CORRESPONDENCE

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Induction of circulating ABCB1 transcripts under platinum-based chemotherapy indicates poor prognosis and a bone micrometastatic phenotype in ovarian cancer patients

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Abstract

The drug efflux transporter P-glycoprotein, encoded by the ABCB1 gene, promotes acquired chemoresistance. We explored the presence and clinical relevance of circulating cell-free ABCB1 transcripts (cfABCB1[tx]) in ovarian cancer patients (173 longitudinal serum samples from 79 cancer patients) using digital droplet PCR. cfABCB1[tx] were readily detectable at primary diagnosis (median 354 mRNA copies/20 μl serum), paralleled FIGO-stage and predicted surgical outcome (p=0.023, p=0.022, respectively). Increased cfABCB1[tx] levels at primary diagnosis indicated poor PFS (HR=2.329, 95%CI:1.374–3.947, p=0.0017) and OS (HR=2.074, 95%CI:1.194–3.601, p=0.0096). cfABCB1[tx] induction under platinum-based chemotherapy was an independent predictor for poor OS (HR=2.597, 95%CI: 1.218–5.538, p=0.013) and paralleled a micrometastatic phenotype, shaped by the presence of disseminated tumor cells in the bone marrow. A strong correlation was observed between cfABCB1[tx] and circulating transcripts of the metastasis-inducer MACC1, which is the transcriptional activator of ABCB1. Combined assessment of cfABCB1[tx] and circulating cell-free MACC1 transcripts (cfMACC1[tx]) resulted in an improved prognostic prediction, with the cfABCB1[tx]-high/cfMACC1[tx]-high phenotype bearing the highest risk for relapse and death. Conclusively, we provide proof of principle,

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Schwarz *et al. Molecular Cancer*   (2024) 23:174

Page 2 of 7

that ABCB1 transcripts are readily traceable in the liquid-biopsy of ovarian cancer patients, advancing a new dimension for systemic monitoring of ABCB1/P-glycoprotein expression dynamics.

Keywords Ovarian cancer, ABCB1, Liquid biopsy, Platinum-resistance

To the editor

Ovarian cancer is the leading cause of death among gynaecological malignancies. Standard treatment of advanced ovarian cancer consists of primary surgical debulking, aiming at macroscopic complete tumor resection, followed by adjuvant platinum/paclitaxel-based chemotherapy. Despite modern anti-angiogenic treatment with bevacizumab and PARP-inhibition in patients with homologous recombination deficiency (HRD), the overall prognosis of advanced ovarian cancer remains poor with the majority of advanced ovarian cancer patients experiencing relapse. Although 80% of patients initially respond to platinum/paclitaxel-based chemotherapy, primary platinum-resistance typically develops over time and constitutes a major clinical challenge. Therefore, the identification of blood-based predictive and prognostic biomarkers is a clinical priority. A known risk factor for poor survival in ovarian cancer is the early dissemination of occult micrometastases, shaped by the presence of single disseminated tumor cells (DTCs) in the bone marrow [1]. While a minor fraction of DTCs are known to provide metastasis initiating capacity, DTCs frequently enter a state of dormancy [2]. However, the exact biological determinants for their reactivation as drug resistant cells with metastatic outgrowth are poorly understood.

Among a variety of molecular mechanisms, platinum resistance typically arises in tumor cells by increased drug export, which can be mediated by ATP binding cassette (ABC) transporters [3]. ABC transporters constitute an ubiquitous superfamily of integral membrane proteins and confer ATP-mediated translocation of substrates across membranes. P-glycoprotein, encoded by the ATP Binding Cassette Subfamily B Member 1 *(ABCB1)* gene, is one of the most relevant ABC transporters with a wide range of efflux substrates, including chemotherapeutic agents or tyrosine kinase inhibitors. It promotes acquired resistance to a variety of structurally unrelated anti-cancer drugs, a phenomenon known as multidrug resistance (MDR) [4]. A recent meta-analysis on 8607 patients confirmed that *ABCB1* overexpression, either on mRNA or protein level, is associated with chemoresistance and poor prognosis in ovarian cancer patients [5]. However, previous studies were restricted to the analysis of P-glycoprotein or ABCB1 mRNA on the basis of cancer tissue, which is typically non-available in a longitudinal setting during treatment. Whether P-glycoprotein is detectable on ABCB1 transcript level in the blood of ovarian cancer patients and may serve as a liquid-biopsy marker, is completely unknown.

