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***ABCB1* (MDR1) induction defines a common resistance mechanism in paclitaxel- and olaparib-resistant ovarian cancer cells**

¹Aparajitha Vaidyanathan, ¹Lynne Sawers, ¹Anne-Louise Gannon, ²Probir Chakravarty, ¹Alison L Scott, ³Susan E Bray, ⁴Michelle J Ferguson and ¹Gillian Smith

¹Division of Cancer Research, University of Dundee, Ninewells Hospital & Medical School, Dundee DD1 9SY UK; ²Bioinformatics and Biostatistics Service, Cancer Research UK, 44 Lincolns Inn Fields, London WC2A 3PX UK, ³Tayside Tissue Bank, University of Dundee, Ninewells Hospital & Medical School, Dundee DD1 9SY UK and ⁴NHS Tayside, Ninewells Hospital & Medical School, Dundee DD1 9SY UK

Corresponding Author: Dr Gillian Smith, Division of Cancer Research, University of Dundee, Jacqui Wood Cancer Centre, Ninewells Hospital & Medical School, Dundee DD1 9SY UK (g.smith@dundee.ac.uk)

Running title: *ABCB1* influences taxane and PARP inhibitor resistance in ovarian cancer

Abstract

Background Clinical response to chemotherapy for ovarian cancer is frequently compromised by the development of drug-resistant disease. The underlying molecular mechanisms and implications for prescription of routinely prescribed chemotherapy drugs are poorly understood.

Methods We created novel A2780-derived ovarian cancer cell lines resistant to paclitaxel and olaparib, following continuous incremental drug selection. MTT assays were used to assess chemosensitivity to paclitaxel and olaparib in drug-sensitive and drug-resistant cells \pm the *ABCB1* inhibitors verapamil and elacridar, and cross-resistance to cisplatin, carboplatin, doxorubicin, rucaparib, veliparib and AZD2461. *ABCB1* expression was assessed by qRT-PCR, copy number, Western blot and immunohistochemical analysis and *ABCB1* activity assessed by Vybrant™ and P-glycoprotein-Glo™ assays.

Results Paclitaxel-resistant cells were cross-resistant to olaparib, doxorubicin and rucaparib, but not veliparib or AZD2461. Resistance correlated with increased *ABCB1* expression, and was reversible following treatment with the *ABCB1* inhibitors verapamil and elacridar. Active efflux of paclitaxel, olaparib, doxorubicin and rucaparib was confirmed in drug-resistant cells and in *ABCB1*-expressing bacterial membranes.

Conclusion We describe a common *ABCB1*-mediated mechanism of paclitaxel and olaparib resistance in ovarian cancer cells. Optimal choice of PARP inhibitor may therefore limit the progression of drug resistant disease, while routine prescription of first-line paclitaxel may significantly limit subsequent chemotherapy options in ovarian cancer patients.

Keywords: ovarian cancer, drug resistance, chemotherapy, paclitaxel, olaparib, PARP inhibitor, *ABCB1*, MDR1, P-glycoprotein

Introduction

Ovarian cancer, the most common and most lethal gynaecological malignancy, is commonly initially treated with combination chemotherapy, most frequently combining the platinum drug carboplatin with the taxane paclitaxel ((SIGN), 2013). While a minority of ovarian cancer patients are inherently drug resistant, development of chemotherapy-induced adaptive drug resistance is a common treatment-limiting complication in the majority of patients – understanding the molecular events which lead to the development of drug-resistant disease is therefore a clinical priority (Vaughan *et al*, 2011). Increased understanding of causal molecular mechanisms may facilitate biomarker development to predict and monitor the onset of drug-resistant disease in individual patients, and inform the most rational prescription of alternative second-line chemotherapy, where a variety of drugs are currently being evaluated in clinical trials, including the PARP inhibitors olaparib, veliparib (ABT-888) and rucaparib (Sonnenblick *et al*, 2015).

PARP inhibitors (PARPi) are drugs which primarily inhibit *PARP1* (poly (ADP-ribose) polymerase), an enzyme with a key role in single-strand break DNA repair, and were rationally designed to promote selective toxicity through exploitation of synthetic lethality resulting from targeting alternative or compensatory DNA repair pathways (Bryant *et al*, 2005; Farmer *et al*, 2005). Individuals inheriting relatively rare mutations (5-10% of breast and ovarian cancer patients) in the tumour suppressor genes *BRCA1* or *BRCA2*, proteins which play an essential role in homologous recombination (HR)-mediated repair of DNA

double strand breaks, have increased risk of developing breast and ovarian cancer, but are particularly sensitive to olaparib and similar PARPi drugs (Audeh *et al*, 2010; Tutt *et al*, 2010). Olaparib has recently been licensed for maintenance treatment of ovarian cancer patients following first-line chemotherapy (Oza *et al*, 2015), while increased understanding of the molecular basis of the “BRCA-like” or “BRCAness” phenotype may lead to future extension of current PARPi licensing (Rouleau *et al*, 2010). Before PARP inhibitors can be routinely prescribed, however, it is clearly important that PARPi-induced resistance mechanisms are better understood, and that the potential for treatment-limiting resistance mechanisms common both to PARP inhibitors and conventional chemotherapy is evaluated.

The majority of recent studies on PARPi-induced drug resistance mechanisms have focused on adaptive changes in the DNA damage response, for example describing restoration of HR function following the acquisition of secondary BRCA mutations (Edwards *et al*, 2008; Sakai *et al*, 2008), HSP-90 mediated stabilisation of mutant *BRCA1* (Johnson *et al*, 2013), *RAD51* up-regulation (Martin *et al*, 2007), loss of *REV7* (Xu *et al*, 2015) or promotion of alternative error-prone non-homologous end joining (NHEJ) DNA repair (Patel *et al*, 2011). Similarity in patient chemosensitivity profiles following treatment with platinum drugs and PARP inhibitors and common resistance mechanisms, where *in vitro* selection in one drug promotes cross-resistance to the other have been described (Edwards *et al*, 2008; Sakai *et al*, 2008; Tutt *et al*, 2010), while *BRCA1* revertant mutations have been reported in carboplatin-resistant ovarian cancer patients (Norquist *et al*, 2011; Swisher *et al*, 2008).

In contrast, increased expression of the ATP-dependent efflux pump *ABCB1* (MDR1) which encodes the membrane drug transporter P-glycoprotein, is a well-described resistance mechanism for doxorubicin, paclitaxel and related taxane drugs (Fojo *et al*, 1987; Parekh *et al*, 1997; Shen *et al*, 1986). Increased *ABCB1* copy number resulting from a chromosomal amplification event at 7q11.2-21 has been correlated with increased P-glycoprotein expression in paclitaxel-resistant cells from various cancers including ovarian cancer (Wang *et al*, 2006), with resulting drug-resistant phenotypes confirmed in *ABCB1* over-expression and P-glycoprotein inhibitor studies (Lawlor *et al*, 2014). Intriguingly, recent reports have described increased expression of the homologous murine *Abcb1a* and *Abcb1b* genes in an olaparib resistant mouse breast cancer model (Rottenberg *et al*, 2008) and in mouse and human lymphocytes (Dumitriu *et al*, 2004). A functional role for murine ATP transporters in oral bioavailability and brain accumulation of rucaparib has recently been described (Durmus *et al*, 2015), although the role of *ABCB1* in olaparib resistance in ovarian cancer has not been investigated.

We now describe the creation of a novel olaparib-resistant A2780-derived ovarian cancer cell line (A2780olapR), and highlight an *ABCB1*-mediated resistance mechanism common to paclitaxel, doxorubicin and rucaparib. Our findings are highly clinically relevant, as our data suggests that prescription of specific PARP inhibitors as second-line or maintenance treatment may not be appropriate in the majority of ovarian cancer patients where clinical response is compromised by paclitaxel-induced drug resistance.

Materials and Methods

Cell lines

The ovarian cancer cell line A2780 was obtained from the European Collection of Cell Culture (ECACC, <https://www.phe-culturecollections.org.uk/collections/ecacc.aspx>). Olaparib-resistant (A2780olapR) and paclitaxel-resistant (A2780pacR) cells were created following continuous incremental drug selection. MTT cytotoxicity assays (Mosmann, 1983) were used as described below to assess olaparib and paclitaxel sensitivity in A2780 cells, and to identify initial drug concentrations toxic to approximately 50% of cells (1 μ M olaparib and 3nM paclitaxel, respectively). Drug concentrations were then incrementally increased to final concentrations of 20 μ M (A2780olapR) to mimic typical steady-state plasma concentrations in ovarian cancer patients receiving 400mg olaparib daily by oral administration (Dean *et al*, 2012), and 2 μ M paclitaxel (A2780pacR), the concentration at which A2780 cells became maximally stably resistant to paclitaxel. The additional ovarian cancer cell lines A1847 (Eva *et al*, 1982) and PE-01 (Langdon *et al*, 1988) and derivative cell lines were a generous gift from Professor Roland Wolf, Division of Cancer Research, University of Dundee. All cell lines were maintained in RPMI1640 medium containing 10% fetal bovine serum and 1% penicillin/streptomycin (\pm selection drugs), with additional supplementation of 1% sodium pyruvate and 1% L-glutamine for PE-01 lines, in 37^oC incubators, supplemented with 5% CO₂.

siRNA-mediated *ABCB1* gene knockdown

A2780pacR and A2780olapR cells (2.5×10^5 cells per well) were seeded in six-well plates and incubated for 24 h before transient transfections using Lipofectamine RNAi MAX (Thermo Fisher Scientific, Loughborough, UK) with a final concentration of 25 nM *ABCB1*-specific siRNA (Dharmacon SMARTpool ON-TARGETplus; GE Healthcare Life Sciences, Little Chalfont, UK) or negative control (sterile water) in serum-free medium. The extent of *ABCB1* knockdown was assessed by qRT-PCR analysis 24 h, 48 h and 72 h following transfection, as described below.

MTT chemosensitivity assays

MTT assays (Mosmann, 1983) were used to compare the chemosensitivity of A2780, A2780olapR and A2780pacR cells and the additional cell lines described above to the chemotherapy drugs cisplatin, carboplatin, paclitaxel, doxorubicin, olaparib, AZD2461, veliparib and rucaparib and the *ABCB1* inhibitors verapamil and elacridar. Each cell line was plated in a 96-well plate (5000 cells/well or 3000 cells/well for A1847 and PE-01 cell lines) and treated in triplicate with serial dilutions of each drug, at concentrations selected (where possible) to mimic typical peak plasma levels in ovarian cancer patients (range 0-200% [peak plasma]; cisplatin 0-25 μ M, carboplatin 0-85 μ M, paclitaxel 0-32 μ M, doxorubicin 0-5.5 μ M, olaparib 0-50 μ M, AZD2461 0-20 μ M, veliparib 0-20 μ M and rucaparib 0-20 μ M)(Konecny *et al*, 2000). For *ABCB1* inhibitor studies, cells were initially treated with a dose range from 0-

100µM verapamil or elacridar to identify non-toxic inhibitor concentrations for use in future combination treatment studies, then subsequently combining 5µM and 10µM verapamil or elacridar with serial dilutions of paclitaxel or olaparib. Cells were drug treated for 72 hours, media removed and 100µl of a 0.5mg/ml MTT solution (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide in phenol red free DMEM) added and cells incubated at 37°C for 3 hours. The resulting formazan crystals were solubilised in DMSO, quantitated spectrophotometrically at 570nm and the percentage of viable cells remaining following each drug treatment calculated (assigning a value of 100% to vehicle-treated cells). IC₅₀ values were calculated from log dose response curves using Prism 6 software (GraphPad Software Inc, La Jolla, USA).

