Multiple ABCB1 transcriptional fusions in drug resistant high-grade serous ovarian and breast cancer

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ABCB1 encodes Multidrug Resistance protein (MDR1), an ATP-binding cassette member involved in the cellular efflux of chemotherapeutic drugs. Here we report that ovarian and breast samples from chemotherapy treated patients are positive for multiple transcriptional fusions involving ABCB1, placing it under the control of a strong promoter while leaving its open reading frame intact. We identified 15 different transcriptional fusion partners involving ABCB1, as well as patients with multiple distinct fusion events. The partner gene selected depended on its structure, promoter strength, and chromosomal proximity to ABCB1. Fusion positivity was strongly associated with the number of lines of MDR1-substrate chemotherapy given. MDR1 inhibition in a fusion positive ovarian cancer cell line increased sensitivity to paclitaxel more than 50-fold. Convergent evolution of ABCB1 fusion is therefore frequent in chemotherapy resistant recurrent ovarian cancer. As most currently approved PARP inhibitors (PARPi) are MDR1 substrates, prior chemotherapy may precondition resistance to PARPi.
Our previous whole-genome analysis of post-treatment high-grade serous ovarian cancer (HGSC) and breast cancer samples identified a transcriptional fusion between ABCB1 and the upstream gene SLC25A40 associated with up-regulation of ABCB1 expression, while leaving the predicted MDR1 protein unaltered\textsuperscript{1,2}. Here, we sought to determine the frequency and circumstances in which ABCB1 fusions arise in a large cohort of recurrent HGSC and a smaller series of breast cancer patients.

**Results**

Prevalent over-expression of ABCB1 in recurrent HGSC. We analysed relapse ascites samples from 108 HGSC patients who had received a median of 2 (range: 0–9) lines of chemotherapy (Table 1 and Supplementary Data 1). We began by measuring ABCB1 expression using quantitative polymerase chain reaction (Q-RT-PCR) and SLC25A40–ABCB1-specific PCR\textsuperscript{1}. SLC25A40–ABCB1 fusions were seen in 15.7% of recurrent HGSC samples (Fig. 1a). When rank ordered for ABCB1 expression, fusions were distributed over 64 (59%) of the highest expressing samples.

**Table 1 Clinical characteristics of recurrent HGSC and breast cancer cohorts**

| Source                          | AOCs          | University of Utah | kConFab |
|--------------------------------|---------------|--------------------|---------|
| **Age at diagnosis**           |               |                    |         |
| Mean                           | 59.6          | Male               | 0       | 1      |
| Range                          | 24.4-81.8     | Female             | 13      | 19     |
| **Primary site**               |               |                    |         |
| Ovary                          | 77            | Mean               | 54.6    | 45.2   |
| Peritoneum                     | 24            | Range              | 34-83   | 33-61  |
| **Female genital tract**       | 4             | Grade              |         |        |
| **Endometrium**                | 1             |                    |         |        |
| **Unknown**                    | 2             |                    |         |        |
| **Grade**                      | 3             |                    |         |        |
| **2**                          | 12            | NA                 | 6       | 0      |
| **3**                          | 71            | Phenotype at diagnosis | 1 | 4 |
| **Unknown**                    | 22            | HR−, HER2−         | 1       | 4      |
| **Stage**                      |               | HR+, HER2+         | 0       | 4      |
| I                              | 1             |                    | 12      | 2      |
| II                             | 2             | HR−, HER2 NA       | 0       | 4      |
| III                            | 80            | HR+, HER2 NA       | 0       | 1      |
| IV                             | 13            | HR NA, HER2−       | 0       | 1      |
| **Unknown**                    | 12            | HR NA, HER2+       | 0       | 1      |
| **Residual disease**           |               |                    |         |        |
| No macroscopic disease         | 20            | Progression free survival | 13\textsuperscript{a} | 20 |
| Macroscopic disease ≤1 cm      | 36            | Number of events   | 29      | 44     |
| Macroscopic disease >1 cm      | 29            | Median months      | 8-50    | 18-90  |
| Macropscopic disease, size unknown | 10          | 95% CI of median   | 34-172  | 43-139 |
| Tumour not resected            | 6             | NA                 | 1       | 0      |
| **Unknown**                    | 7             | Overall survival   |         |        |
| **Progression free survival**  |               | Number of events   | 13      | 18     |
| Number of events               | 108           | Median months      | 72      | 76     |
| 95% CI of median               | 9.63-13.08    | 95% CI of median   | 34-172  | 43-139 |
| 95% CI of median               |               | Germline mutation  |         |        |
| **Overall survival**           |               |                    |         |        |
| Number of events               | 99            | BRCA1              | 0       | 4      |
| Median months                  | 31.3          | BRCA2              | 0       | 3      |
| 95% CI of median               | 26.24-38.89   | NA                 | 13      | 13     |
| 95% CI of median               |               | Sample collection procedure | 0 | 1 |
| **Germline mutation**          |               | Biopsy             | 0       | 1      |
| BRCA1                          | 11            | Excision           | 0       | 11     |
| **BRCA2**                      | 2             | Autopsy            | 0       | 8      |
| **Lines of prior chemotherapy**|               | Thoracentesis      | 13      | 0      |
| Median                         | 2             | Prior treatment    |         |        |
| Range                          | 0-9           | Median lines chemotherapy | 5 | 1 |
| **Lines of prior MDR1 substrate chemotherapy** | | Range lines chemotherapy | 1-8 | 0-4 |
| Median                         | 2             | Median lines radiotherapy | 2 | 1 |
| Range                          | 0-6           | Median lines targeted therapy | 0 | 0 |
| **Lines of targeted therapy**  |               | Range lines targeted therapy | 0-6 | 0-2 |
| Median                         | 2             | Median lines hormone therapy | 0 | 0 |
| Range                          | 0-5           | Range lines hormone therapy | 0-5 | 0-1 |

