JBUON 2020; 25(1): 40-50
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
Email: editorial_office@jbuon.com

REVIEW ARTICLE

Circulating tumor cells in gynaecological malignancies

Imrich Kiss[1,2,3], Katarina Kolostova[1], Ireneusz Pawlak[4], Vladimir Bobek[1,4,5]

[1]Department of Laboratory Genetics, Laboratory Diagnostics, University Hospital Kralovske Vinohrady, Srobarova 50, Prague, Czech Republic; [2]Department of Gynecology, Military University Hospital and the 3rd Faculty of Medicine, Charles University, U Vojenské nemocnice 1200, Prague, Czech Republic; [3]Charles University, 1st Faculty of Medicine, Katerinska 32, Prague, Czech Republic; [4]Department of Thoracic Surgery, Lower Silesian Oncology Centre, Plac Hirszfelda 12, Wroclaw, Poland; [5]Department of Thoracic Surgery, Socialni pece 3316/12A, Krajska zdravotni a.s. Hospital, Usti nad Labem, Czech Republic and 3rd Department of Surgery, University Hospital FN Motol and 1st Faculty of Medicine, Charles University, V Uvalu 85, Prague, Czech Republic and Department of Histology and Embryology, Wroclaw Medical University, Wybrzeze Ludwika Pasteura 1, 50-367 Wroclaw, Poland

Summary

New non-invasive approaches have developed for diagnosis and treatment of malignant diseases. Cells shed from the primary tumor circulating in the bloodstream with metastasis potential are called Circulating Tumor Cells (CTCs). These cells are easily acquired from the peripheral blood of patients, while several enrichment and isolation methods are available nowadays with different benefits and positive detection rates. A brief characterization of three major categories of detection is described (nucleic acid-based, physical properties-based, antibody-based). In this review we concentrate on gynecological malignancies and how CTCs could be used in the diagnosis of cancer, treatment management and its effective prognosis and early recurrence detection. Presence of CTCs in endometrial cancer patients show worse overall survival, while gene analysis could identify patients in need of systemic therapy after surgical treatment to prevent metastasis and recurrence. Based on the influence of human papillomavirus (HPV) in the etiology of cervical cancer, viral oncogene transcripts could be used as an ideal marker for cervical cancer cells detection. In ovarian cancer, CTCs could help in the differentiation from benign adnexal masses and show a high independence from other biomarkers such as CA125 and HE4. While detection of CTC after complete cytoreductive surgery could indicate invisible lesions, combination of tumor associated genes rises the specificity of CTC detection.

Key words: *biomarker, cervical cancer, circulating tumor cells, endometrial cancer, liquid biopsy, ovarian cancer*

Introduction

In the last two decades, big effort and hopes are put into the discovery of new non-invasive methods for diagnosis and understanding the pathophysiology of malignant diseases. Further development of these tools could help in diagnosis, prognosis, personalized therapy and evaluation of its effectiveness or even alert for recurrences in patients in the follow up period. Liquid biopsy which is easily acquired from patients allows to study the molecular architecture and behaviour of tumors in real time [1]. The tumor material is composed most often by circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), circulating tumor miRNA, proteins and exosomes and besides blood they could be present in several body fluids such as saliva, urine, cerebrospinal fluid, uterine aspirates, pleural effusions or even stool [2,3]. This review analyses the momentary state of circulating tumor cells in the malignancies of the female genital system. The studies used in this review are listed in Table 1.

CTCs are shed from the primary tumor into the bloodstream with potential ability of metastasis (Figure 1). Positive isolation and detection of CTCs have been validated as a prognostic factor in metastatic breast cancer and several other solid

Corresponding author: Vladimir Bobek, MD, PhD. University Hospital, Kralovske Vinohrady, Department of Laboratory Genetics, Srobarova 50, 100 34 Prague, Czech Republic.
Tel: +420 26716 3578, Email: vbobek@centrum.cz
Received: 09/04/2019; Accepted: 14/05/2019