RNA and DNA extraction from cell lines

Cells were grown to 80% confluency in 75cm² flasks, harvested by trypsinisation, counted using a haemocytometer, and 1x10⁷ cells used for RNA extraction using RNeasy mini kits (Qiagen, Manchester, UK), following the Manufacturer's protocol for mammalian cells, with additional on column DNase digestion (RNase free DNase kit, Qiagen). RNA yield and integrity were assessed from absorbance readings at 260nm and 280nm using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Loughborough, UK), according to the Manufacturer's guidelines. Similarly, DNA was extracted from 3 x 10⁶ cells using a DNeasy Blood and Tissue kit (Qiagen), following the Manufacturer's protocol. DNA yield was assessed from absorbance readings at 260nm using a Nanodrop 1000 spectrophotometer, as described above.

Quantitative real-time PCR (qRT-PCR) analysis

RNA was reverse transcribed into cDNA (200ng RNA per 50µl RT reaction) using Taqman Reverse Transcription Reagents kits (Life Technologies, Paisley, UK), according to the Manufacturer's instructions, and the expression of *ABCB1* (MDR1) (Taqman probe ID Hs00184500_m1), *ABCC1* (*MRP1*) (Taqman probe ID Hs01561502_m1), PARP1 (Taqman probe ID Hs00242302_m1) and the loading control *18S ribosomal RNA* (Taqman probe ID Hs99999901_s1) assessed by qRT-PCR analysis, as previously described (Smith *et al*, 2012), where 20µl individual reaction mixes (per well) contained 10µl Taqman Universal PCR Master Mix (Life Technologies), 1µl gene-specific Taqman probe, 1µl cDNA, and 8µl sterile water. Each reaction was performed in triplicate and run on the Standard Real Time PCR program on a 7900 Taqman real-time PCR system (Life Technologies) using pre-defined thermal cycling conditions (50°C for 2 minutes, 94.5°C for 10 minutes, and then 40 cycles of 97°C for 30 seconds, 59°C for 1 minute). Analysis of gene expression was performed using SDS 2.3 software (Life Technologies); optimal experimental baselines and thresholds were chosen for each gene, and gene expression in each cell line quantitated by cycle threshold (Ct) values. Relative expression values were determined by comparing the expression of each target gene with the invariant "loading control" *18S ribosomal RNA*, as previously described (Smith *et al*, 2012). All samples were analysed in triplicate and gene expression calculated relative to 18S ribosomal RNA \pm compound error $((SD \text{ target gene})^2 + (SD \text{ 18S ribosomal RNA})^2)^{1/2}$, where SD=standard deviation of the mean of triplicate replicates.

Copy number analysis

ABCB1 (MDR1) copy number was assessed in A2780, A2780pacR and A2780olapR cells using a quantitative Taqman gene copy number assay (Taqman assay ID Hs04962504_cn), where *ABCB1* copy number was compared to the copy number of the endogenous control gene *RNAse P* (Taqman Copy Number Reference Assay) by the comparative Ct method, and relative quantitation values obtained using CopyCaller Software (Life Technologies). As additional controls, *ABCB1* and *RNAse P* copy numbers were assessed in peripheral blood samples (n=2) obtained from healthy volunteers, and copy numbers of 2 confirmed.

Western blotting

Cells for protein extraction were harvested by trypsinisation as described above and protein extracts for *ABCB1* analysis prepared in RIPA buffer (50mM Tris-HCl pH 8, 150mM NaCl, 2mM EDTA, 0.1% SDS, 0.5% sodium deoxycholate, 50mM NaF, 1% Nonidet P-40) supplemented with EDTA-free protease inhibitor (Roche, Burgess Hill, UK) and for *PARP1* analysis in 2X SDS protein sample buffer (100mM Tris-HCl pH 6.8, 4% SDS, 0.04% Bromophenol blue, 20% Glycerol, 200mM DTT) followed by sonication of samples when required. Following 30 minutes incubation on ice with vortexing at regular intervals, lysed cells were centrifuged (14000rpm, 10 minutes, 4°C) to pellet cell debris, and protein concentrations of the resulting cell supernatants determined by Bradford Assay (*ABCB1*) or DC Protein Assay (*PARP1*) (BioRad, Hemel Hempstead, UK), relative to a standard curve

prepared from serial dilutions of bovine serum albumin (0-4mg/ml), with absorbance readings at 595nm or 750nm respectively.

ABCB1 and *PARP1* expression was analysed in protein extracts from each cell line by Western blotting, following SDS-PAGE. Each protein sample (15µg) was diluted in equal volumes of 2 x protein sample buffer (125mM Tris-HCl (pH 6.8), 4% SDS, 0.02% bromophenol blue, 0.2M DTT, 20% glycerol), denatured and separated by 12% SDS-PAGE gels in Tris-glycine buffer (25mM Tris pH 8.3, 250mM glycine, 0.1% SDS). Following electrophoresis, proteins were transferred to nitrocellulose membranes in Tris-glycine-methanol buffer (48mM Tris pH8.3, 39mM glycine, 0.037% SDS, 20% methanol) and non-specific antibody binding blocked by incubation for 2 hours in TBST (25mM Tris-HCl pH 7.6, 150mM NaCl, 0.05% Tween-20) containing 5% milk powder. Membranes were then incubated overnight with a mouse monoclonal *ABCB1* primary antibody (SC-55510, Santa Cruz Biotechnology, Heidelberg, Germany, diluted 1:1000), *PARP1* (#9542, Santa Cruz Biotechnology, diluted 1:1000) or a mouse monoclonal β -*actin* antibody (sc-47778, Santa Cruz Biotechnology, diluted 1:1000), washed in PBST (PBS supplemented with 0.05% Tween-20) and incubated for 1 hour with a goat anti-mouse polyclonal secondary antibody (*ABCB1*; 170-6516, BioRad, diluted 1:1000) or HRP-conjugated goat anti-rabbit polyclonal secondary antibody (*PARP1*; 170-6515; Biorad, diluted 1:3000). Immunoblots were developed using an ECL-chemiluminescence kit (Merck Millipore, Watford, UK), according to the Manufacturer's instructions.

Immunohistochemical analysis

A2780, A2780pacR or A2780olapR cells for immunohistochemical analysis were grown in 10 cm² dishes, harvested by scraping, centrifuged (1000rpm, 5min, RT) and re-suspended in 50µl Normal Pooled Plasma (CCN-10, Alpha Laboratories, Eastleigh, UK). Suspended cells were pelleted by adding 50µl Bovine Thrombin (BTUD293, Diagen Reagents Ltd, Thame, UK) and cell pellets immersed in 10% neutral buffered formalin prior to overnight processing on a Leica Peloris II processor using an 8hr xylene standard protocol. Processed cells were then embedded into paraffin wax. Sections (4 µM) were cut onto Superfrost plus slides (Thermo Scientific) and dried for 1 hr at 60°C. Antigen retrieval and de-paraffinisation was performed using DAKO EnVision™ FLEX Target Retrieval solution (high pH) buffer in a DAKO PT Link (Dako, Ely, UK). Immunostaining using DAKO EnVision™ FLEX system on a DAKO Autostainer Link48 was carried out according to manufacturer's protocol. Sections were incubated with 1/2000 dilution of *ABCB1* primary antibody (SC-55510, Santa Cruz Biotechnology) for 30 mins. DAKO substrate working solution was used as a chromogenic agent for 2 x 5mins and sections were counterstained in EnVision™ FLEX hematoxylin. Negative controls were prepared by replacing the primary antibody with DAKO antibody diluent.

P-glycoprotein activity assays

Assessment of drug efflux activity in A2780, A2780pacR and A2780olapR cells and the influence of the *ABCB1* inhibitor verapamil was performed using the Vybrant™ Multidrug

Resistance Assay Kit (Life Technologies) which estimates drug transporter activity by quantitative assessment of intracellular calcein accumulation. Cells were treated with the non-fluorescent prodrug calcein AM which crosses the cell membrane by passive diffusion and is cleaved in the cytoplasm by endogenous esterases, releasing fluorescent calcein. In *ABCB1*-expressing A2780pacR and A2780olapR cells, calcein AM is effluxed prior to esterase cleavage, resulting in reduced intracellular calcein fluorescence. A2780, A2780pacR and A2780olapR cells were plated in triplicate in cell culture medium in 96-well microplates (5 x 10⁵ cells/well) and incubated with 0.25µM calcein AM ± verapamil (0-25µM) for 15 minutes at 37°C. Microplates were then centrifuged 3 times for 5 minutes at 200 x g, the cell supernatants removed and cells re-suspended in 200µl ice-cold cell medium. Intracellular calcein fluorescence was then measured using a fluorescence microplate reader, with absorption maximum=494nm and emission maximum=517nm. Relative calcein AM efflux in paired drug-sensitive and drug-resistant cell lines, and the influence of individual *ABCB1* inhibitors was assessed by plotting log₁₀dose-response curves of calcein retention (fluorescence of inhibitor treated cells/fluorescence of untreated cells).

Independent assessment of the potential of olaparib to act as either a substrate or inhibitor of P-glycoprotein was performed using the P-glycoprotein-Glo™ Assay System (Promega, Southampton, UK), according to the Manufacturer's guidelines. The P-glycoprotein-Glo™ Assay System uses recombinant P-glycoprotein membranes to assess potential drug interactions based on ATP-dependent generation of a luminescent signal following activation of firefly luciferase, and therefore avoids potential confounding contributions

from additional drug transporters present in cell line analyses. Baseline P-glycoprotein membrane ATPase activity was first compared with the activity of membranes treated with the selective P-glycoprotein inhibitor sodium orthovanadate (final concentration 0.1mM) to quantify any minor non-P-glycoprotein-dependent ATPase activity in the recombinant membrane preparations. Membranes were then incubated with verapamil (final concentration 0.2 μ M; positive control) or with various concentrations of paclitaxel (8 and 16 μ M), olaparib (12.5-50 μ M), veliparib (25 and 50 μ M), rucaparib (25 and 50 μ M), AZD2461 (25 and 50 μ M), and ATPase activity assessed by quantitative assessment of luminescence (increased in the presence of P-glycoprotein substrates and decreased in the presence of inhibitors, relative to the activity of sodium orthovanadate-treated control samples).