\textsuperscript{a}One patient progressed but no date available
NA information not available
Identification of multiple fusion partners with \textit{ABCB1}. In between samples with an SLC25A40–\textit{ABCB1} fusion on the barchart were other tumours with high \textit{ABCB1} expression without the SLC25A40–\textit{ABCB1} fusion, suggesting that additional structural changes may deregulate \textit{ABCB1}. We, therefore, characterised 25 recurrent HGSC samples with high levels of \textit{ABCB1} using a modified 5′ Rapid Amplification of cDNA Ends (RACE) assay called FusionPlex\textsuperscript{4}. We also performed whole-genome sequencing (WGS) and transcriptome analyses in ten samples that partially overlapped those subject to FusionPlex (Supplementary Data 1). We identified 16 novel \textit{ABCB1} fusion partners (Supplementary Data 3). Twelve had the same general structure as the SLC25A40–\textit{ABCB1} event, involving breakage in intron 1 of \textit{ABCB1} and splicing of non-coding 5′ exon(s) to exon 2 of \textit{ABCB1} but leaving the predicted coding region unaltered (Fig. 1a, b, Table 2, Supplementary Data 3). Most of the novel fusions occurred in patients where a fusion with SLC25A40 was also observed (Fig. 1a). Amongst the novel fusions only CNOT4–\textit{ABCB1} was identified in more than one patient, suggesting that we had not screened to saturation and that other partners could be detected if further tumours were analysed. Strikingly, Patient 7 had \textit{ABCB1} fusions involving five different partners (Fig. 1a, Supplementary Data 3). In addition, as the breakpoints in \textit{ABCB1} are unique to each translocation event we
Analysis of RNAseq data showed strong but without a detectable fusion. We previously found a ABCB1 that patient samples carrying ostensibly the same in a given tumour, suggested a strong selective pressure for a fusion partners, and multiple instances of the same fusion event HGSC ascites samples tested. The presence of multiple different (Table 2). Patient 9

