarian cancer patients who have failed standard frontline combination platinum/taxane chemotherapy. IGROVCDDP is not resistant to veliparib or CEP-8983. Therefore, these agents could be useful for the second-line treatment of platinum/taxane resistant ovarian cancer.

The response rates of single-agent olaparib in relapsed platinum/taxane pre-treated ovarian cancer range from 12- 40% (1, 46-49). Response rates are higher in platinum-sensitive BRCA1/2-mutated ovarian cancer patients and range from 41-62% (1, 48, 49). Platinum sensitivity (relapse > 6 months after chemotherapy) is the most consistent predictive factor of response amongst salvage chemotherapy regimens in a pre-treated ovarian cancer (50-52). Therefore, platinum-sensitive patients who are also BRCA1/2 mutation carriers, have the best possible chance of responding to parp inhibitors. Conversely, pre-treated patients who are platinum-resistant (relapse ≤ 6 months after chemotherapy) and BRCA1/2 wild-type patients have a much lower response rate to olaparib, 3.9% (48).

Only one study has been published using veliparib for the treatment of relapsed platinum/taxane pre-treated ovarian cancer, which reported a response rate of 45% (n = 11). However, this study used veliparib in combination with cyclophosphamide and the small

cohort were all BRCA2 mutation carriers which could contribute to the higher response rate (30). It may be that there is a limited difference in response rate between olaparib and veliparib in the clinical treatment of relapsed ovarian cancer, which suggests that the impact of P-gp is limited in this setting. Unfortunately, P-gp was not examined as a marker in any of the olaparib and veliparib clinical studies.

The maximal tolerated doses and the peak-plasma levels of olaparib are higher than veliparib in cancer patients (43, 44). Olaparib is also a more potent drug in vitro, the average IC_{50} in a panel of 17 BRCA1/2 wild-type ovarian cancer cell lines was 4.05 µM, compared to veliparib, average IC_{50} was 44.64µM (53). Olaparib may therefore be a more successful in the clinical treatment of cancer than veliparib by being a more potent drug that has a higher maximal tolerated dose in patients regardless of its P-gp substrate status. However, the combination of agents with differing mechanisms of cytotoxic action is routine in clinical cancer therapy. If a PARP inhibitor is to be combined with another class of agent which is a P-gp substrate, with other factors such as toxicity being equal, then veliparib or CEP-8983 could be superior to olaparib.

Conclusions

Olaparib appears to be a P-glycoprotein substrate. In contrast, veliparib and CEP-8983 do not appear to be substrates. Veliparib and CEP-8983 may therefore be more useful in combined chemotherapy regimens with other P-gp substrates or as salvage chemotherapy after exposure to P-gp substrates. Veliparib and CEP-8983 may be useful in the treatment of platinum and taxane resistant ovarian cancer.

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Figure Legends

Figure 1 – Cytotoxicity of olaparib. A) IGROV-1 and IGROVCDDP and B) KB-3-1 and KB-8-5-11. Open bars indicate parental cell lines, grey bars indicate resistant cell lines. Diagonally striped bars indicate treatment with 0.25µM elacridar. Vertically striped bars indicate treatment with 1.5 µM zosuquidar. Checked bars indicate treatment with 0.25 µM or 31.25 nM valspodar (IGROVCDDP and KB-8-5-11 respectively). Graphs show means and standard deviation of a minimum of n = 3 biological repeats. * Indicates a significant difference of the resistant cell line from the parent cell line $p \leq 0.05$ student's t-test. # Indicates a significant difference on the addition of a P-gp inhibitor; $p \leq 0.05$ student's t-test.

Figure 2 – P-gp and PAR protein expression in response to treatment with olaparib or veliparib. IGROV-1, IGROVCDDP, KB-3-1 and KB-8-5-11 cells were treated for 72 hours with an IC_{50} dose of olaparib or veliparib and compared to a drug-free control. Western blots are shown for A and B) P-gp and C and D) PAR. Representative images of n = 4 biological replicates are shown.

Figure 3 – Molecular structure of parp inhibitors used in the study. A) Olaparib, B) Veliparib and C) CEP-8983.