Whole-genome microarray mRNA analysis in A2780, A2780pacR and A2780olapR cells

RNA was prepared from A2780, A2780pacR and A2780olapR cells and integrity assessed as described above. Each RNA sample was converted to biotinylated amplified cRNA using an Illumina TotalPrep RNA Amplification kit (Life Technologies) according to the manufacturer's guidelines, and cRNA quality and concentration confirmed on an Agilent Bioanalyzer 2100 (Agilent Technologies, Wokingham, UK). cRNA samples were hybridised in triplicate on Illumina Human HT-12 BeadChip Arrays (Illumina, Little Chesterford, UK) using standard protocols optimised by the Genetics Core, Wellcome Trust Clinical Research Facility, University of Edinburgh.

Bioinformatics analysis

Gene expression data were analysed using Bioconductor 2.7 (<http://bioconductor.org>), running on R 2.12.1. Normalised probeset expression measures were calculated using log₂ transformation and quantile normalisation using the 'Lumi' package. To identify significant differences in gene expression in A2780, A2780pacR and A2780olapR cells, moderated Student's t-tests were performed using empirical Bayes statistics in the 'Limma' package, and resulting P-values adjusted for multiple testing using the false discovery rate (FDR) Benjamini and Hochberg method (Smyth, 2004); probe sets with adjusted P-value FDR $q < 0.05$ were called differentially expressed.

Results

Creation of ovarian cancer cell lines resistant to paclitaxel and olaparib

To investigate common mechanisms of paclitaxel and olaparib resistance we created A2780-derived paclitaxel-resistant (A2780pacR) and olaparib-resistant (A2780olapR) cell lines as described in Materials and Methods, with drug concentrations selected to mimic typical treatment protocols in ovarian cancer patients. A2780pacR cells were more than 7-fold resistant to paclitaxel (7.49-fold increase in $IC_{50}(\text{paclitaxel})$, ($p < 0.0001$; Figure 1A), while A2780olapR cells were 36-fold resistant to olaparib ($p < 0.0001$; Figure 1B). A2780pacR cells were significantly cross-resistant to olaparib (36.95-fold increase in $IC_{50}(\text{olaparib})$, ($p < 0.0001$; Figure 1C), although A2780olapR cells were not cross-resistant to paclitaxel (Figure 1D). Similarly, paclitaxel-resistant A1847TX1 and PE-01TX5 ovarian cancer cell lines were cross-resistant to olaparib (Supplementary Figure 1), supporting our hypothesis that resistance may arise by a common mechanism. Neither A2780pacR nor A2780olapR cells were cross-resistant to either cisplatin or carboplatin (data not shown).

Olaparib and paclitaxel resistance leads to increased expression of *ABCB1*, the “multidrug resistance” drug efflux pump

Increased *ABCB1* expression has previously been reported in multiple paclitaxel-resistant cell lines and has been convincingly shown to influence paclitaxel chemosensitivity. We therefore used qRT-PCR analysis to confirm lack of *ABCB1* mRNA expression in A2780 cells, but abundant expression in both A2780pacR, A2780olapR cells (Figure 2A) and 1847TX1 and

PEO1TX5 cells (Supplementary Figure 1). We then used quantitative gene copy number assays to investigate whether increased mRNA expression could be attributed to amplification of the *ABCB1* gene. Consistent with more abundant *ABCB1* mRNA expression, only A2780pacR cells were found to have amplified *ABCB1*, with 2 gene copies detected in DNA extracted from whole blood samples obtained from healthy volunteers, drug-sensitive A2780 cells and A2780olapR cells, but 4 gene copies detected in A2780pacR cells (Figure 2B). Changes in *ABCB1* gene expression and copy number were reflected in *ABCB1* protein expression, assessed both by Western blotting (Figure 2C) and immunohistochemical analysis (Figure 2D). Although increased P-glycoprotein expression was clearly present in both A2780pacR and A2780olapR cells, expression was highest in A2780pacR cells, consistent with increased *ABCB1* copy number. As increased expression of the alternative multidrug resistance protein *ABCC1* (*MRP1*) has also been associated with paclitaxel resistance in various cancer cell lines (Allen *et al*, 2000) we additionally used qRT-PCR analysis to compare *ABCC1* levels in A2780, A2780pacR and A2780olapR cells, but found no differences in expression (data not shown). To extend our analysis of differential ABC transporter expression, we use whole genome microarray analysis (Agilent HT-12 arrays) to perform an unbiased comparison of gene expression in A2780, A2780pacR and A2780olapR cells, as described in Materials and Methods. *ABCB1* was the only drug transporter with significantly increased expression in resistant cells (Supplementary Table 1); *ABCB1* probe sets were the first and second most abundantly induced probe sets from 12, 590 probe sets significantly altered in A2780pacR cells, and the 4th and 6th most abundantly induced probe sets from 11, 182 probe sets significantly altered in A2780olapR cells. It was also interesting to note that the expression of several ABC transporters (A2780pacR: *ABCA1*, *ABCA2*, *ABCB7*,

ABCB9, ABCC4, ABCC5, ABCC6, ABCC10 and *ABCG4*; A2780olapR: *ABCB6, ABCB9, ABCC4, ABCD3, ABCE1, ABCF2* and *ABCG4*) was significantly decreased in resistant cells.

Paclitaxel and olaparib resistance can be reversed by the *ABCB1* inhibitors verapamil and elacridar

To further test our hypothesis that resistance to both paclitaxel and olaparib is influenced by increased *ABCB1*-mediated drug efflux, we treated A2780pacR and A2780olapR cells with the *ABCB1* inhibitors verapamil and elacridar. Verapamil, a calcium channel-blocker and anti-arrhythmic drug, is a competitive *ABCB1* inhibitor (Cornwell *et al*, 1987; Safa, 1988), while elacridar does not directly compete with *ABCB1* substrates for active site binding but regulates protein function through allosteric inhibition (Hyafil *et al*, 1993). Initial MTT assays were performed with both inhibitors in A2780, A2780pacR and A2780olapR cells to identify non-toxic doses (5 μ M and 10 μ M) for use in subsequent combination treatment experiments (data not shown). Dose-dependent increases in chemosensitivity were subsequently observed following combination treatment, with a complete reversal of paclitaxel resistance in both A2780pacR cells (Figure 3A) and A2780olapR cells (Figure 3B) treated with paclitaxel, following combination treatment with 10 μ M verapamil. Verapamil was a less efficient competitive inhibitor in olaparib-resistant cells, although a >80% re-sensitisation to olaparib was observed in A2780olapR cells (Figure 3C). Consistent with these findings, combination treatment with the more potent *ABCB1* inhibitor elacridar resulted in complete reversal of both paclitaxel and olaparib resistance (Figures 3D-F). Further, re-sensitisation to paclitaxel and olaparib was additionally observed in A2780pacR and

A2780olapR cells where *ABCB1* expression was disrupted following transient siRNA knockdown (Supplementary Figure 2). As *ABCB1* expression is approximately 10-fold higher in A2780pacR than in A2780olapR cells (Panels A and B) as a consequence of a gene duplication event, it is not surprising that siRNA disruption was less effective in A2780pacR than in A2780olapR cells.

Paclitaxel and olaparib are actively effluxed from drug-resistant A2780pacR and A2780olapR cells

To investigate whether paclitaxel and olaparib are actively effluxed from drug-resistant cells, we compared the ability of A2780, A2780pacR and A2780olapR cells to efflux the model substrate CalceinAM. CalceinAM is a prodrug which is cleaved to fluorescent calcein by intracellular esterases; ATP-dependent CalceinAM efflux is increased in the presence of *ABCB1*, thus limiting esterase cleavage and fluorescent calcein production. Consistent with increased *ABCB1* activity, calcein fluorescence was significantly reduced in A2780pacR and A2780olapR cells (6.18-fold $p=0.005$ and 4.49-fold, $p=0.03$; Figures 4A). We then assessed intracellular calcein fluorescence in the presence of increasing concentrations of verapamil. Verapamil treatment did not influence CalceinAM efflux in drug-sensitive A2780 cells which do not express *ABCB1*, but caused a dose-dependent decrease in Calcein AM efflux (assessed by increased intracellular calcein fluorescence) in both A2780pacR and A2780olapR cells (Figure 4B).

To further confirm that both paclitaxel and olaparib are P-glycoprotein substrates, we used luminescent P-glycoprotein-Glo™ assays as described in Materials and Methods, where the influence of paclitaxel and olaparib on recombinant P-glycoprotein activity was evaluated in cell membrane fractions. Similar to verapamil (positive control), the addition of either paclitaxel or olaparib lead to a dose-dependent increase in ATPase activity (indirectly assessed by relative luciferase activity), confirming a direct protein/substrate interaction (Figure 4C).

Paclitaxel and olaparib-resistant cells are cross-resistant to doxorubicin and rucaparib, but not to the alternative PARP inhibitors AZD2461 and veliparib

Liposomal doxorubicin is commonly prescribed as second-line chemotherapy to ovarian cancer patients with relapsed platinum/taxane resistant disease. The first description of *ABCB1*-mediated drug resistance in various cancer cell lines reported both increased *ABCB1* mRNA expression and copy number in adriamycin (also known as doxorubicin)-resistant A2780 cells (Shen *et al*, 1986), while numerous subsequent studies have confirmed that doxorubicin is a P-glycoprotein substrate. We therefore predicted that paclitaxel and olaparib-resistant cells may also be cross-resistant to doxorubicin – as expected, both A2780pacR (Figure 5A) and, to a lesser extent, A2780olapR (Figure 5B) cells were significantly more resistant to doxorubicin than A2780 cells. Similar paclitaxel/doxorubicin cross-resistance was observed in A1847TX1 and PEO1TX5 cells (Supplementary Figure 1).

To investigate whether *ABCB1*-mediated drug resistance could be avoided by prescription of an alternative PARP inhibitor, we next investigated whether A2780pacR (Figure 5C) or A2780olapR (Figure 5D) cells were cross-resistant to AZD2461, a novel PARP inhibitor with low affinity for P-glycoprotein (Jaspers *et al*, 2013). No cross-resistance was observed, confirming that AZD2461 is a poor P-glycoprotein substrate and suggesting that the prescription of an alternative PARP inhibitor may avoid the treatment-limiting complication of *ABCB1*-induced drug resistance. Similarly, A2780pacR and A2780olapR cells were not cross-resistant to the PARP inhibitor veliparib (Figure 5E and 5F), but were significantly cross-resistant to rucaparib (Figure 5G and 5H). To further confirm that doxorubicin and rucaparib are P-glycoprotein substrates, we repeated the P-glycoprotein-Glo™ assays described above and demonstrated dose-dependent increases in ATPase activity following incubation with doxorubicin and rucaparib, but not with any of the other PARP inhibitors tested (Figure 6). We additionally confirmed that altered chemosensitivity did not result from altered *PARP1* expression in A2780pacR or A2780olapR cells, assessed both by qRT-PCR analysis and Western blotting (Supplementary Figure 3) and in our whole genome microarray analysis, described above, where we did not observe significant differences in *PARP1* mRNA expression in A2780, A2780pacR and A2780olapR cells (data not shown).