| Case ID | Patient ID | Fusion | RT-PCR | WGS | RNAseq | FusionPlex |
|---------|------------|--------|--------|-----|--------|------------|
| HGSC 15245 | 1 | SLC25A40:ABCB1 | Y | NT | NT | NT |
| HGSC 65612 | 2 | SLC25A40:ABCB1 | Y | NT | NT | NT |
| HGSC 65554 | 4 | MATR3:ABCB1 | NT | NT | NT | Y |
| HGSC 65554 | 4 | PRRC2C:ABCB1 | Y | NT | NT | Y |
| HGSC 65554 | 4 | SLC25A40:ABCB1 | Y | NT | NT | N |
| HGSC 15224 | 6 | SLC25A40:ABCB1 | Y | NT | NT | N |
| HGSC 15340 | 7 | ARPC1B:ABCB1 | Y | Y | Y | Y |
| HGSC 15340 | 7 | CNOT4:ABCB1 | NT | N | N | Y |
| HGSC 15340 | 7 | GTF2I:ABCB1 | N | Y | Y | Y |
| HGSC 15340 | 7 | KMT2E:ABCB1 | N | N | N | N |
| HGSC 15340 | 7 | PHTF2:ABCB1 | NT | N | N | N |
| HGSC 15340 | 7 | WRN:ABCB1 | N | N | N | Y |
| HGSC 15272/AOCS-135 | 10 | SLC25A40:ABCB1 | Y | N | N | N |
| HGSC 15260 | 11 | CALU:ABCB1 | Y | NT | NT | Y |
| HGSC 15260 | 11 | CNOT4:ABCB1 | Y | NT | NT | Y |
| HGSC 15260 | 11 | SLC25A40:ABCB1 | Y | NT | NT | Y |
| HGSC 15335 | 15 | SLC25A40:ABCB1 | Y | NT | NT | Y |
| HGSC 15292 | 16 | JTGBB:ABCB1 | NT | NT | NT | N |
| HGSC 15292 | 16 | NAP1L1:ABCB1 | NT | NT | NT | N |
| HGSC 10335 | 17 | SLC25A40:ABCB1 | Y | Y | Y | Y |
| HGSC 10083/AOCS-117 | 18 | SLC25A40:ABCB1 | Y | N | N | N |
| HGSC 9954/AOCS-092 | 22 | SLC25A40:ABCB1 | Y | Y | Y | N |
| HGSC 12159 | 23 | SLC25A40:ABCB1 | Y | NT | NT | N |
| HGSC 65632 | 26 | SLC25A40:ABCB1 | Y | NT | NT | N |
| HGSC 11424 | 27 | SLC25A40:ABCB1 | Y | NT | NT | N |
| HGSC 10336 | 32 | CLOCK:ABCB1 | Y | Y | Y | Y |
| HGSC 10336 | 32 | SLC25A40:ABCB1 | Y | Y | Y | Y |
| HGSC 15239 | 33 | SLC25A40:ABCB1 | Y | NT | NT | N |
| HGSC 15209/AOCS-150 | 40 | SLC25A40:ABCB1 | Y | Y | Y | N |
| Breast | 14 | NRF1:ABCB1 | NT | NT | NT | Y |
| Breast | 19 | SLC25A40:ABCB1 | Y | NT | NT | Y |
| Breast | 21 | TPX2:ABCB1 | NT | NT | NT | Y |

Y fusion identified, N fusion not identified, NT not tested

could determine that in Patient 17 two independent SLC25A40 fusions to ABCB1 had occurred (Fig. 1c, Supplementary Data 2). Overall, ABCB1 fusions were identified in 18.5% of recurrent HGSC ascites samples tested. The presence of multiple different fusion partners, and multiple instances of the same fusion event in a given tumour, suggested a strong selective pressure for a convergent resistance phenotype in HGSC patients. It is notable that patient samples carrying ostensibly the same SLC25A40–ABCB1 fusion event differed in the overall level of ABCB1 expression, suggesting that fewer tumour cells carried the fusion event in ascites with lower ABCB1 expression.

Structural variants (SV) involving ABCB1 were detected in all ten samples subject to WGS (Supplementary Data 2), and verified three of the novel fusions identified by FusionPlex in two patients (Table 2). Patient 9’s tumour very strongly expressed ABCB1 but without a detectable fusion. We previously found a 95 kb insertion of part of the ABCA4 gene in intron 1 of ABCB1 of this tumour1. Analysis of RNAseq data showed strong expression from an alternative transcriptional start site in exon 2 of ABCB1, possibly due to the juxtaposition of regulatory elements from ABCA4 (Fig. 1d). Therefore, SV in patient samples that did not result in predicted transcriptional fusion events may nevertheless alter ABCB1 expression.

