Detection of circulating tumor cells in platinum-resistant ovarian cancer patients enrolled in the GANNET53 study

E. Obermayr[1], E. Braicu[2] , E. Taube[3], J. Sehouli[2] , I. Vergote[4] , E. Pujade-Lauraine[5], I. Ray-Coquard[6], P. Harter[7], P. Wimberger[8], F. Joly- Lobbedez[9], S. Mahner[10], U.M. Moll[11], N. Concin[12], R. Zeillinger[1]

[1]Molecular Oncology Group, Department of Obstetrics and Gynecology, Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria; [2]Department of Gynecology, European Competence Center for Ovarian Cancer; Campus 3 Virchow Klinikum, Charité, Universitätsmedizin Berlin, Berlin, Germany; [3]Institute of Pathology, Campus Charité Mitte, Charité, Universitätsmedizin Berlin, Berlin, Germany; [4]Division of Gynecological Oncology, Department of Obstetrics and Gynecology, University Hospitals Leuven, Katholieke Universiteit Leuven, Leuven, Belgium; [5]Assistance Publique – Hôpitaux de Paris, Hôpital Tenon, Paris, France; [6]Centre Anticancereux Léon Bérard, University Claude Bernard Lyon, GINECO Group, Lyon, France; [7]Department of Gyneacologic Oncology, Kliniken Essen Mitte, Evang. Huyssens-Stiftung/Knappschaft GmbH, Essen, Germany; [8]Klinik und Poliklinik für Frauenheilkunde und Geburtshilfe, Gynäkologisches Krebszentrum, University Hospital Carl Gustav Carus Dresden, Technische Universitaet Dresden, Dresden, Germany; [9]Centre François Baclesse, Centre de Lutte Contre le Cancer (CLCC) de Caen, Caen, France; [10]Department of Gynecology, University Medical Center Hamburg-Eppendorf, AGO, Hamburg, Germany; [11]Universitätsmedizin Göttingen, Georg-August-Universität Göttingen, Göttingen, Germany; [12]Department of Obstetrics and Gynecology, Innsbruck Medical University, Innsbruck, Austria

* eva.obermayr@muv.ac.at

The project has received funding from the EU 7th Framework Programme for research, technological development and demonstration (grant agreement no 602602), and was supported by Angle plc. (UK) by an in-kind contribution of Parsortix™ devices and microfluidic separation cassettes.

Background

The GANNET53 clinical trial (EUDRACT Number: 2013-003868-31) combats metastatic platinum-resistant ovarian cancer with a novel drug strategy targeting stabilized mutant p53 protein, the central driver of aggressiveness and metastatic ability of the disease. Mutp53 proteins depend on folding support by the Hsp90 chaperone, while Hsp90 blockade by Ganetespib induces degradation of mut53 and increased sensitivity to chemotherapeutics.
In the course of the Phase II trial, patients were randomized in a 2:1 manner to receive Paclitaxel (P) + Ganetespib (G) or P alone (Figure 1). In the present translational study, we asked whether circulating tumour cells (CTCs) might be suitable for monitoring patients and determining their response to therapy.

Methods

Peripheral blood was taken after written informed consent at start of treatment cycle 1 (C1D1) and 24 hours later (C1D2), of cycle 2 (C2), of cycle 3 (C3), and at every other cycle thereafter (C3, C5,…) until disease progression occurred. The blood was drawn in Cell-free DNA blood collection tubes (Streck), shipped to the lab, and processed within 24 hours using a two-step protocol consisting of density gradient centrifugation and enrichment using the microfluidic Parsortix™ technology (Angle plc, UK; Figure 2).

The total RNA was extracted from the enriched cells (RNeasy Micro Kit, Qiagen) and converted into cDNA (SuperScript VILO, Invitrogen). After a specific pre-amplification (TaqMan PreAmp Master Mix, Life Technologies) of all 28 target genes (Table 1), qPCR was performed in duplicates (ViiA7 Real-Time PCR System, Life Technologies).

At each cycle of treatment, the samples were stratified into two groups: samples with a mean Ct-value of <35 were assigned as positive for the respective gene transcript, and samples with a mean Ct-value ≥35 as negative. Progression-free survival (PFS) was defined as the time from the date of the blood draw at each respective cycle to the date of last contact or to the date of progression. For each gene, the association of the two groups (positive vs. negative) and PFS was assessed at every time-point of blood draw using Kaplan-Meier curves and log-rank (Mantel-Cox) tests.