Discussion

The development of chemotherapy-induced drug resistance is a major treatment-limiting complication in the treatment of ovarian cancer (Vaughan *et al*, 2011). Many drug resistance mechanisms involve adaptation of drug metabolism or drug disposition pathways in resistant cancer cells, which normally function to protect healthy cells from exposure to toxic chemicals. One of the most well characterised examples of cellular adaptation is increased activity of the membrane-bound ATP-dependent “multidrug resistance” drug efflux pump P-glycoprotein, encoded by the *ABCB1* (MDR1) gene (Kathawala *et al*, 2015). Increased P-glycoprotein activity, often as a result of a chemotherapy-induced gene amplification event, has been associated with the development of drug resistance in a variety of human solid tumours and haematological cancers (Thomas & Coley, 2003). *ABCB1*-mediated drug resistance mechanisms are of particular relevance to ovarian cancer as paclitaxel, used routinely in combination with carboplatin in first-line chemotherapy protocols, is one of the most well characterised P-glycoprotein substrates which is efficiently effluxed from drug resistant cells (Sikic *et al*, 1997).

Our data confirms many previous reports of an *ABCB1*-mediated adaptive resistance mechanism in paclitaxel-resistant ovarian cancer cell lines and xenograft models (e.g. (Ahmed *et al*, 2007; Boesch *et al*, 2015; Ingemarsdotter *et al*, 2015; Syed *et al*, 2011), and describes a common mechanism of resistance to PARP inhibitors, including olaparib and rucaparib. *ABCB1*-mediated resistance to PARP inhibitors is a novel finding in ovarian cancer,

and is of particular clinical relevance as olaparib and related drugs are currently being evaluated in multiple clinical trials as second-line or maintenance chemotherapy options, often in paclitaxel pre-treated patients. It is therefore particularly important that our data suggests that some (e.g. rucaparib) but not all (e.g. veliparib, AZD2461) alternative PARP inhibitors are P-glycoprotein substrates and are actively effluxed from paclitaxel-resistant cells – optimal PARP inhibitor choice, particularly in paclitaxel-resistant patients, may therefore be an important determinant of clinical response. Consistent with this hypothesis, comparison of olaparib with the novel PARP inhibitor AZD2461 in a mouse mammary tumour model, showed greater efficacy following administration of AZD2461, which we and others have shown is a poor P-glycoprotein substrate (Jaspers *et al*, 2013), while recent advances in PARP inhibitor development have prioritised drug candidates, for example, novel 7-azaindole-1-carboxamide derivatives, which are not P-glycoprotein substrates (Cicinelli *et al*, 2014). Although our A2780olapR cells were not cross-resistant to paclitaxel, we believe this to be an *ABCB1* threshold effect, and highlight the potential for olaparib and rucaparib to additionally induce P-glycoprotein-mediated resistance, particularly if prescribed for an extended period, for example as long-term maintenance treatment. It is also important to note that doxorubicin, frequently prescribed to ovarian cancer patients in a liposomal formulation as second-line chemotherapy for recurrent disease is also a well-known P-glycoprotein substrate (Shen *et al*, 1986), which is also actively effluxed from paclitaxel-resistant cells.

The first suggestion that clinical response to olaparib may be compromised by altered drug efflux was proposed by Rottenberg *et al* in 2008, describing increased murine *Abcb1a/b* expression in a drug-resistant mouse mammary tumour model, where resistance was reversible with the P-glycoprotein inhibitor tariquidar (Rottenberg *et al*, 2008). Subsequently, increased *Abcb1b* expression has been reported in a murine BRCA2-dependent breast cancer model following exposure to olaparib, docetaxel or doxorubicin (Jaspers *et al*, 2015), while rucaparib has recently been reported to be a substrate for both *ABCB1* and *ABCG2* (Durmus *et al*, 2015). Intriguingly, experiments in mouse embryonic fibroblasts have suggested that doxorubicin resistance is caused by increased P-glycoprotein expression and activity in *PARP1* null cells (Wesierska-Gadek, 2005; Wurzer *et al*, 2000), while Dumitriu *et al* demonstrated that PARP inhibitors increase P-glycoprotein activity in both mouse and human lymphocytes (Dumitriu *et al*, 2004). If a similar mechanism is present in ovarian cancer cells, sequential paclitaxel and PARP inhibitor treatment could therefore further potentiate the development of *ABCB1*-mediated drug resistance.

Our data suggests that both paclitaxel and olaparib-induced resistance is reversible following combination treatment with a P-glycoprotein inhibitor. Early clinical trials of P-glycoprotein inhibitors have been disappointing, however, due in part to verapamil cardiotoxicity (Ozols *et al*, 1987), although a hyaluronic acid based CD44-targeted MDR1 siRNA was recently shown to successfully reverse paclitaxel resistance in SKOV 3 cells (Yang *et al*, 2015) and a more recent clinical trial of tariquidar in docetaxel-treated patients demonstrated P-glycoprotein inhibition *in vivo* (Kelly *et al*, 2011). A variety of novel P-

glycoprotein inhibitors are currently in development, including CB-PIC (Yun *et al*, 2015) and the bisbenzylisoquinoline CBT-1, a novel inhibitor which inhibits P-glycoprotein without influencing paclitaxel pharmacokinetics (Kelly *et al*, 2012).

In addition to the obvious potential to target P-glycoprotein therapeutically, assessment of *ABCB1* copy number or P-glycoprotein mRNA or protein expression may also have clinical utility as drug resistance biomarkers in cancer patients. Consistent with our own data, *ABCB1* copy number changes or increased P-glycoprotein expression (without associated copy number changes) have been widely reported in paclitaxel and docetaxel-resistant ovarian cancer cell lines e.g. (Wang *et al*, 2006) and in recurrent ovarian cancers (Zajchowski *et al*, 2012), suggesting that clinical assessment of changes in *ABCB1* expression or activity may have greatest prognostic potential as patients progress through chemotherapy. It is also interesting to note, however, that analysis of public domain ovarian cancer datasets from The Cancer Genome Atlas (TCGA) identifies a subset (approximately 2%) of chemotherapy-naïve ovarian cancer patients with *ABCB1* gene amplifications and a similar number of patients with high basal *ABCB1* mRNA expression, suggesting that these patients may be inherently resistant to paclitaxel, doxorubicin and selected PARP inhibitors. Consistent with this finding, amplification of regions of chromosome 7q21 spanning the *ABCB1* gene locus have frequently been described in ovarian cancer (Micci *et al*, 2014; Partheen *et al*, 2004; Tapper *et al*, 1997). High basal *ABCB1* expression has also been described in subsets of inherently resistant ovarian cancers (Arao *et al*, 1994; Holzmayer *et al*, 1992), and in a series of chemo-naïve ovarian tumours where P-glycoprotein expression

was inversely correlated with survival (Materna *et al*, 2004). Although the majority of previous studies are cell line based, it is interesting to note that *ABCB1* is one of several genes with increased expression in chemo-resistant breast cancers (Krech *et al*, 2012) and in paclitaxel-resistant ovarian cancers (Penson *et al*, 2004), while a recent whole-genome sequence based comparison of drug-sensitive and drug-resistant ovarian cancers described a novel gene promoter fusion in which *ABCB1* is fused with *SLC25A40* in chemotherapy resistant cancers, resulting in increased P-glycoprotein expression (Patch *et al*, 2015). Unfortunately, our ability to evaluate *ABCB1* expression in ovarian cancer patients before and following PARPi-based chemotherapy is limited by the current lack of clinical material outwith the clinical trial setting.

Should our cell line data be confirmed *in vivo*, we further highlight the potential for inherited differences in *ABCB1* activity to modify clinical response. Although not all published studies are consistent (e.g. (Johnatty *et al*, 2013; Marsh *et al*, 2007), inheritance of the *ABCB1* G2677T/A allele has been shown to influence paclitaxel clearance (Fransson *et al*, 2011; Green *et al*, 2006), the C2005T polymorphism to influence substrate specificity (Liu *et al*, 2010) and various *ABCB1* alleles to influence survival in both breast (Chang *et al*, 2009) and ovarian (Johnatty *et al*, 2013; Kim *et al*, 2009) paclitaxel-treated patient cohorts. It is therefore particularly important to note that only one third of ovarian cancer patients responded to second-line olaparib treatment in recent clinical trials (Audeh *et al*, 2010), when majority of enrolled patients had failed to respond to previous chemotherapy and almost all patients had been pre-treated with paclitaxel (median of 3 previous

chemotherapy regimens) – it is therefore tempting to speculate that non-responders had altered *ABCB1* expression, and we encourage testing of this hypothesis in clinical trial cohorts.

Our experimental models, combining novel drug-resistant cell lines with functional drug transport assays provide a useful system for the evaluation of additional PARP inhibitors, and more detailed analysis of potential drug/drug interactions - studies with additional PARP inhibitors are ongoing in our laboratory. We were particularly interested to learn of a recent report describing synergistic effects of olaparib and cediranib (Liu *et al*, 2014), particularly as cediranib was previously shown to be a potent P-glycoprotein inhibitor (Tao *et al*, 2009), and hypothesised that clinical response may result from cediranib-induced inhibition of *ABCB1*-mediated olaparib efflux. Experiments in our drug-resistant cell lines did not confirm this hypothesis (data not shown), however, consistent with a recent report suggesting that there may be an immunological basis for response to combination treatment (Lee *et al*, 2015).

In summary, our data describes a novel mechanism of PARP inhibitor resistance in ovarian cancer and highlights the importance of evaluating *ABCB1* expression in ongoing and future clinical trials. If our hypotheses are confirmed in extended clinical series, our data strongly suggests that optimal choice of PARP inhibitor may limit the progression of drug resistant

disease, and further suggests that routine prescription of first-line paclitaxel may significantly limit subsequent chemotherapy options in ovarian cancer patients.

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Figure Legends

Figure 1: Cross-resistance of paclitaxel and olaparib-resistant ovarian cancer cell lines.