Here, we investigated the presence and potential clinical relevance of circulating cell-free ABCB1 RNA transcripts (cfABCB1^{tx}) in 173 longitudinal serum samples from 79 ovarian cancer patients using droplet digital polymerase chain reaction (ddPCR; Supplementary Table 1, Supplementary Methods). At primary diagnosis, cfABCB1^{tx} was detectable with a mean of 354 mRNA copies/20 µl serum (range 172–730 copies), which was significantly higher than in healthy controls (ED = 441 mRNA copies/20µl; $p < 0.0001$; Fig. 1A). cfABCB1^{tx} levels were significantly elevated in patients with advanced (Fédération Internationale de Gynécologie et d'Obstétrique (FIGO) III + IV) vs. early (FIGO I + II) ovarian cancer (estimated difference (ED) = 425 mRNA copies/20 µl serum, $p = 0.023$; Fig. 1B). Moreover, preoperative cfABCB1^{tx} levels from patients with any residual tumor, left after primary debulking, were significantly increased compared to patients with a macroscopic

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Fig. 1 Clinical relevance of cfABCB1^{tx} levels in ovarian cancer patients. Box plots comparing cfABCB1^{tx} levels at primary diagnosis with regard to (**A**) healthy controls, (**B**) FIGO-stage, (**C**) surgical outcome and (**D**) *BRCA1/2* mutational status. Statistical analysis was performed using the Mann-Whitney test or the Kruskal-Wallis test with Dunn's correction (**** $p < 0.0001$; * $p < 0.05$). **E** Kaplan-Meier analysis comparing PFS and OS among median-stratified cfABCB1^{tx} levels (cfABCB1^{tx}-low vs. cfABCB1^{tx}-high) in ovarian cancer patients. Statistical analysis was performed using the Log-rank (Mantel-Cox) test (** $p < 0.01$). **F** Graphical illustration of the longitudinal blood sampling strategy. From each patient, five longitudinal plasma samples were obtained in the course of primary treatment, framed by primary diagnosis and the completion of adjuvant platinum-based chemotherapy. The illustration was created using Biorender.com. **G** Boxplot comparing cfABCB1^{tx} levels during the course of treatment. Statistical analysis was performed using a mixed effects analysis with Dunnet correction (** $p_{adj} < 0.01$; **** $p_{adj} < 0.0001$). Representative examples for a longitudinal progression pattern of cfABCB1^{tx} levels with (**H**) an induction of cfABCB1^{tx} (referred to as "*cfABCB1^{tx}-induction-pattern*") or (**I**) a cfABCB1^{tx} decline under adjuvant platinum-based chemotherapy (referred to as "*cfABCB1^{tx}-decline-pattern*"). **J** Kaplan-Meier analysis comparing PFS and OS among ovarian cancer patients with a *cfABCB1^{tx}-induction-pattern vs.* a *cfABCB1^{tx}-decline-pattern*. Statistical analysis was performed using the Log-rank (Mantel-Cox) test (* $p < 0.05$)
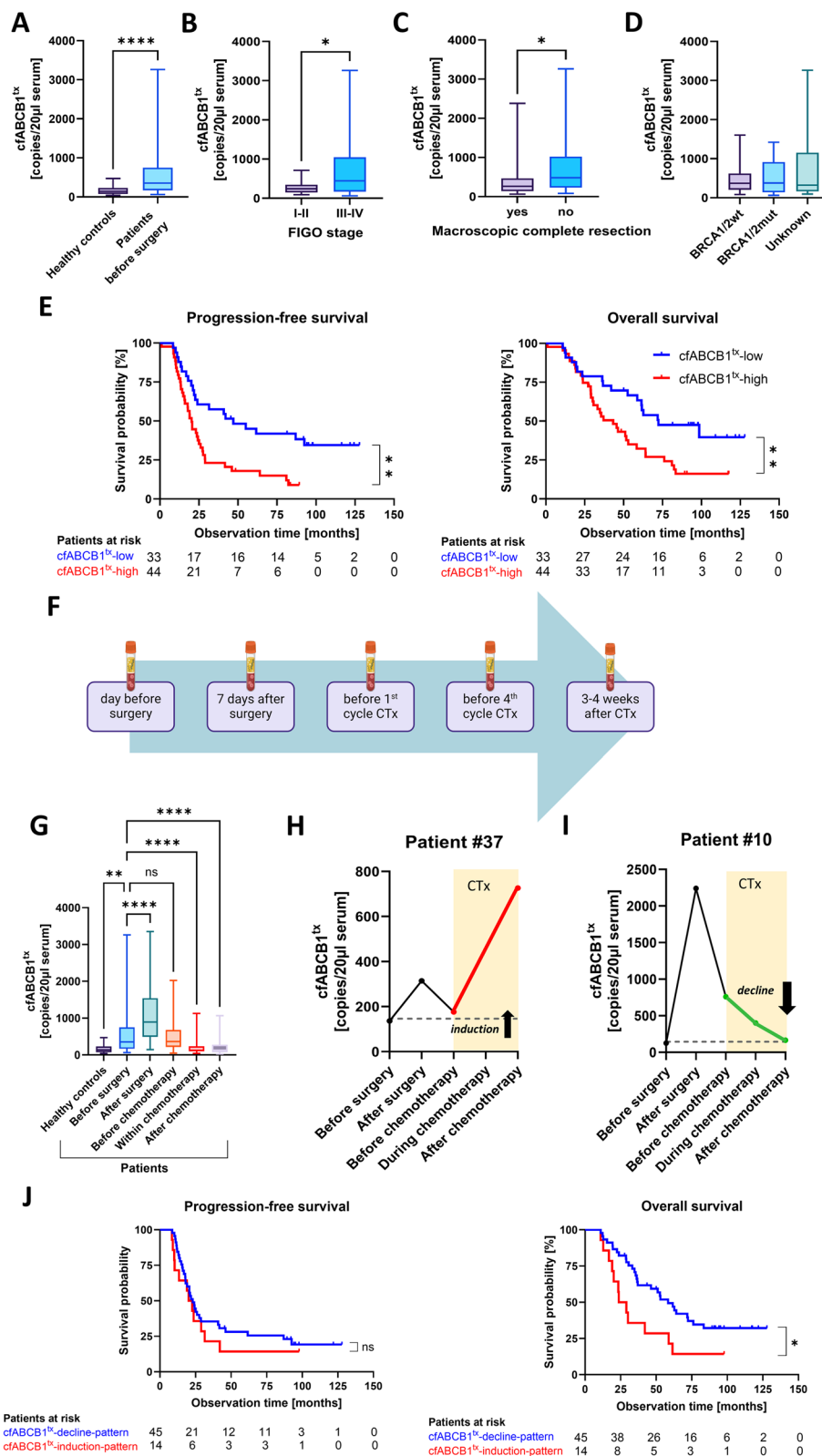
Schwarz *et al. Molecular Cancer*   (2024) 23:174