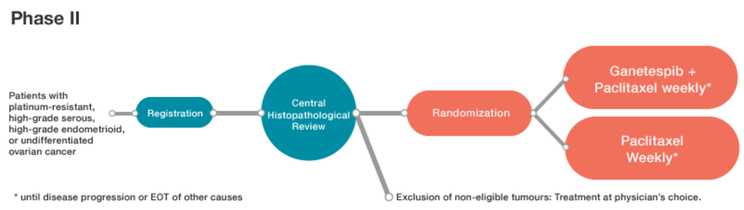

Figure 1: Gannet53 patient recruitment strategy.

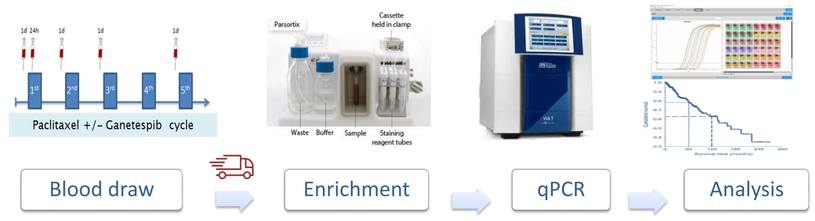

Figure 2: Flowchart of blood sample collection and processing.

AGR2	CK19	ESR1	MAL2	SCGB2A2
CCNE2	EMP2	FN1	PGR	TFF1
CDH1	EPCAM	FXYD3	PLAT	TUSC3
CDH2	ERBB2	GPX8	PPIC	VIM
CDH3	ERBB3	HJURP	PRAME	
CDH5	ERCC1	LAMB1	S100A16	

Table1: 28 Genes markers for qPCR

Results

Patients and bio-banking

A total of 133 patients were enrolled, with 90 patients assigned to the G+P, and 43 patients to the G group. The median OS was 10.0 months, and PFS in the G+P group was not statistically different from the P group (3.5 vs. 5.3 months, p=0.16) [1]. In total, 522 blood samples were taken. On average four (range 1-9) blood samples per patient were available for the analysis of CTC-related transcripts. Excluding blood samples due to withdrawal of consent or poor RNA quality/quantity resulted in a final number of 114 samples taken at C1D1, 99 samples taken at C2, 78 samples taken at C3, 43 samples taken at C5, and 19 samples at C7.

Candidate gene markers for monitoring disease progression

To answer the question, whether the presence of a specific gene transcript correlated with progressive disease (PD) proven by radiologic imaging, we assessed for each gene marker the proportion of positive and negative findings in all samples at initiation of treatment taken at C1D1 (these are patients with PD per definition) or with radiologically confirmed PD during treatment (total n=160), and in those samples taken at partial remission (PR), stable disease (SD), or complete response (CR; total n=115). From all 28 gene transcripts, ERCC1 ranked on top to indicate PD (Table 2).

Gene symbol	Sensitivity	Specificity	Accuracy	Σ	p
ERCC1	0,725	0,461	0,615	1,800	0.002
CDH1	0,481	0,391	0,444	1,316	0.038
VIM	0,419	0,400	0,411	1,230	0.003
ESR1	0,575	0,217	0,425	1,218	<0.001

Table2: Genes transcripts indicating disease progression.

Prognostic impact of ERCC1 and ESR1 before and during treatment

High ERCC1 gene expression before treatment, and furthermore at initiation of each further cycle of treatment until C5, was associated with a significantly higher risk for progression of the disease. In contrast, ESR1 gene expression was associated with better patient outcome. Similar to ERCC1, the difference in PFS was statistically significant from C1 throughout to C5.

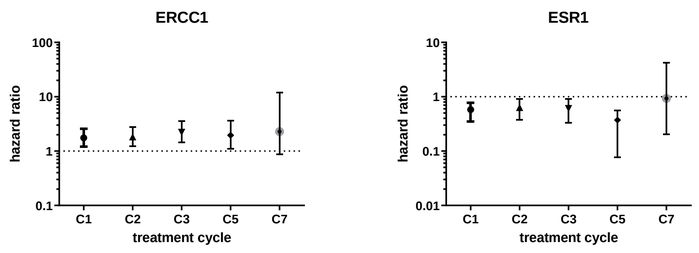

Figure 3: Hazard ratios and 95% CI for PFS of ERCC1- and ESR1-positivity at start and during treatment.