Novel ovarian cancer A2780-derived paclitaxel (A2780pacR) and olaparib (A2780olapR) resistant cell lines were created as described in Materials and Methods. MTT chemosensitivity assays were used to compare cell viability following exposure to serial dilutions of paclitaxel (panels A and D) and olaparib (panels B and C) in A2780 and A2780pacR (panels A and C) and A2780olapR (panels B and D) cells. Chemosensitivity values obtained from triplicate replicate experiments, expressed as IC₅₀ values, are summarised in Panel E.

Figure 2: *ABCB1* (P-glycoprotein) expression is increased in A2780pacR and A2780olapR cells.

ABCB1 mRNA expression (panel A), copy number (panel B), and P-glycoprotein expression (panels C and D) was compared in A2780, A2780pacR and A2780olapR cells by qRT-PCR analysis, Taqman-based copy number analysis, Western blotting and immunohistochemical analysis, respectively, as described in Materials and Methods.

Figure 3: Paclitaxel and olaparib resistance are reversible following combination treatment with the P-glycoprotein inhibitors verapamil and elacridar.

MTT chemosensitivity assays were used as described in Materials and Methods to compare cell viability following exposure to serial dilutions of paclitaxel and olaparib in A2780, A2780pacR and A2780olapR cells in the presence and absence of combination treatments (5 μ M and 10 μ M) with the P-glycoprotein inhibitors verapamil (Panels A-C) and elacridar (panels D-F).

Figure 4: P-glycoprotein mediated drug efflux is altered in paclitaxel and olaparib resistant cells.

(A) Vybrant™ Multidrug Resistance Assays were used, as described in Materials and Methods, to compare P-glycoprotein dependent efflux of the model substrate Calcein-AM in untreated A2780, A2780pacR and A2780olapR cells and (B) in the presence of 0-25 μ M verapamil. Results were confirmed (Panel C) using the P-glycoprotein-Glo™ Assay System, as described in Materials and Methods, to demonstrate dose-dependent increases in luminescence following the incubation of paclitaxel or olaparib with P-glycoprotein containing membranes.

Figure 5: Paclitaxel and olaparib resistant cells are cross-resistant to doxorubicin and rucaparib, but not to AZD2461 or veliparib.

MTT chemosensitivity assays were used as described in Materials and Methods to compare cell viability in A2780, A2780pacR and A2780olapR cells following exposure to serial dilutions of doxorubicin (Panels A and B), AZD2461 (Panels C and D), veliparib (Panels E and F) and rucaparib (Panels G and H). Chemosensitivity values obtained from triplicate replicate experiments, expressed as IC₅₀ values, are summarised in Panel I.

Figure 6: The PARP inhibitors olaparib and rucaparib are P-glycoprotein substrates, while the alternative PARP inhibitors AZD2461 and veliparib are not actively effluxed.

The P-glycoprotein-Glo™ Assay System was used, as described in Materials and Methods, to investigate whether dose-dependent increases in luminescence were observed following the incubation of verapamil (positive control), olaparib, veliparib, rucaparib or AZD2461 with P-glycoprotein containing membranes.

Supplementary Figure 1

qRT-PCR analysis was used as described in Materials and Methods to confirm increased *ABCB1* expression in paclitaxel-resistant (A) 1847TX1 and (B) PEO1TX5 cells, relative to drug-sensitive parental cells. MTT chemosensitivity assays were used as described in Materials and Methods to compare cell viability following exposure to serial dilutions of paclitaxel

(panels C and F), olaparib (panels D and G) and doxorubicin (panels E and H) in matched drug-sensitive and drug-resistant cell line pairs.

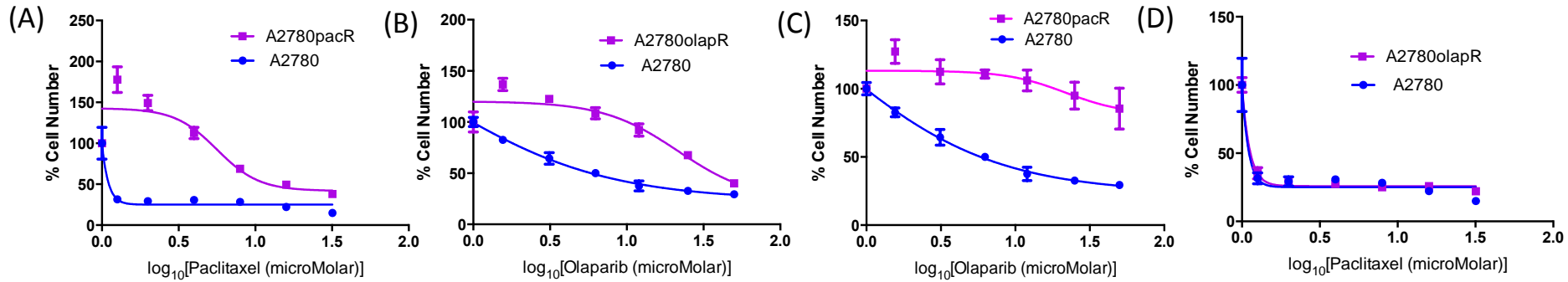
Supplementary Figure 2

ABCB1 expression was disrupted following siRNA transfection in (A) A2780pacR and (B) A2780olapR cells, as described in Materials and Methods (control cells were transfected with sterile water). Gene knockdown is illustrated 24 hours following transfection, although was maintained from 24 -72 hours post transfection. MTT chemosensitivity assays were used as described in Materials and Methods to compare the viability of drug-sensitive, drug-resistant and siRNA knockdown cells following exposure to serial dilutions of (C) paclitaxel and (D) olaparib.

Supplementary Figure 3

(A) qRT-PCR analysis and (B) Western blotting was used as described in Materials and Methods to compare *PARP1* mRNA expression in A2780, A2780pacR and A2780olapR cells.

Figure 1



(E) Summary of chemosensitivity data

	IC ₅₀ paclitaxel (μM)	IC ₅₀ olaparib (μM)	IC ₅₀ olaparib (μM)	IC ₅₀ paclitaxel (μM)
A2780	0.75	0.59	0.59	0.75
A2780olapR		21.56		0.58
A2780pacR	5.63		21.80	
Fold change (p-value)	7.49 (<0.0001)	36.54 (<0.0001)	36.95 (<0.0001)	0.77 (ns)

Figure 2

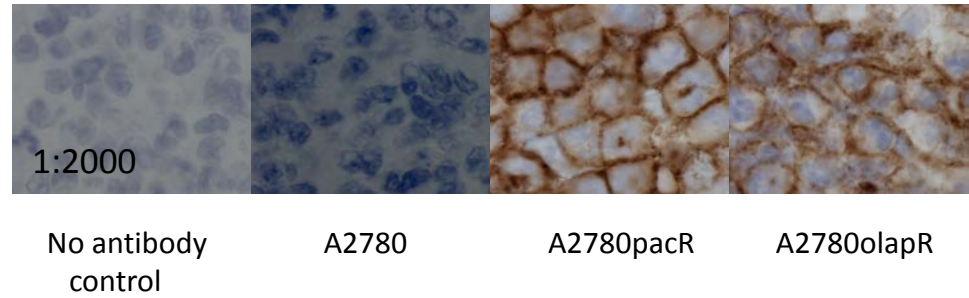
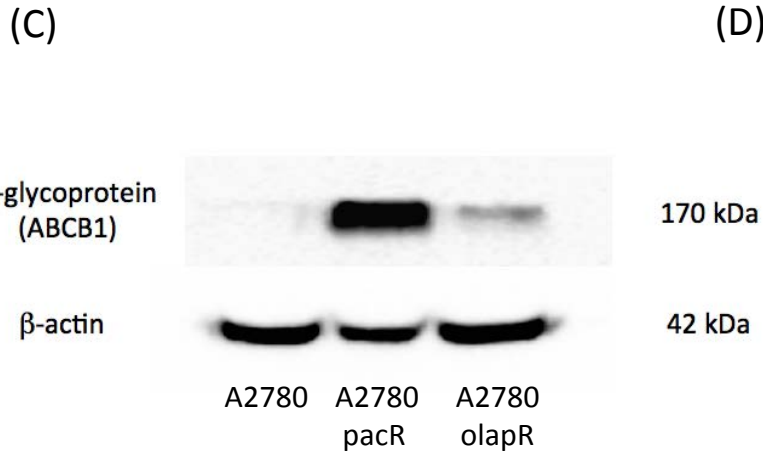
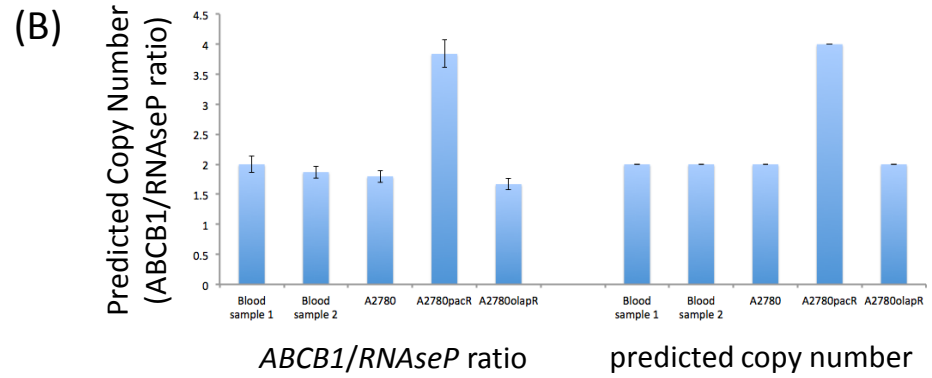
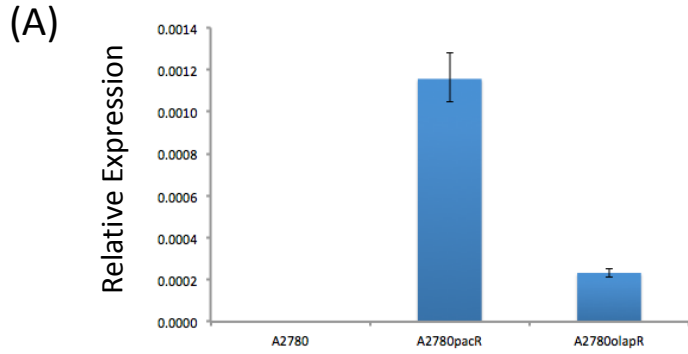