Table 1 – Resistance profile of IGROVCDDP examining P-glycoprotein substrates

Drug (Units)	**IGROV-1 IC_{50}**		**IGROVCDDP IC_{50}**		**Resistant vs Sensitive**		**IGROV-1 +/- Inhibitor**	**IGROVCDDP +/- Inhibitor**
	Mean ± SD	**n**	**Mean ± SD**	**n**	**Fold**	**P-value**	**P-value**	**P-value**
Known P-glycoprotein Substrates								
Doxorubicin (nM)	21.81 ± 3.73	4	86.04 ± 16.18	4	3.94	**2.45E-04**		
+ Elacridar 0.25 μM	12.80 ±0.77	3	12.97 ± 0.92	4	1.01	0.81	**0.01**	**6.07E-04**
+ Zosuquidar 1.5 μM	12.52 ± 2.20	3	8.00 ± 1.27	4	0.64	**0.02**	**0.01**	**7.24E-05**
+ Valspodar 0.25 μM	13.92 ±2.67	5	13.49 ± 1.18	4	0.97	0.80	**7.71E-03**	**1.09E-04**
Vincristine (nM)	8.30 ±1.50	4	26.76 ± 4.24	4	3.22	**1.76E-04**		
+ Elacridar 0.25 μM	1.69 ± 0.14	4	0.26 ± 0.04	5	0.16	**1.14E-07**	**1.21E-04**	**1.60E-05**
+ Zosuquidar 1.5 μM	1.35 ±0.12	4	0.27 ± 0.04	5	0.20	**2.63E-06**	**9.03E-05**	**1.60E-05**
+ Valspodar 0.25 μM	1.48 ±0.20	4	0.55 ± 0.09	3	0.37	**6.59E-04**	**1.04E-04**	**1.38E-04**
Parp Inhibitors								
Olaparib (μM)	1.25 ± 0.11	7	11.17 ± 1.98	8	8.96	**6.88E-09**		
+ Elacridar 0.25 μM	1.17 ± 0.11	4	4.65 ± 0.49	5	3.99	**2.40E-06**	0.27	**2.30E-04**
+ Zosuquidar 1.5 μM	1.90 ± 0.31	5	4.63 ± 0.52	5	2.43	**7.81E-06**	**1.47E-03**	**6.33E-05**
+ Valspodar 0.25 μM	1.45 ± 0.22	5	8.06 ± 1.66	4	5.56	**2.14E-05**	0.06	**0.02**
Veliparib (μM)	54.23 ± 5.38	7	50.55 ± 8.33	9	0.93	0.328		
+ Elacridar 0.25 μM	45.88 ± 4.14	7	46.19 ± 7.83	10	1.01	0.926	**6.92E-03**	**6.33E-02**
+ Zosuquidar 1.5 μM	44.34 ± 1.60	5	48.77 ± 3.42	8	1.10	**0.021**	**2.78E-03**	0.58
+ Valspodar 0.25 μM	38.38 ± 3.41	4	47.13 ± 3.16	7	1.23	**0.002**	**5.25E-04**	0.32
CEP-8983 (μM)	5.69 ± 0.75	8	5.35 ± 0.75	8	0.94	0.372		
+ Elacridar 0.25 μM	5.97 ± 1.07	9	5.48 ± 0.78	8	0.92	0.306	0.55	0.73
+ Zosuquidar 1.5 μM	5.14 ± 0.81	6	4.09 ± 0.60	4	0.80	0.134	0.21	**0.01**
+ Valspodar 0.25 μM	4.45 ±0.42	5	4.31 ± 0.61	5	0.97	0.692	**0.01**	**0.03**
P-gp Inhibitors								
Elacridar (μM)	3.17 ± 0.12	4	1.62 ± 0.03	4	0.51	**1.97E-06**		
Zosuquidar (μM)	5.81 ± 0.64	4	5.72 ± 1.31	6	0.98	0.90		
Valspodar (μM)	4.15 ± 1.01	4	2.77 ± 0.71	6	0.67	**0.03**		