Page 3 of 7

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Schwarz *et al. Molecular Cancer* (2024) 23:174

Page 4 of 7

complete resection (ED = 268 mRNA copies/20 μl serum, $p = 0.022$; Fig. 1C). cfABCB1^{tx} levels were unrelated to the standard serum biomarker cancer antigen 125 (CA125; $r^2 = 0.03525$; $p = 0.1143$) or Breast Cancer 1/2, Early Onset *(BRCA1/2)* mutational status (Fig. 1D). Patients with a cfABCB1^{tx} level > 290 copies/20 μl serum (cABCB1^{tx}-high) had a significantly worse progression-free survival (PFS; hazard ratio (HR) = 2.329, 95% confidence interval (95%CI): 1.374–3.947, $p = 0.0017$) and overall survival (OS; HR = 2.074, 95%CI: 1.194–3.601, $p = 0.0096$) compared to patients with cfABCB1^{tx} level ≤ 290 copies/20 μl serum (cfABCB1^{tx}-low; Fig. 1E; Supplementary Table 2). Since ABC-transporters are not only involved in acquired chemoresistance but also in a variety of other cancer hallmarks, such as proliferation or invasion [6], higher levels of cfABCB1^{tx} may indicate more aggressive tumors with poor prognosis. In our patient cohort, $n = 14$ patients were identified as primary platinum resistant, according to the conventional definition of a documented relapse within the first six months after the completion of adjuvant platinum-based chemotherapy. There was a numerical trend that cfABCB1^{tx} levels at primary diagnosis were elevated in platinum resistant patients (ED = 304 mRNA copies/20 μl serum).