Figure 3

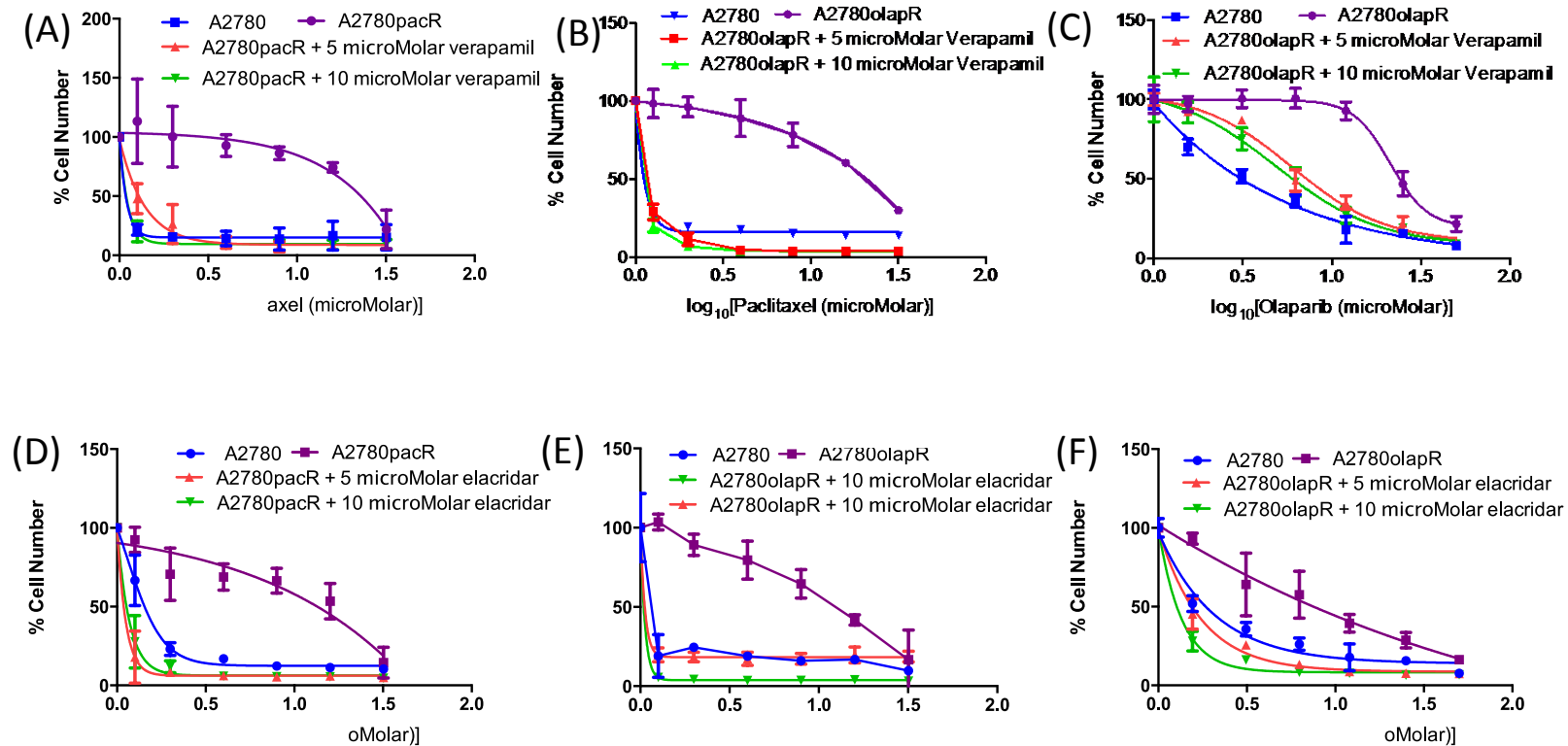


Figure 4

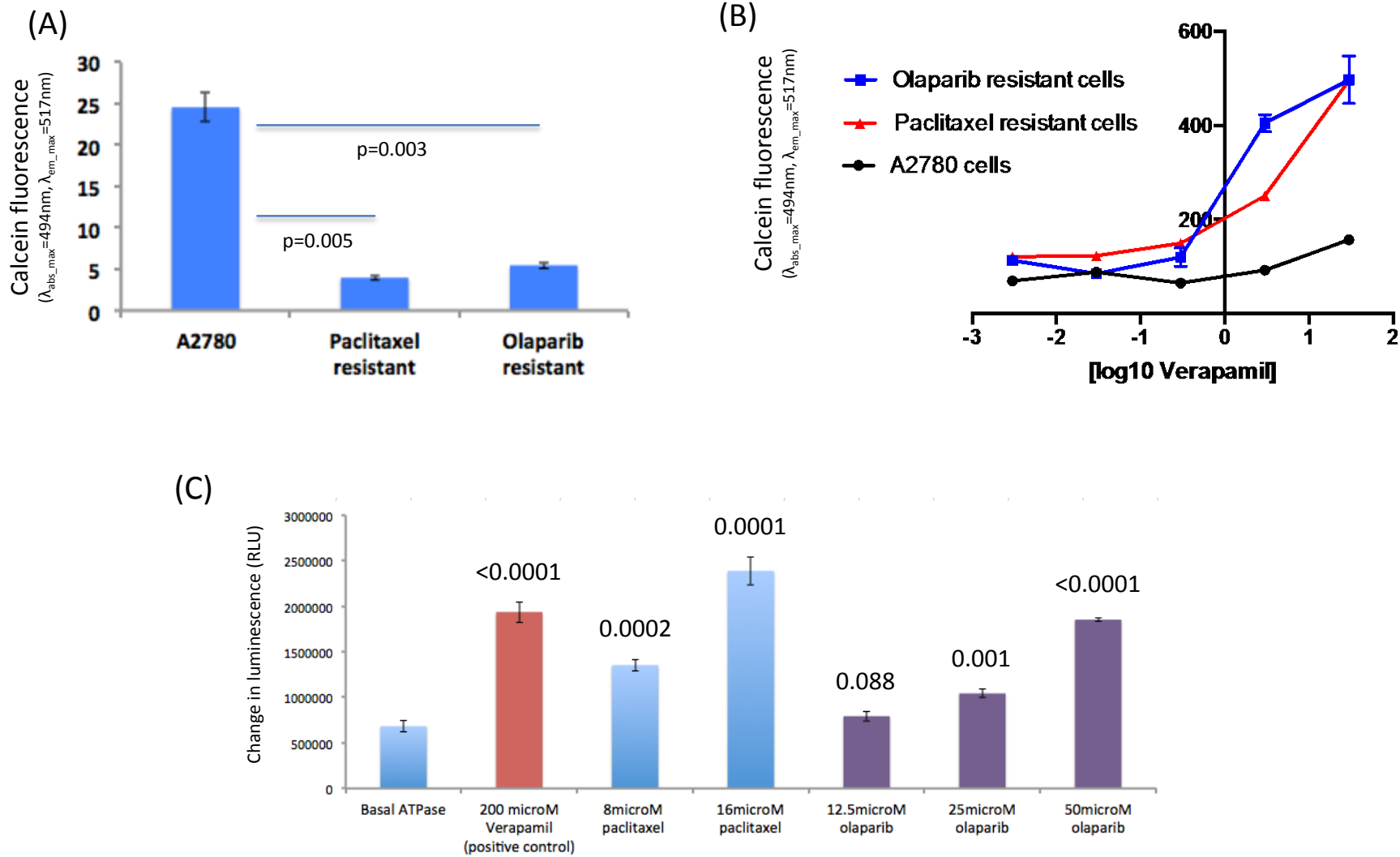
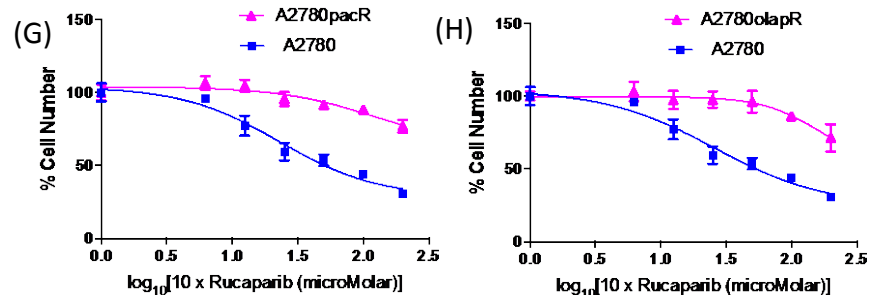
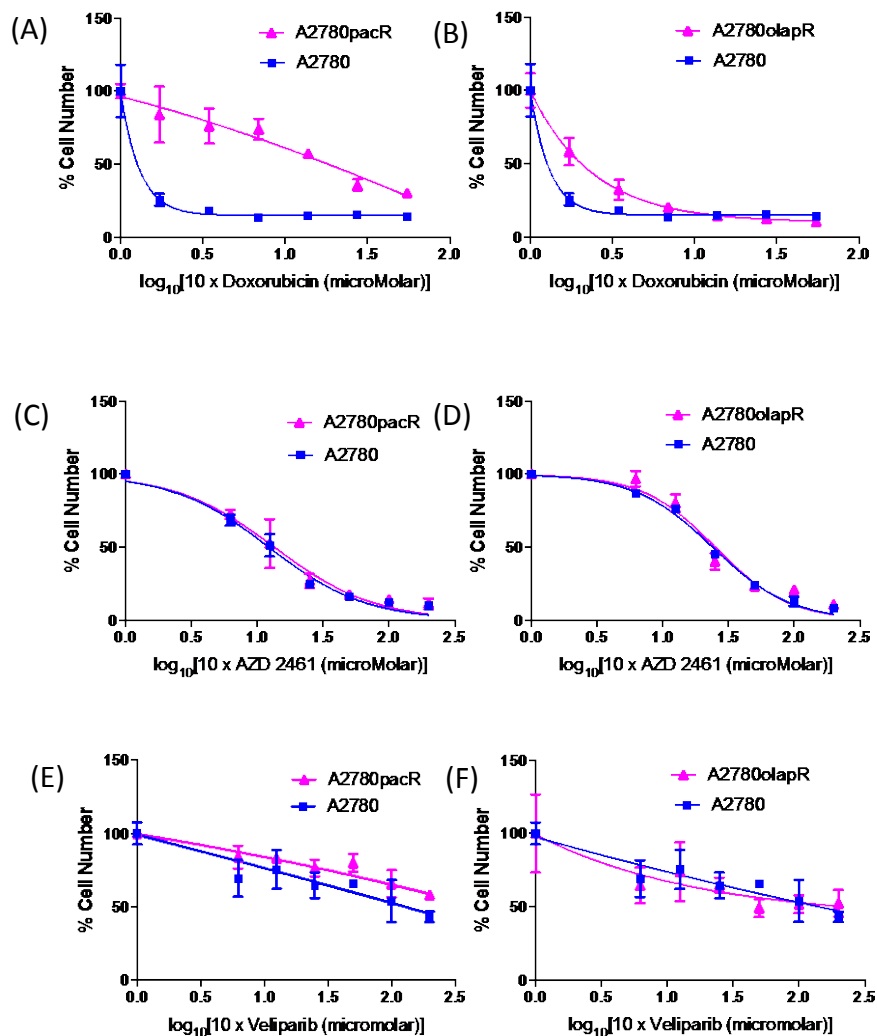