### ABCB1 fusions in end-stage breast cancer patients

We extended our analysis to recurrent and rapid autopsy breast cancer patients (n = 33) (Table 1), a disease that shares common treatment approaches with HGSC. Using a combination of SLC25A40–ABCB1 fusion-specific PCR and FusionPlex assays we detected SLC25A40–ABCB1 fusions in nine patients. An ABCB1 fusion involving NRF1 was found in Patient 14 that co-occurred with an SLC25A40–ABCB1 fusion (Fig. 1e), demonstrating that convergent ABCB1 deregulation also occurs in breast cancer patients. Patient 21 was observed to have a TPX2–ABCB1 fusion by FusionPlex but did not have an SLC25A40–ABCB1 fusion. The use of 18 autopsy samples from 6 fusion positive-breast cancer patients allowed us to obtain a survey of fusion positivity across metastatic deposits (Supplementary Fig. 18). Patient 5 was the only patient where all tested sites were SLC25A40–ABCB1 fusion positive, Patients 1, 2, 7 and 9 had two fusion-positive sites and Patient 4 had only a single site that was positive for the SLC25A40–ABCB1 fusion. The presence of subclonal fusion events suggests that in fusion-negative metastases novel mechanisms of ABCB1 deregulation or other mechanisms conferring resistance are extant.

Amongst the HGSC and breast cancer samples, overall 15 fusions involving addition of non-coding exons 5′ from the partner gene to exon 2 of ABCB1 were identified. Other ABC family transporters have been implicated in chemotherapy resistance2, although the data is less compelling than for ABCB1. We evaluated samples where we had comprehensive WGS, copy number and transcriptome data but found little evidence of deregulation similar to that seen with ABCB1 in other transporters (see Supplementary Information).

### Factors influencing selection of a fusion gene partner

As SLC25A40 was the most common fusion partner, and novel fusions with ABCB1 were mostly found in tumours where an SLC25A40–ABCB1 fusion was also detected, we considered why certain genes became partners to ABCB1. In principle,
transcriptional fusion could result in loss of negative regulation of \textit{ABCB1} and/or acquisition of a constitutively expressed, stronger promoter in the lineage giving rise to HGSC. To assess promoter strength of the fusion partner genes we examined their RNA expression levels in pre-treatment tumour samples and fallopian tube secretory cells, the precursor cell type for HGSC. Consistent with fusion resulting in acquisition of a stronger promoter to drive \textit{ABCB1} expression, the partner genes exceeded \textit{ABCB1} expression in fallopian tube cells, and HGSC and breast cancer tumours obtained prior to chemotherapy exposure (Fig. 2a).

Surprisingly, \textit{SLC25A40} was not the highest expressing gene amongst fusion partners, despite being the most consistently involved, suggesting that other factors must determine its selection. A majority of partner genes (9/15) are located on chromosome 7 (Fig. 2b). Amongst the fusion partner genes, \textit{SLC25A40} was the physically closest downstream of \textit{ABCB1}. While \textit{RUNDC3B} is located closer to \textit{ABCB1} than \textit{SLC25A40} it is in the opposite transcriptional orientation to \textit{ABCB1} and has lower expression than \textit{ABCB1} (Supplementary Fig. 19b). These findings suggest that chromosomal proximity to \textit{ABCB1}, transcriptional level and orientation, and position relative to \textit{ABCB1}, are major determinants in being involved in a productive fusion event, enabling cells to take advantage of simple deletions.

Our findings suggested that intrachromosomal rearrangements provided the highest probability of creating a productive \textit{ABCB1} fusion. We considered whether tumours with a propensity to undergo structural rearrangement may be more likely to have fusion events, but we found no indication of an association between the frequency of SV in a sample and \textit{ABCB1} fusion events, but we found no indication of an association between the frequency of SV in a sample and \textit{ABCB1} fusion events.

Fig. 2 Characteristics of fusion partners. a Mean expression of all genes from normal fallopian tube, primary HGSC and primary breast cancer RNAseq data. Gene expression was rank ordered. Fusion partners are highlighted in red, \textit{ABCB1} in green. b Chromosomal location of fusion partners, with a majority located on chromosome 7 (boxed). c Number of SVs, by type, in recurrent HGSC samples described as \textit{ABCB1} fusion-positive or negative recurrent HGSC samples described as \textit{ABCB1} fusion-positive or negative.
that are known substrates of MDR1, with the probability of a fusion event closely correlated to the number of lines of substrate chemotherapy \( (p \text{ value} < 0.001, \text{Wilcoxon test}) \) (Fig. 3a). Of the 20 patients with fusion-positive tumours, 18 failed at least one line of treatment with a known MDR1 substrate (Supplementary Figure 20).