Table 2 – Resistance profile of KB-8-5-11 examining P-glycoprotein substrates

Drug (Units)	**KB-3-1 IC_{50}**		**KB-8-5-11 IC_{50}**		**Resistant vs Sensitive**		**KB-3-1 +/- Inhibitor**	**KB-8-5-11 +/- Inhibitor**
	Mean ± SD	**n**	**Mean ± SD**	**n**	**Fold**	**P-value**	**P-value**	**P-value**
Known P-glycoprotein Substrates								
Doxorubicin (nM)	2.61 ± 0.19	3	142.72 ± 21.23	4	54.70	**1.01E-04**		
+ Elacridar 0.25 μM	2.53 ± 0.37	5	7.05 ± 7.05	4	2.79	**2.13E-05**	0.75	**1.41E-05**
+ Zosuquidar 1.5 μM	1.69 ± 0.15	3	2.53 ± 2.53	4	1.50	**0.03**	**2.77E-03**	**1.16E-05**
+ Valspodar 31.25 nM	3.03 ± 0.46	3	9.79 ± 9.79	3	3.23	**9.84E-04**	0.22	**1.31E-04**
Vincristine (μM)	3.32E-08 ± 9.71E-09	4	5.61E-02 ± 8.91E-03	6	1.69E-06	**0.04**		
+ Elacridar 0.25 μM	2.40E-09 ± 8.86E-10	3	2.74E-09 ± 1.06E-09	3	1.14	0.69	**3.06E-03**	**1.52E-05**
+ Zosuquidar 1.5 μM	6.65E-11 ± 1.81E-11	3	3.72E-10 ± 1.18E-10	3	5.60	**0.01**	**2.19E-03**	**1.52E-05**
+ Valspodar 31.25 nM	5.29E-10 ± 2.31E-11	3	2.35E-05 ± 2.03E-06	4	4.45E-04	**6.38E-06**	**2.34E-03**	**1.75E-05**
PARP Inhibitors								
Olaparib (μM)	17.98 ± 3.26	5	46.54 ± 6.82	6	2.59	**1.38E-04**		
+ Elacridar 0.25 μM	16.19 ± 1.80	5	5.32 ± 1.08	5	0.33	**4.73E-05**	0.56	**6.33E-05**
+ Zosuquidar 1.5 μM	15.39 ± 2.49	8	9.39 ± 1.12	4	0.61	**1.14E-03**	0.25	**1.05E-04**
+ Valspodar 31.25 nM	16.38 ± 0.92	5	4.59 ± 0.58	5	0.28	**9.08E-09**	0.55	**6.78E-06**
Veliparib (μM)	52.97 ± 4.05	4	51.43 ± 1.03	4	0.97	0.490		
+ Elacridar 0.25 μM	53.06 ± 7.90	6	45.49 ± 4.23	5	0.86	0.088	0.98	**0.03**
+ Zosuquidar 1.5 μM	54.84 ± 9.97	5	44.01 ± 4.91	4	0.80	0.089	0.83	**0.03**
+ Valspodar 31.25 nM	46.31 ± 3.68	4	38.37 ± 5.75	6	0.83	**0.042**	**0.05**	**5.46E-04**
CEP-8983 (μM)	73.86 ± 11.86	4	96.71 ± 11.23	4	1.31	**0.031**		
+ Elacridar 0.25 μM	30.66 ± 2.01	3	32.37 ± 1.09	4	1.06	0.246	**3.69E-04**	**2.02E-04**
+ Zosuquidar 1.5 μM	110.02 ± 23.06	3	99.08 ± 10.73	4	0.90	0.944	**0.02**	0.77
+ Valspodar 31.25 nM	71.82 ± 19.25	4	93.04 ± 14.03	4	1.30	0.125	0.86	0.70
P-gp Inhibitors								
Elacridar (μM)	41.50 ± 1.50	4	37.20 ± 5.54	4	0.90	0.18		
Zosuquidar (μM)	7.36 ± 0.30	3	5.55 ± 0.94	4	0.75	**0.02**		
Valspodar (μM)	4.33 ± 0.43	5	4.69 ± 0.66	3	1.08	0.38		

Table S1 – Concentration ranges of chemotherapy drugs and inhibitors for cytotoxicity assays