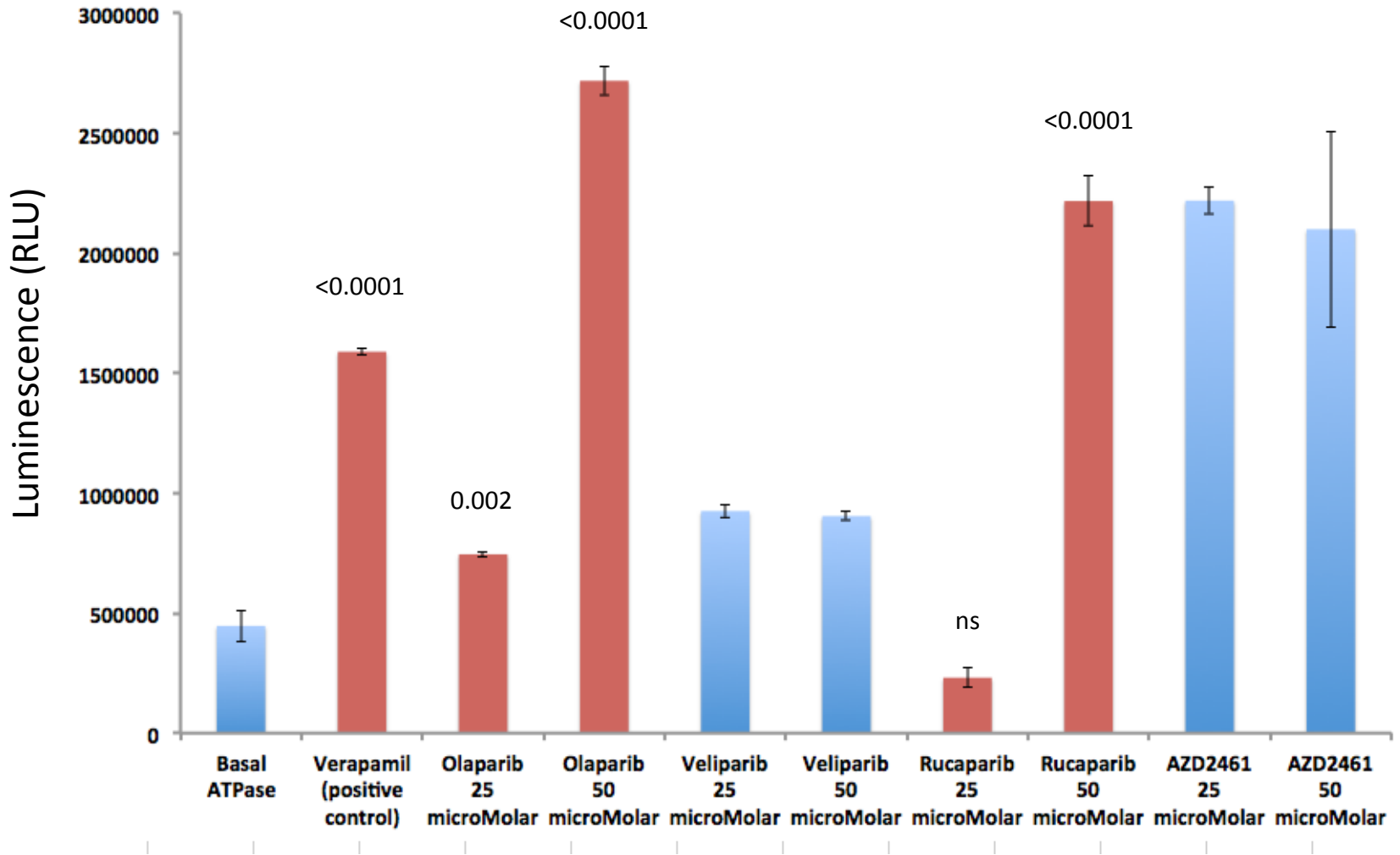
Figure 5



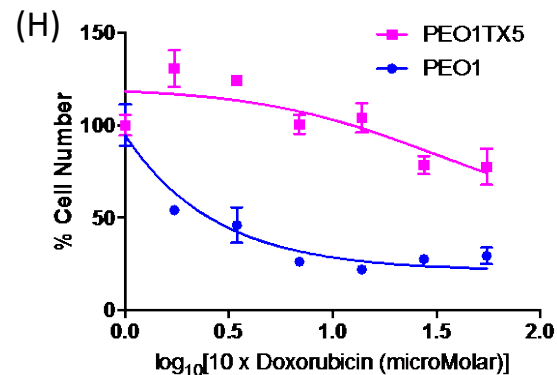
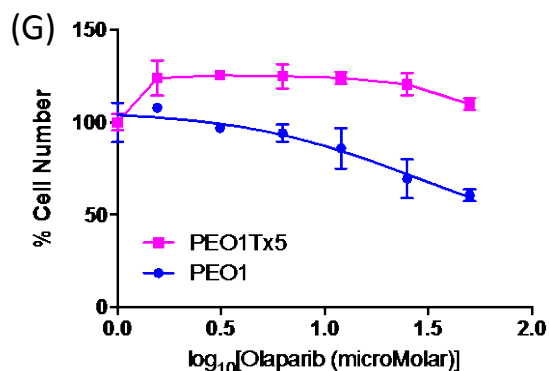
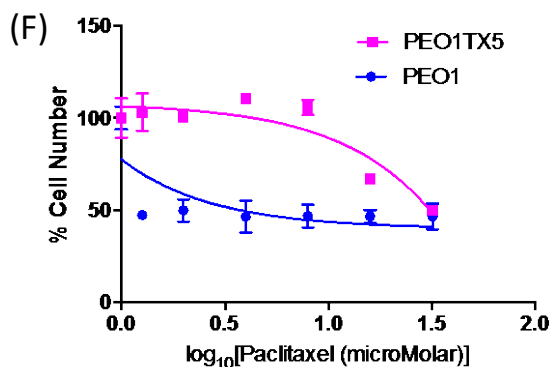
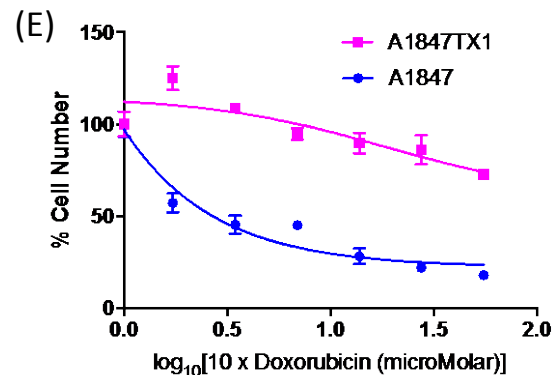
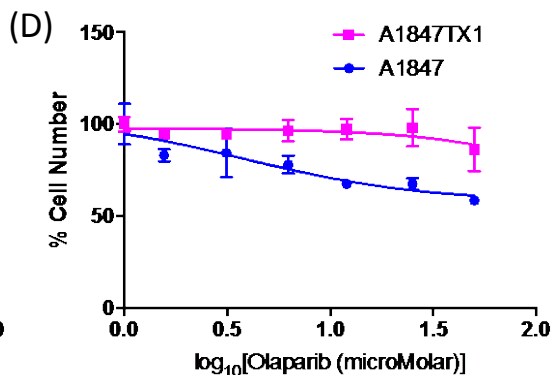
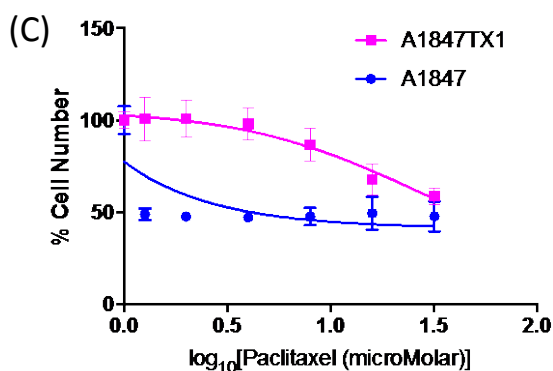
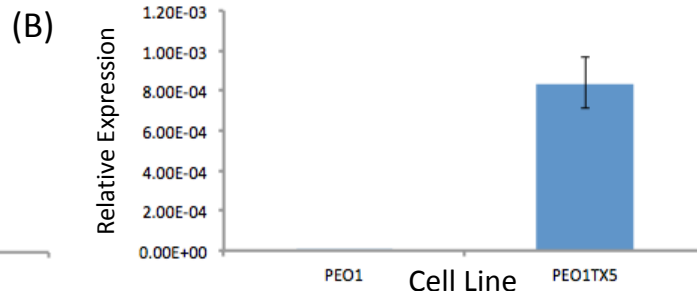
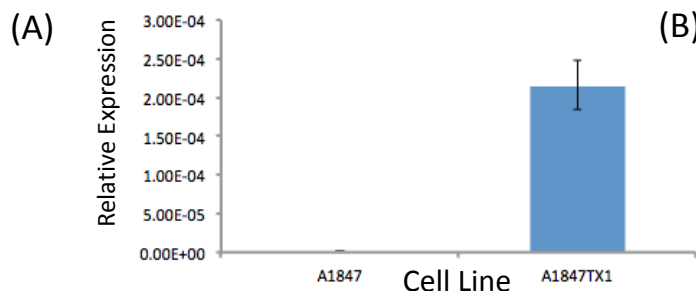
(I) Summary of chemosensitivity data

Cell line/Drug treatment	Doxorubicin IC ₅₀ (μM)	AZD2461 IC ₅₀ (μM)	Veliparib IC ₅₀ (μM)	Rucaparib IC ₅₀ (μM)
A2780	Pac: 0.03 Olap: 0.06	Pac: 1.23 Olap: 2.40	6.18	Pac: 2.31 Olap: 2.58
A2780pacR	14.23	1.35	20.09	11.68
A2780olapR	0.18	2.56	5.86	16.49
Fold change (relative to A2780)	PacR: 536.98 p<0.001 OlapR: 2.80 p=0.003	PacR: 1.09 p=0.715 OlapR: 1.05 p=0.729	PacR: 3.25 p=0.123 OlapR: 0.95 p=0.876	PacR: 5.06 p<0.001 OlapR: 6.39 p<0.001