**Fusion positivity is associated with substrate chemotherapy.** We note that fusions were seen in two-thirds of patients who had received 3 lines of paclitaxel, and rose from 12 to 26% of patients following at least one line of liposomal doxorubicin (Fig. 3b); both drugs are MDR1 substrates and are known to induce chemoresistance by over-expression of MDR1\(^3\). However, given that...
patients frequently received more than one line of a known MDR1-substrate chemotherapy prior to sample collection, it was difficult to determine which agent(s) were most likely to select for presence of a fusion. We, therefore, sought patients where samples were collected sequentially (Fig. 3c). We observed the appearance of a fusion-positive sample in Patient 10 following a line of carboplatin and gemcitabine followed by three cycles of topotecan. Carboplatin and gemcitabine are not effluxed by MDR1 but topotecan is a known MDR1 substrate6, and therefore most likely to have resulted in the selection of the fusion in Patient 10. Consistent with the strong association of fusion positivity with number of lines of paclitaxel (Fig. 3b), we observed transition from negative to fusion positive in Patients 7 and 15 following paclitaxel treatment (Fig. 3c).

**Inhibition of MDR1 resensitizes to paclitaxel.** To further characterise the relationship between fusion positivity and paclitaxel sensitivity, and preclinically validate ABCB1 fusions as a therapeutic target, we isolated a patient-derived HGSC cell line with the SLC25A40–ABCB1 fusion (Fig. 4a). Western blot shows MDR1 expression in fusion-positive line AOCS18.5 but not in fusion-negative line AOCS21.2. MDR1 expression in AOCS18.5 by immunofluorescence. Inset shows antibody isotype control. d IC50 plots in AOCS18.5 and non-fusion AOCS21.2 cell lines, showing a fusion-specific increase in paclitaxel sensitivity, but sensitivity to non-substrate cisplatin, in the presence of the MDR1 inhibitor elacridar (n = 3 replicates). Error bars indicate ± SEM. For some points, error bars are shorter than the height of the symbol and are not shown.

**Discussion**

Our finding of frequent transcriptional fusions involving ABCB1 in recurrent HGSC and breast cancer has important implications for chemotherapy choice in disease relapse and the clinical development of targeted agents. To our knowledge all currently clinically approved PARPi are MDR1 substrates and preclinically over-expression of MDR1 has been associated with olaparib resistance in cell line and animal models8,9. A requirement that patients should have received two to three lines of chemotherapy before being eligible for access to a PARPi may be unintentionally preconditioning tumours for resistance to these agents. Evaluation of fusion status may be particularly important in patients who have received dose-dense paclitaxel10,11. The clinical
development of novel targeted agents in HGSC should take into account whether the new agent is an MDRI substrate and if so, determination made of fusion status prior to enrolment on trials of patients with recurrent disease. Future clinical trials could explore the benefit of stratifying patients based on fusion positivity and then directing fusion-positive patients to subsequent non-MDRI substrate chemotherapy. Finally, although clinical findings with MDRI inhibitors were largely disappointing, little or no patient stratification occurred\cite{35}, and it may be timely to reconsider previously abandoned MDRI inhibitors\cite{36}.

**Methods**

**Patients.** Ethics board approval was obtained at all institutions for patient recruitment. A sample collection and research studies (Peter MacCallum Cancer Centre & University of Utah). Written informed consent was obtained from all participants in this study. Tables 1 and 2 summarises the clinical characteristics of each cohort.

The ovarian cancer cohort (n = 108) consisted of women diagnosed with epithelial ovarian, primary peritoneal or fallopian tube cancer since 1992. The women were treated at hospitals across Australia and were recruited through the Australian Ovarian Cancer Study (AOCS)\cite{13}. All patients were diagnosed with serous carcinoma of high-grade (grade 2 or grade 3) and advanced stage (FIGO stage III or IV, International Federation of Gynaecology and Obstetrics) and received platinum-based chemotherapy as part of primary treatment. All samples utilised in the study were collected at relapse as ascites. Six of the 108 patients were previously described\cite{11}. Summary treatment information is provided in Supplementary Data 1. The KConFab cohort consisted of 20 breast cancer patients who were recruited to KConFab, a consortium for the study of familial breast cancer\cite{14}. Patients have a strong family history of breast and/or ovarian cancer, 7 have germline BRCA1/2 mutations. Post-treatment samples were collected through biopsies (n = 1), excision (n = 11), or during rapid autopsy (n = 8).