Agent	**IGROV-1 and IGROVCDDP**	**KB-3-1 and KB-8-5-11**
Doxorubicin	31.57 pM – 2.07 μM	331.03 fM – 33.10 μM
Vincristine	48.49 fM – 484.87 nM	216.68 fM – 21.67 μM
Olaparib	617.51 pM – 40.48 μM	315.67 nM – 80.99 μM
Veliparib	78.13 nM – 144.06 μM	1.12 μM – 288.11 μM
CEP-8983	156.25 nM – 40 μM	1.5 μM – 384.22 μM
Elacridar	156.25 nM – 40 μM	312.5 nM – 80 μM
Zosuquidar	61.02 nM – 15.62 μM	122.0 nM – 31.25 μM
Valspodar	312.5 nM – 80 μM	312.5 nM – 80 μM

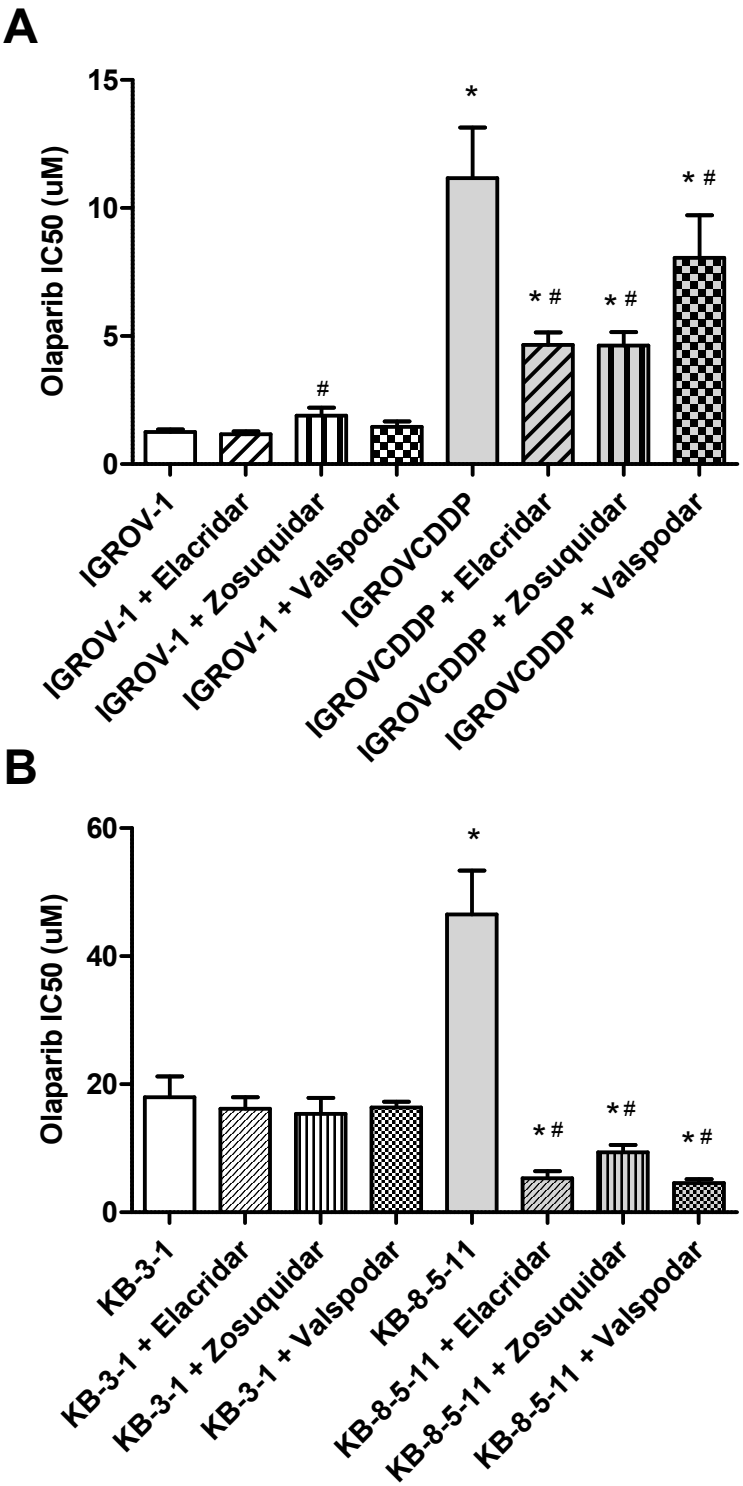
Table S2 – Antibodies for western blotting