Table 1. List of studies used in this review

Type of cancer	*Authors*	*Year*	*Method*	*Number of patients*	*CTC isolated*	*CTC positivity rate*	*Notes*
Endometrial carcinoma	Kiss et al.	2018	Size based isolation (MetaCell)	92	69	75%	Independent from stage, grade, lymph node involvement
	Bogani et al.	2015	Immunomagnetic selection, immunofluorescence staining (CellSearch)	28	2	7%	Associated with myometrial invasion, lymph node positivity
	Ni et al.	2016	CellSearch	40	6	15%	Associated with cervical involvement
	Lemech et al.	2016	Detection of EpCAM (CellSearch)	30	18	60%	Stathmin expression as a biomarker for treatment response
	Kolbl et al.	2016	RNA isolation, cDNA - qPCR	6 cell lines 10 control	NA	NA	Suitable markers: Cytokeratin 19, claudin 4
	Obermayr et al.	2010	Microarray technology	25 EC, 25 CxCa, 23 CaOv	NA	44% CxCa, 64 EC, CaOv 19%)	CCNE2, DKFZp762E1312, EMP2, MAL2, PPIC, and SLC6A8
	Zhang et al.	2016	Flow cytometry	78	NA	NA	TTF-1 positive CTCs correlated with TNM stating, vascular infiltration, lymphatic metastasis
	Alonso-Alconada et al.	2014	EpCAM based immunoisolation, RTqPCR	34	NA	NA	EMT markers ETV5, NOTCH1, SNAI1, TGFB1, ZEB1 and ZEB2
Cervical carcinoma	Pfitzner et al.	2014	Digital-Direct-RT-PCR	10	3	30%	1 local and 2 systemic diseases
	Takakura et al.	2017	Conditionally replicative adenovirus targeting telomerase-positive cells	23	6	26%	CTCs negative for cytokeratins
	Wen et al.	2018	Magnetic beads separation, CEP8+/ DAPI+/CD45-	99	45	45.9%	Combination of CTC and SCC-Ag a significant predictive marker
Ovarian carcinoma	Suh et al.	2017	TSF - physical deformability	87	49	56.3% (44.2% benign, 100% early stage, 66.7% advanced stage)	Better than other modalities in detecting early stage
	Chebouti et al.	2017	AdnaTest ovarian cancer	91	NA	Before surgery (18% Epithelial, 30% EMT-like), after CHT (14% and 52%)	Emerge of PI3Ka and Twist
	Kuhlmann et al.	2014	AdnaTest ovarian cancer	143	20	14%	ERCC-1 Positive CTCs predict platinum resistance
	Obermayr et al.	2013	Microarray analysis, RT-qPCR	200	49	24.5%	PPIC gene correlates with poor DFS, OS and platinum resistance
	Kolostova et al.	2015	Metacell	118	77	65.2%	MUC1, EPCAM, KRT18, KRT 19 overexpressed
	Obermayr et al.	2017	Multimarker immunostaining, FISH	102	27	26.5%	CTC positivity associated with higher risk of death after optimal surgery
	Marth et al.	2001	Microbead coated with MOC-31 antibody	90	11	12%	PFS nit associated with CTCs in peripheral blood or bone marrow
	Kolostova et al.	2016	MetaCell	56	32	58%	KRT7, WT1, EPCAM, MUC16, MUC1, KRT18 and KRT19 overexpressed
	Judson et al.	2003	Anti-cytokeratin 8, 18, TFS-2, CK-7, CK-20, EGFR mad MiniMACS	64	12	18.7%	CTC positive patients had more grade 3 tumors, follow up had no correlation
	Lee et al.	2017	Electronically conductive chip	54	54	98.1%	CTC positive cluster associated with diminished OS in recurent disease and chemoresistance
	Lou et al	2017	EpCAM+, CK+, DAPI+, CD45 negat	49 (35 malignancy)	9	18.4% (malignant grup)	Only 17.5% positivity in OC, 80% in non-ovarian origin - metastatic tumours to ovary

tumors such as prostate, colorectal and lung cancer [4]. The main limitation of CTC is the low quantity of cells in the blood of cancer patients. The quantity of cells detected differs widely also by the method of isolation.

The broad heterogeneity of CTCs in cancer patients may play a dominant role in therapy resistance and recurrence of disease [5]. Disseminated and CTCs may undergo a broad range of biochemical changes and reversibly acquire fibroblastoid or mesenchymal traits described as epithelial-to-mesenchymal-transition (EMT) already published for breast cancer [6]. This mechanism is a key for malignant progression and is referred to as Oncogenic EMT. This allows tumor cells to gain invasive properties, develop metastatic growth characteristics and defend them during dissemination. Metastatic cells can, after reaching the distant organ, change back to their original epithelial phenotype, mesenchymal-epithelial-transition (MET), to support colonization [7].

CTC detection and isolation: methods and devices

To fully been able to benefit from CTCs, high purity isolation of viable CTCs and their detection is necessary. Isolation is the process when CTCs are separated from all other cells in the sample, while detection is the direct or indirect identification of tumor cells. The enrichment process may precede, when the majority of blood cells are removed from the sample to enhance relative CTC concentration. The most common methods are density gradient centrifugation, red blood cell lysis, positive or negative immunomagnetic separation and sized-based filtration [8]. Based on their working principles, isolation and detection could be classified in three major categories.

1. Nucleic acid-based methods for CTC detection

This method directly or indirectly detects the presence of CTCs by identifying specific DNA or mRNA molecules in the sample. Specific primers are enrolled on polymerase chain reaction (PCR) to target DNA or mRNA molecules known to be associated with cancer cells. CTC detection using mRNA is more effective due to short period of presence in the circulation (unstable molecule with rapid degradation) which means capture of living CTCs, while free DNA could deliver false positive result by capturing molecules released by necrotic or apoptotic cancer cells circulating longer period [9]. Nowadays, multiplex reverse transcription followed by primer specific PCR is widely used, in which expression of multiple transcripts