Thirteen additional breast cancer patient samples were ascertained from Huntsman Cancer Institute, University of Utah. Samples were collected post-treatment during paracentesis.

**Nucleic acid isolation.** DNA was isolated from peripheral lymphocytes or lymphoblastoid cell lines using the salting out method for germline DNA. Tumour cells were isolated from ovarian cancer patient ascites using Dynabeads Epithelial Enrich (Invitrogen/Life Technologies), followed by DNA and RNA extractions. For breast cancer patients in the Bild cohort, cells from pleural effusions were collected through centrifugation and for 9 of 18 samples, CD45-, CD90+ and PDPN+ normal cells were depleted from the population using the Miltenyi quadrroMACS as per manufacturer's protocol. Frozen tumour samples were cyrosectioned, 5 × 30 mm, for DNA and RNA extractions.

DNA extractions were performed using the DNeasy blood and tissue kit (QIAGEN). DNA was subsequently quantified using the Qubit dsDNA BR assay (ThermoFisher Scientific). For ovarian cancer and KConFab samples, RNA was isolated using the mirVana miRNA Isolation kit (Ambion/Life Technologies). The RNasey Mini Kit (QIAGEN) was used to isolate RNA fromBlood breast pleural effusions. The Qubit RNA HS assay was used to assess RNA quantity.

**SNP arrays, tumour cellularity and copy number analysis.** Tumour and matched normal DNA was assayed with the Illumina HumanOmniExpress arrays as per manufacturer’s instructions at the Australian Genome Research Facility (AGRF, Melbourne Australia). Single-nucleotide polymorphism (SNP) arrays were scanned on an iScan (Illumina), data was processed using the Genotyping module (v.1.9.8) in GenomeStudio v.2011.1 (Illumina) to calculate b-allele frequencies and logR ratios. Tumour cellularity was assessed using ASCAT\cite{15} and qPUR\cite{16}, tumour ploidy was also calculated using ASCAT. Median tumour content was 96% (range: 70–99%).

**Whole-genome sequencing.** WGS libraries were generated from 1 µg of genomic DNA using TruSeq DNA PCR-free sample preparation protocol (Illumina, San Diego). Sequencing on a HiSeq X Ten (Illumina at GenomeOne, Kingshorn Comprehensive Cancer Centre, Sydney Australia) was performed to a minimum average of 30-fold base coverage for germline samples and 60-fold coverage for ascites samples (Supplementary Data 2). Each lane of sequencing data underwent alignment to the Genome Reference Consortium human genome assembly (GRCh37) using BWA-MEM\cite{17}. Optical duplicate reads were marked using Picard MarkDuplicates (https://broadinstitute.github.io/picard/).

The Genome Modelling System (GMS)\cite{48} was utilised to perform end to end analysis of WGS data to call somatic SVs. The GMS workflow was configured to utilise a combination of three complementary algorithms—Breakdancer\cite{19} and Squaredancer (https://github.com/ding-lab/squaredancer) followed by a read assembly step using TIGRA (http://bioinformatics.mdanderson.org/main/TIGRA). Concurrently, Genome Rearrangement Identification Software Suite (GRIDSS)\cite{20} was utilised for its ability to detect SV at low cellular prevalence. GRIDSS was also run for the six samples previously described\cite{35}. All SVs in ABC21 were manually reviewed in integrative genomics viewer (IGV)\cite{21}.

**Transcriptome sequencing.** One microgram of total RNA was used for library preparation using the TruSeq RNA Sample Preparation kit (Illumina) as per the manufacturer’s low-throughput protocol. All libraries were sequenced as paired-end 100 bp on a HiSeq2500 (Illumina, at AGRF), generating 100 million paired reads per sample.

**Fusion detection.** A minimal criteria of 21 one split read support was imposed for filtering of predicted ABC21 fusion events to facilitate the discovery of fusion transcripts with low representation in the transcriptome. All fusions involving ABC21 were manually reviewed in IGV.