We further monitored cfABCB1^{tx} levels along primary treatment in a subset of 59 patients, from whom longitudinal serum samples were available (Fig. 1F and G; Supplementary Fig. 1). cfABCB1^{tx} levels showed a particular pattern of progression in the course of treatment with transient rise of median cfABCB1^{tx} levels after primary debulking (ED = 464 mRNA copies/20 μl serum, $p_{adj} < 0.0001$), followed by a strong decrease in the subsequent course of adjuvant platinum-based chemotherapy (Fig. 1G). After chemotherapy, median cfABCB1^{tx} levels significantly dropped below baseline level (ED = 403 mRNA copies/20 μl serum, $p_{adj} < 0.0001$; Fig. 1G). However, there was a subgroup of patients ($n = 14$) with a relative increase of cfABCB1^{tx} under chemotherapy, referred to as "*cfABCB1^{tx}-induction-pattern*" (Fig. 1H). This was opposed by a decline of cfABCB1^{tx} under chemotherapy ("*cfABCB1^{tx}-decline-pattern*"), observed in remaining majority of patients (Fig. 1I ($n = 45$)). While

mean cfABCB1^{tx} level in the *decline-pattern* dropped after chemotherapy towards the level of healthy controls, it remained significantly elevated in patients with an *induction-pattern* (ED = 214 mRNA copies/20 μl; $p = 0.0001$; Supplementary Fig. 2). Interestingly, induction of cfABCB1^{tx} under chemotherapy was prognostically informative and was an independent predictor for a poor OS (HR = 2.597, 95%CI = 1.218–5.538; $p = 0.013$: Fig. 1J, Supplementary Table 3 and 4). Furthermore, we observed the numerical trend, that patients with a *cfAB-CB1^{tx}-induction-pattern* were more likely to be primary platinum resistant compared to patients with a *cfAB-CB1^{tx}-decline-pattern* (28.6% (4/14) vs. 15.6% (7/45)). Although previous studies suggested an association between high P-glycoprotein expression and chemoresistance in ovarian cancer patients [5], only paclitaxel [7] but not platinum [8] is supposed to be a direct P-glycoprotein substrate, so that the functional contribution of P-glycoprotein to platinum/paclitaxel resistance is not entirely understood. Conceivably, cfABCB1^{tx} induction under chemotherapy in our patients could be explained by (i) an increased ABCB1 expression in the tumor in terms of a generalized cellular stress response [8], (ii) an unspecific cfABCB1^{tx} release due to increased cell-death under chemotherapy or (iii) a specific response of tumor cells to platinum exposure as part of acquired chemoresistance. Considering the limited number of platinum resistant patients, our results call for a large-scale validation with independent patient cohorts, which was beyond the scope of this present study.

A total of 64/79 of our patients consented to bilateral bone marrow aspiration and analysis for the presence of DTCs on a single cell level, using pan-cytokeratin (CK) epitopes (8/18 and 8/19 heterodimers) as immunocytochemical selection marker. At primary diagnosis of ovarian cancer, 17/64 (26.6%) patients were DTC positive with the presence of at least one CK positive cell. In the remaining 47/64 patients, no DTCs could be detected (DTC negative; Fig. 2A and B).

Median cfABCB1^{tx} levels at primary diagnosis, after surgery and during chemotherapy were statically indistinguishable between DTC-positive vs. DTC-negative