Figure 6

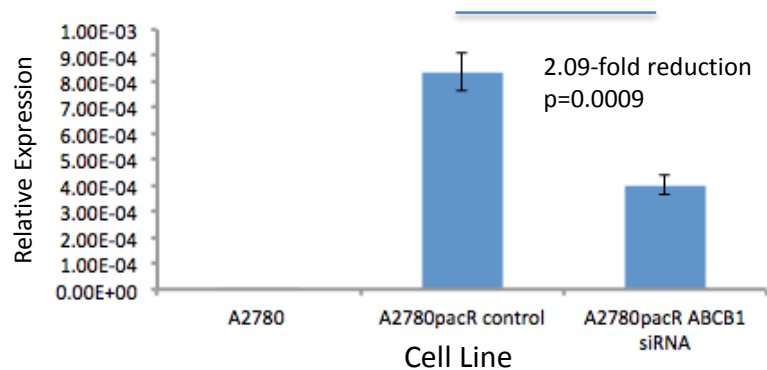


Supplementary Figure 1

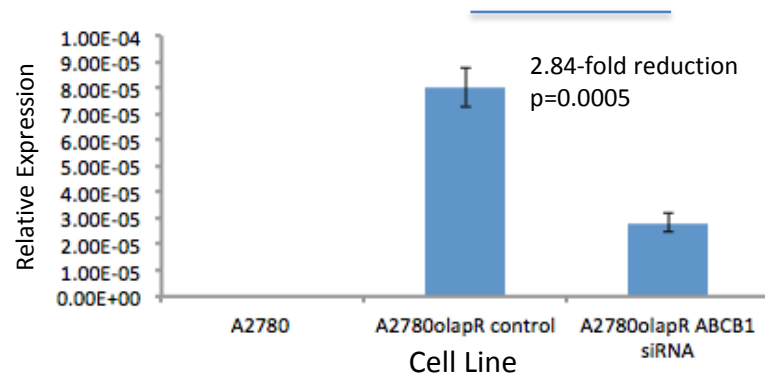


Supplementary Figure 2

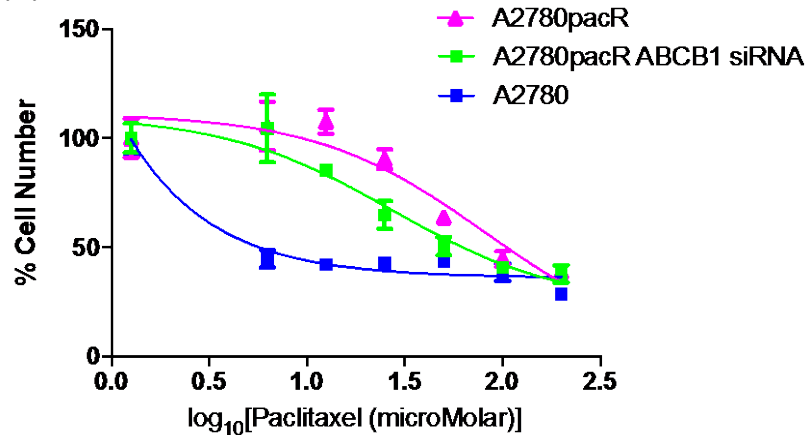
(A)



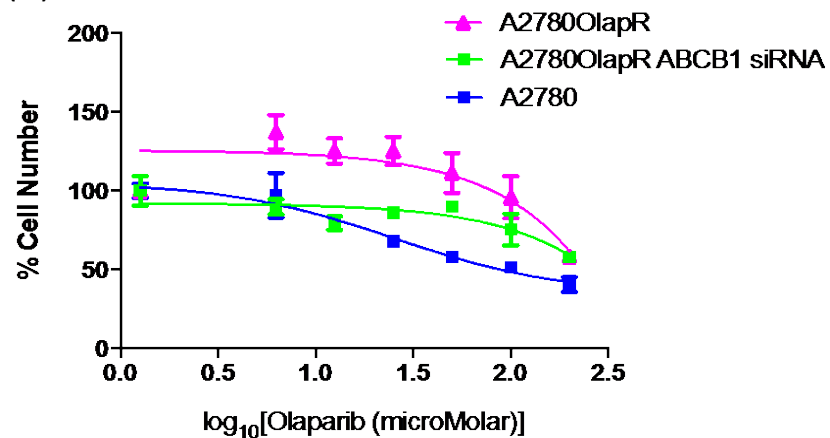
(B)



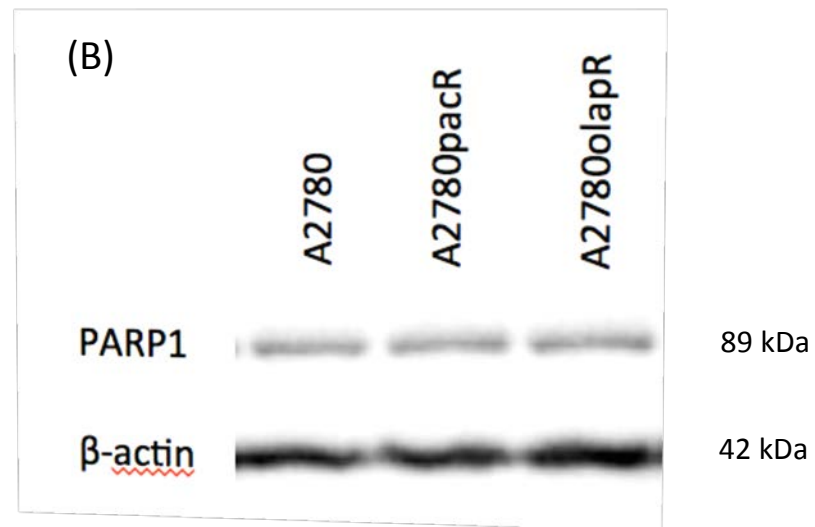
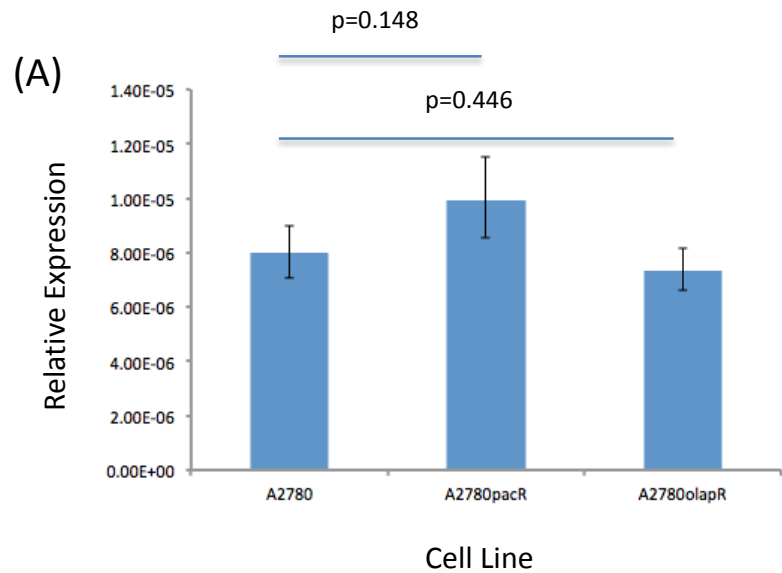
(C)



(D)



Supplementary Figure 3



GENE	ALIAS	FOLD CHANGE probe set 1	p-value (adjusted)	FOLD CHANGE probe set 2	p-value (adjusted)
Fold increase in A2780pacR cells (relative to A2780)					
<i>ABCA1</i>	ABC1, CERP	-2.29	3.55E-10		
<i>ABCA2</i>	ABC2	-25.49	1.17E-03		
<i>ABCA3</i>	ABC3	0.10	1.26E-05		
<i>ABCA7</i>	ABCX	-0.65	4.66E-11		
<i>ABCA11P</i>	none	0.05	8.54E-04		
<i>ABCB1</i>	MDR1, PGY1	35.94	2.16E-22	45.52	1.53E-22
<i>ABCB7</i>	ABC7	-3.38	1.15E-05		
<i>ABCB9</i>	none	-2.09	1.11E-09	-5.13	3.55E-06
<i>ABCB10</i>	MABC2, MTABC2	1.13	2.72E-11		
<i>ABCC3</i>	MRP3, CMOAT2	0.02	1.26E-02		
<i>ABCC4</i>	MRP4, MOATB	-28.19	1.36E-03		
<i>ABCC5</i>	MRP5, MOATC	-17.33	8.17E-04	0.02	1.69E-03
<i>ABCC6</i>	MRP6, ARA	-64.47	1.58E-02	0.02	3.97E-03
<i>ABCC8</i>	SUR, SUR1	0.05	3.96E-04		
<i>ABCC10</i>	MRP7	-15.33	3.10E-05		
<i>ABCD3</i>	PXMP1, PMP70	0.14	2.14E-06		
<i>ABCE1</i>	RNS4I	0.08	3.94E-03	0.13	1.22E-05
<i>ABCF1</i>	ABC50	0.03	4.30E-02		
<i>ABCF2</i>	HUSSY18	0.11	1.65E-06		
<i>ABCF3</i>	none	0.02	1.11E-02		
<i>ABCG4</i>	WHITE2	-3.01	4.92E-10		

Fold increase in A2780olapR cells (relative to A2780)

<i>ABCA2</i>	ABC2	0.13	2.03E-06		
<i>ABCA3</i>	ABC3	0.04	2.87E-03		
<i>ABCA17P</i>	none	0.02	8.02E-03		
<i>ABCB1</i>	MDR1, PGY1	15.38	3.18E-20	17.14	3.18E-20
<i>ABCB6</i>	MTABC3	-11.14	4.09E-04		
<i>ABCB7</i>	ABC7	0.09	5.97E-03		
<i>ABCB9</i>	none	-39.60	4.16E-02	0.04	2.16E-03
<i>ABCB10</i>	MABC2, MTABC2	0.20	2.80E-06		
<i>ABCC3</i>	MRP3, CMOAT2	0.34	6.65E-10		
<i>ABCC4</i>	MRP4, MOATB	-15.59	8.03E-04	-18.70	2.33E-04
<i>ABCC5</i>	MRP5, MOATC	0.14	8.84E-08		
<i>ABCC6</i>	MRP6, ARA	0.03	3.97E-03		
<i>ABCC8</i>	SUR, SUR1	0.12	3.58E-06		
<i>ABCD1</i>	ALDP	0.09	1.09E-06		
<i>ABCD3</i>	PXMP1, PMP70	-14.31	1.56E-04		

<i>ABCE1</i>	RNS4I	-12.20	4.89E-03	-14.27	4.47E-04
<i>ABCF1</i>	ABC50	-1.49	8.03E-10	-1.02	1.74E-10
<i>ABCF2</i>	HUSSY18	-3.24	3.46E-09	-48.85	3.77E-02
<i>ABCF3</i>	none	0.04	9.38E-04		
<i>ABCG1</i>	ABC8, WHITE	0.11	1.64E-05		
<i>ABCG4</i>	WHITE2	-7.03	1.82E-07		

ABCB1 is the first and second most abundantly induced probe set (from 12,590 probe sets significantly altered) in A2780pacR cells, and the 4th and 6th most abundantly induced probe set (from 11,182 probe sets significantly altered) in A2780olapR cells.
 Genes with fold changes less than a pre-defined threshold of 2 are greyed out, as they are not considered to be functionally significant.