**Archer FusionPlex.** A custom Archer FusionPlex assay (ArcherDX, Colorado) was utilised to interrogate 5 fusion partners for ABC21. Up to 200 ng of RNA was utilised to generate sequencing libraries, as per manufacturer’s protocol. Libraries were sequenced on a MiSeq (Illumina) generating approximately 2 million reads per sample. Summary treatment information is provided in Supplementary Table 1.

Quantitative reverse transcription PCR (Q-RT-PCR) was performed to measure ABC21 transcript abundance. Q-RT-PCR was performed in triplicate to examine ABC21 expression, with GAPDH or HPRT and ACTB for normalisation. Primer sequences are listed in Supplementary Table I. The ΔΔCT method was used to calculate expression levels compared to ABC21 expression in the SKOV3 cell line. A custom Archer FusionPlex assay (ArcherDX, Colorado) was performed on 20 cell lines, and one fusion-positive line was sequenced on the CLS25A40-ABCB1 fusion transcript using qRT-PCR with primers to exons 1 of CLS25A40 and exon 3 of ABC21, an assay described previously\cite{41}. Primer sequences are listed in Supplementary Table I. Briefly, PCR conditions were as follows: 98 °C for 30 s, 30 cycles of 98 °C for 10 s, 60 °C for 30 s, 72 °C for 10 s and 72 °C for 10 min. PCR product from first PCR was purified using QIAquick PCR purification kit (QIAGEN), for use as template in the second PCR. The PCR product from the second PCR was run on a 2% agarose gel.

In vitro studies. Patient-derived cell lines AOC818.5 and AOC821.2 were established from AOCs patient ascites. Approximately, 2 mL of ascites fluid collected at recurrence was centrifuged at 1500 rpm for 5 min to create a cell pellet. Supernatant was removed and the cell pellet was resuspended in 10 mL of complete RPMI media (RPMI 1640, 10% FBS, 50 µ/mL penicillin and 50 mg/mL streptomycin) and transferred into a standard humidi-
Cytopsins of AOC518.5 cells were generated and fixed in 4% PFA for 1 h at RT. Cells were permeabilised using 0.1% Triton-X in 50 mM Tris-HCl pH 7.6 for RT for 10 min, before washing in phosphate-buffered saline (PBS). Peroxidase activity was quenched with 3% H2O2 (5 min at RT), followed by PBS rinses. Slides were blocked for 30 min at RT in TNR Blocking Buffer (Anti-Rabbit Immunopex Kit, Vector Labs). Anti-MDR1 antibody (1:400) (D3H1Q1, Cell Signalling Technology) and Rabbit IgG Isotype control (1:145.5) in TNB Blocking Buffer were incubated on slides for 1 h at RT in a humid chamber. Slides were washed in TBST and incubated with PE Anti-Rabbit HRP (1:1000) for 30 min, before washing in TBST. Slides were incubated for 6 min with TSA reagent with 1:100 dilution of Tyramide fluor in PE Amplification Diluent, then washed in TBST. DAPI was added prior to coverslipping and imaging on the Vectors.

AOC518.5 and AOC521.2 cells were seeded in triplicate at 7.5 × 10^3 cells per well in 96-well black-walled plates 24 h prior to drug treatment. Cells were treated for 72 h with a 10-point dilution series of paclitaxel or cisplatin with or without 250 nM elacridar (Selleckchem) in antibiotic-free media. Cell viability was determined via DAPI staining and high content imaging on the Cellomics ArrayScan VTI platform. Briefly, cells were fixed with 4% PFA then permeabilized with 0.2% Triton X-100 and stained with DAPI (1:1000 dilution). IC50 doses of paclitaxel and cisplatin were approximated by fitting a four-parameter dose–response curve (Hill equation) and all parameters used in curve comparison using Prism 7 (GraphPad)31.

**Reporting summary.** Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**
The whole genome and transcriptome sequencing data will be deposited in the European Genome-phenome Archive (EGA). Most of the data will be publicly available, the germline data will not be publicly available due to restraints imposed by the ethics committee, requests for further data can be made to the EGA Data Access Committee (DAC).

The Patch et al. data is available from the EGA repository under the accession code EGAD00001000877. Primary triple negative breast cancer expression data (n = 123) was obtained from cbioPortal ([http://www.cbioportal.org/]), Breast Invasive Carcinoma (TCGA, Nature 2012).

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