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Fig. 2 Association of cfABCB1^{tx} with a bone micrometastatic phenotype and cfMACC1^{tx}. **A** Representative images of pan-cytokeratin positive (CK$^+$) disseminated tumor cells in the bone marrow (DTCs). **B** Bar chart showing the patients' DTC-positivity rate at primary diagnosis of ovarian cancer. Boxplots comparing cfABCB1^{tx} levels of DTC-positive vs. DTC-negative patients (**C**) at primary diagnosis (**D**) after debulking surgery (**E**) before adjuvant platinum-based chemotherapy (**F**) after the first three cycles of chemotherapy (**G**) after the completion of chemotherapy. Statistical analysis was performed using unpaired t-test (* $p < 0.05$). **H-M** Correlation of longitudinal cfABCB1^{tx} and cfMACC1^{tx} levels during the course of primary ovarian cancer treatment, assessed by (**H-L**) linear regression analysis and (**M**) Pearson correlation matrix. Above mentioned longitudinal time-points cfABCB1^{tx} and cfMACC1^{tx} detection are indicated in the correlation matrix by Arabic the numbers 1–5. Kaplan-Meier analysis for (**N**) PFS and (**O**) OS among combined biomarker assessments (cfABCB1^{tx} and cfMACC1^{tx}). Statistical analysis was performed using the Log-rank (Mantel-Cox) test (** $p < 0.01$, * $p < 0.05$)
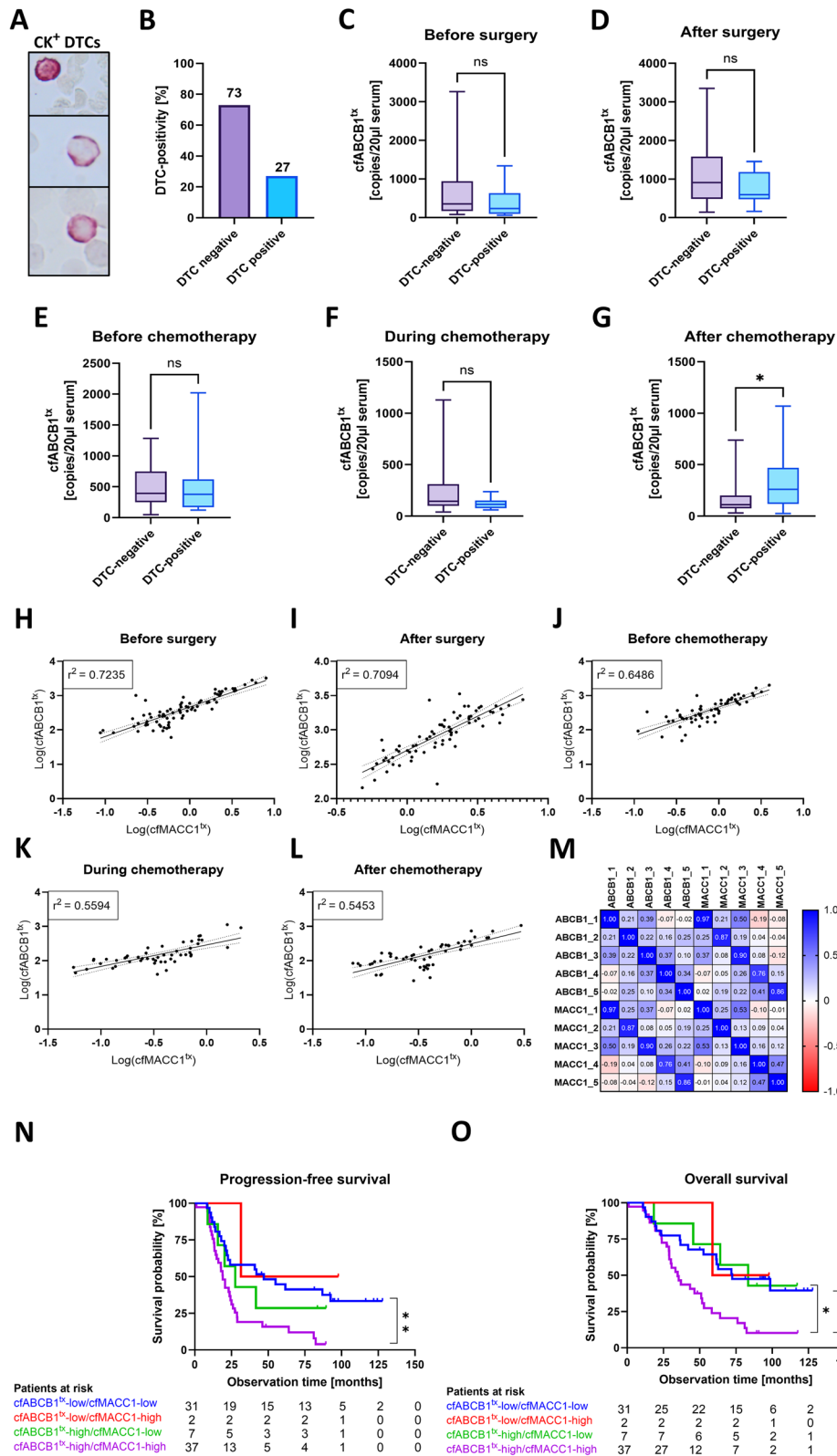
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Schwarz *et al. Molecular Cancer* (2024) 23:174

Page 6 of 7

patients (Fig. 2C-F). After the completion of chemotherapy, however, cfABCB1^{tx} levels diverged towards a significantly increased level in DTC-positive patients (ED = 156 mRNA copies/20 µl serum; $p = 0.018$; Fig. 2G). In line with this finding, there was a striking association between cfABCB1^{tx} dynamics and DTC-status. In DTC-positive patients, there was a significantly higher proportion of patients with an *cfABCB1^{tx}-induction-pattern* compared to DTC-negative patients (58.33% (7/12) vs. 8.33% (3/36); $p = 0.0009$). This result is of particular interest, as it shows for the first time an association between cfABCB1^{tx} dynamics and a bone micrometastatic phenotype. Our data suggest that *ABCB1* expression could be related to the reactivation of dormant (potentially drug resistant) DTCs in the bone marrow.