ERCC1+/ESR1- is related with the most unfavorable prognosis

Next we evaluated the combined effect of ERCC1-positivity and absence of ESR1 expression on outcome. Compared to each single marker, the combination had the best accuracy to predict PD (sensitivity 0.825, specificity 0.417; accuracy 0.655; p<0.001).

A landmark analysis comparing the outcome of the four groups ERCC1+/ESR1+, ERCC1-/ESR1-, ERCC1+/ESR1-, and ERCC1-/ESR1+, indicates that the ERCC1-/ESR1+ group survives longest without progression of the disease (Figure 4).

The donut plots in Figure 4 show that the percentage of ERCC1-/ESR1+ samples increases with the number of treatment cycles received, suggesting that patients who survive longer and receive more cycles of treatment are more likely to be ERCC1-/ESR1+. The heat map below also demonstrates that patients who survive longer without PD are very likely ERCC1-/ESR1+ throughout all cycles of treatment (indicated by arrows)

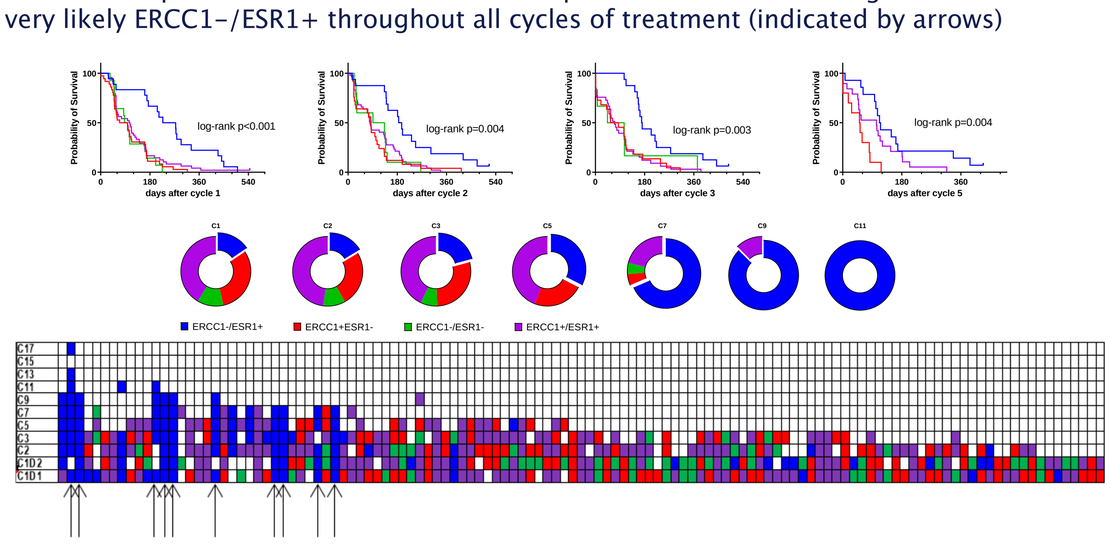

Figure 4: Kaplan-Meier plots of PFS stratified by ERCC1 and ESR1 gene expression at cycle 1, 2, 3 and 5 of treatment. The donut plots show that the percentage of ERCC1-/ESR1+ samples increases with the cycles administered. The heat map depicts the distribution of the four groups per patient. Arrows point to patient assessed as being ERCC1-/ESR1+ throughout all cycles of treatment.

Conclusions

Our results strongly suggest that CTCs before and during treatment are suitable for monitoring platinum-resistant ovarian cancer patients and determining their response to therapy. Beyond enumeration, the molecular characterization of these cells generates valuable knowledge on prognostic and predictive markers, such as ERCC1[2]. Among the CTC-positive patients, ESR1 may downregulate DNA damage response, indicated by the lack of ERCC1 gene expression, and contribute to a better survival. In contrast, ERCC1-positive CTCs may point to an increased capacity to remove platinum-induced DNA damage and thus to drug resistance and poor survival.

References

1. Concin N. et al., J Clin Oncol 36, 2018 (suppl; abstr 5567)
2. Kuhlmann JD et al., ERCC1-positive circulating tumor cells in the blood of ovarian cancer patients as a predictive biomarker for platinum resistance. Clin Chem. 2014;60(10):1282-9.