Protein	**kDa**	**Host**	**Supplier**	**Catalogue#**	**Dilution Western**
β-Actin	42	Mouse	Sigma	A5441	1:10,000
P-glycoprotein	170	Mouse	Alexis	ALX-801-002-C100	1:250
PAR	116-200	Rabbit	BD Pharmingen	551813	1:1000
Anti-Mouse HRP	N/A	Sheep	Sigma	A6782	1:1000
Anti-Rabbit HRP		Goat	Sigma	A4914	1:1000
Anti-Mouse AP		Rabbit	Sigma	A4312	1:1000

Table S3 – ABC transporter TLDA array results IGROV-1 vs IGROVCDDP and KB-3-1 vs KB-8-5-11

		IGROVCDDP				**KB-8-5-11**			
Gene	**Common Name/s**		**Mean Fold Change**	**SD**	**P-value**		**Mean Fold Change**	**SD**	**P-value**
ABCB1	Pgp, MDR1	↑↑	11.38	0.45		↑↑	8041.65	947.6	
ABCC1	MRP1	↓	-1.43	0.02	0.000	-	1.06	0.07	0.532
ABCC2	MRP2, cMOAT	↓↓	-3.27	0.07	0.001	↑	1.82	0.35	0.013
ABCC3	MRP3, MOAT-D	↓↓	-4.27	0.09	0.001	-	1.68	0.12	0.087
ABCC4	MRP4, MOAT-B	↓↓	-4.30	0.02	0.000	↓	-1.82	0.08	0.009
ABCC5	MRP5, MOAT-C	↓	-1.22	0.02	0.037	-	-1.14	0.03	0.275
ABCC6	MRP6, MOAT-E	-	1.12	0.45	0.718	↓↓	-3.50	0.11	0.021
ABCG2	BCRP	↓↓	-2.17	0.19	0.030	-	-1.10	0.05	0.660