We have previously demonstrated, that Metastasis-Associated in Colon Cancer protein 1 (MACC1) is a key metastatic driver and prognostic biomarker in a variety of solid cancers [9]. By its function as a transcription factor, MACC1 promotes the expression of metastasis-associated genes. Moreover, MACC1 induces drug-efflux mediated chemoresistance by transcriptional activation of *ABCB1* [10] and genetic knockdown of the *MACC1* gene reverts platinum resistance in ovarian cancer cells [11]. We moreover demonstrated that increased levels of circulating cell-free MACC1 transcripts (cfMACC1^{tx}) indicate poor prognosis in glioblastoma, colorectal-, gastric-, and ovarian cancer [12–15]. However, whether the interdependence between *MACC1* and *ABCB1* expression in a cellular context [10] is projected into the liquid biopsy and whether this could be exploited for diagnostic purposes in cancer patients, particularly ovarian cancer, has not yet been established. Therefore, we compiled longitudinal cfABCB1^{tx} with cfMACC1^{tx} levels, which we had previously measured in the same serum samples of our patient cohort [15]. In fact, there was a remarkably strong correlation between cfMACC1^{tx} and cfABCB1^{tx} in serum samples of healthy donors ($r^2 = 0.9077$, $p < 0.0001$) and in the patients at primary diagnosis of ovarian cancer ($r^2 = 0.7235$, $p < 0.0001$), strongly supporting the previously reported MACC1-ABCB1 link in the cellular context [10]. Although gradually lowering, this correlation was sustained along the entire course of treatment (Fig. 2H-M). According to Kaplan-Meier analysis, the cfABCB1^{tx}-high/cfMACC1^{tx}-high vs. cfABCB1^{tx}-low/cfMACC1^{tx}-low phenotype was associated with greatest difference in PFS (HR = 2.584, 95% CI: 1.466–4.555, $p = 0.001$; Fig. 2N) and OS (HR = 2.498, 95% CI: 1.380–4.520, $p = 0.0025$; Fig. 2O). This suggests that the collinearity of the transcription factor MACC1 and its transcriptional target gene *ABCB1*, as observed in a

cellular context [10], is likely to be projected into the liquid biopsy of ovarian cancer patients, conceivably due to a proportional release of both transcripts into the circulation.

Conclusion

We for the first time provide proof of principle that *ABCB1* transcripts are readily traceable in the liquid-biopsy of ovarian cancer patients, advancing a new dimension for systemic monitoring of ABCB1/P-glycoprotein expression dynamics. We propose that cfABCB1^{tx} induction under chemotherapy predicts poor survival and parallels a bone micrometastatic phenotype. Ultimately, our results provide rationale for a combined cfABCB1^{tx} and cfMACC1^{tx} assessment in ovarian cancer patients and encourage further investigation on cfABCB1^{tx} levels as a possible response predictor of a MACC1/P-glycoprotein directed therapy.

Abbreviations

HRD	homologous recombination deficiency
DTCs	disseminated tumor cells
ABC	ATP-binding cassette transporters
MDR	multidrug resistance
ABCB1	ATP Binding Cassette Subfamily B Member 1
cfABCB1^{tx}	circulating cell-free ABCB1 RNA transcripts
ddPCR	droplet digital polymerase chain reaction
FIGO	Fédération Internationale de Gynécologie et d'Obstétrique
ED	estimated difference
CA125	cancer antigen 125
BRCA1/2	Breast Cancer 1/2, Early Onset
PFS	progression-free survival
HR	Hazard ratio
CI	confidence interval
OS	overall survival
MACC1	Metastasis-Associated in Colon Cancer protein 1
cfMACC1^{tx}	circulating cell-free MACC1 transcripts

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12943-024-02087-8.

Supplementary Material 1.

Supplementary Material 2.

Availability of data and materials

No datasets were generated or analysed during the current study.

Schwarz *et al. Molecular Cancer*   (2024) 23:174

Page 7 of 7

Declarations

Ethics approval and consent to participate
This study strictly adheres to good clinical practice guidelines, national laws and the Declaration of Helsinki. Informed written consent was obtained from all patients and the study had been approved by the Local Research Ethics Committee (Ethikkommission an der Technischen Universität Dresden) in Dresden, Germany (reference number; EK74032013; EK236082012).

Competing interests
The authors declare no competing interests.

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