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


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Background

The GANNET53 clinical trial (EUDRACT Number: 2013-003868-33) 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. MutyB3 proteins depend on folding support by the Hsp90 chaperone, while Hsp90 blockade by Ganetespib induces degradation of muts 3 and increased sensitivity to chemotherapeutics. In the course of the Phase II trial, patients were randomized in a 2:1 manner to receive Pacitaxel (P) + Ganetespib (G) or P alone (Figure 1). In the present translational study, we asked whether circulating tumour cells (CTC) 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 (CD1) and 24 hours later (CD2), cycle 2 (C2), 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 (Bectec), 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 (Angleik, UK; Figure 2). The total RNA was extracted from the enriched cells (RNaseasy 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 (viAria Real-Time PCR System, Life Technologies).

At the end of each treatment cycle, the samples were stratified into two groups: samples with a mean C5-value of <35 were assigned as positive for the respective gene transcript, and samples with a mean C5-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 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.

Results

Patients and bio-banking

A total of 133 patients were enrolled, with 90 patients assigned to the G+P group 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. 3.3 months, p = 0.16). In total, 52 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 CD1, 99 samples taken at C3, 78 samples taken at C5, 43 samples taken at C7, and 19 samples at C9.

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 CD1 (these are patients with PD per definition) or with radiologically confirmed PD during the whole time 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).

Table 2: Genes transcribing indicating disease progression.

Prognostic impact of ERCC1 and ESRI before and during treatment

High ERCC1 gene expression before treatment, and furthermore at initiation of each further cycle of treatment until C7, was associated with a significantly higher risk for progression of the disease. In contrast, ESRI gene expression was associated with better patient outcome. Similar to ERCC1, the difference in PFS was statistically significant from C1 throughout to C5.

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. Among the CTC-positive patients, ESRI may downregulate DNA damage response, indicated by the lack of ERCC1 gene expression, and contribute to a better survival. In contrast, ERCC1-posititive CTCs may point to an increased capacity to remove platinum-induced DNA damage and thus to drug resistance and poor survival.

References