↑↑ Significantly increased expression, greater than 2-fold, ↓↓ significantly decreased expression, greater than 2-fold
↑ Significantly increased expression, less than 2-fold, ↓ significantly decreased expression, less than 2-fold
- no change in expression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KB-3-1
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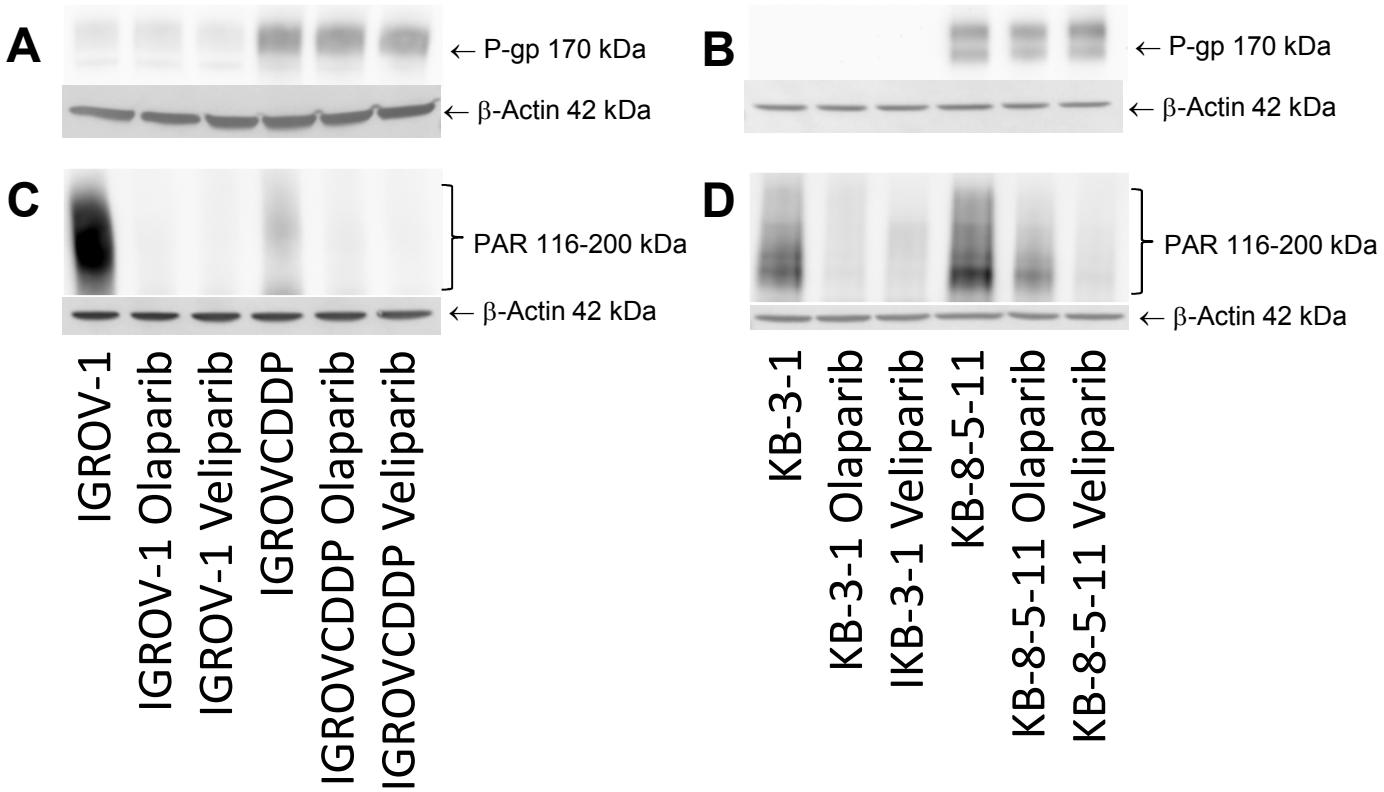
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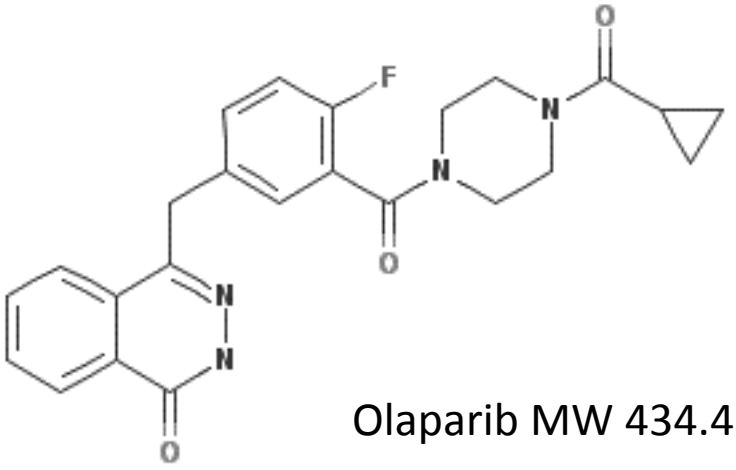

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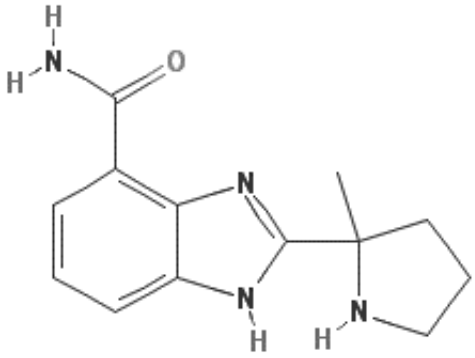

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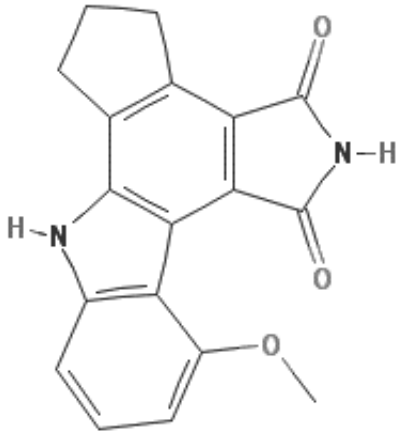

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