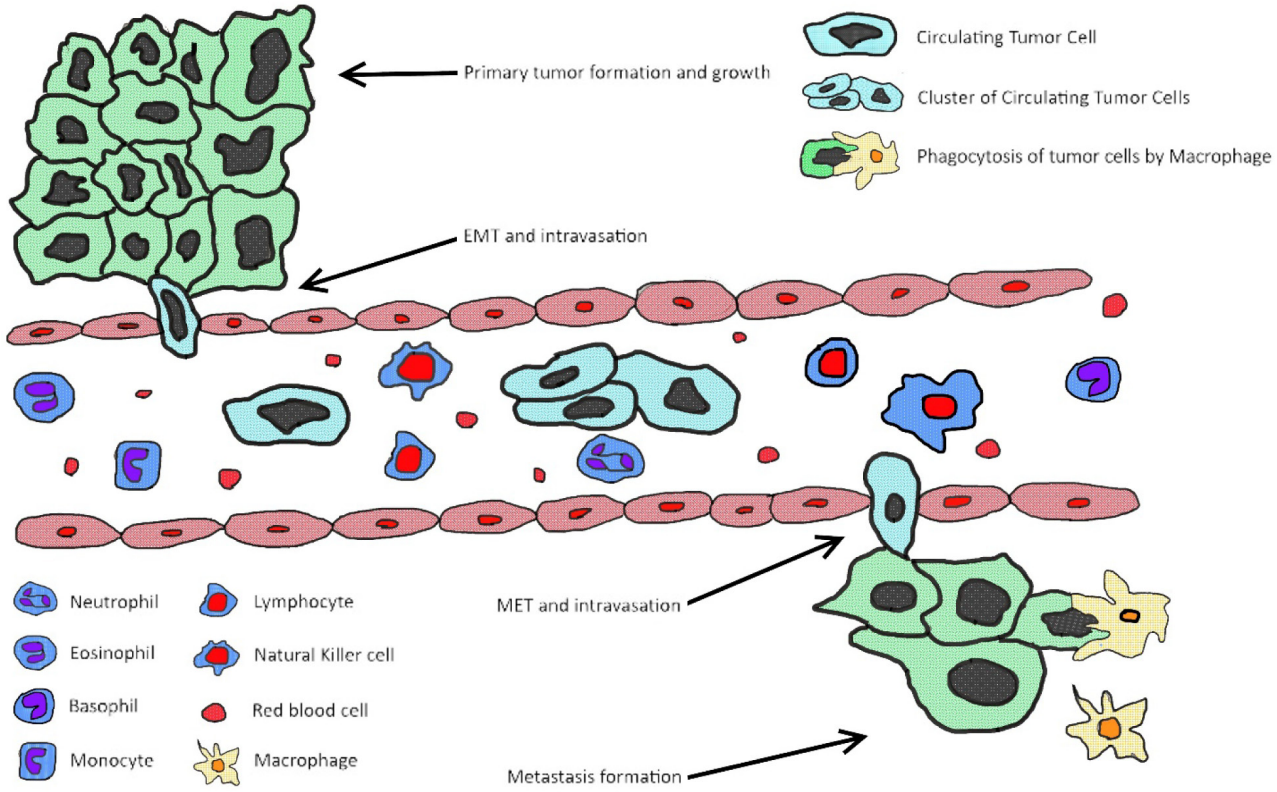

Figure 1. Presentation of potential metastasis: CTCs are shed from the primary tumor into the circulation via EMT process. After intravasation CTCs undergo MET and extravasation with metastasis formation.

could be measured providing improved sensitivity and specificity rated of heterogenic CTCs (commercially available AdnaTest kits – AdnaGen, Germany) [10]. Generally, thanks to the amplification principle of PCR, nucleic acid-based method could effectively pick out the signal from an extremely small amount of marker in a large sample (1 CTC in 5-10×10^6 nucleated blood cells or more than 5 mL of blood) thus offers the highest sensitivity for CTC detection [11]. The essential factor to reach this high sensitivity is the specificity of selected markers. Common markers of epithelial specific genes, such as cytokeratins or EpCAM, are widely used as they constitute malignancies and normally are absent in peripheral blood. Organ-specific markers, such as PSA, MUC1 or tumor specific markers, such as CAE, HER2, could help specify the correct cancer diagnosis [12]. The downside of this approach is possible false-positive result from tissue- and organ-specific markers originating from non-cancer cells that enter the bloodstream due to inflammation or invasive diagnostic biopsies [13]. Moreover, none of the recent markers used are entirely CTC-specific. The major drawback is the fact that CTCs must be lysed before the PCR process, making impossible for further analysis as observation or enumeration.

2. Physical properties-based methods for CTC isolation

These methods use the physical characteristics of cancer cells like density, size, mechanical plasticity and dielectric properties that could be used to isolate CTCs from samples.

a. Isolation of CTCs based on size and mechanical plasticity

This approach considers that cancer cells are larger than normal blood cell, thus it is selected throughout the filtration [14,15]. The simplest method is using track-edged filters or microfilters which are a porous membrane with 8 μm diameter holes that allow the blood cross but capture the bigger CTCs (ISET – Rarecells, France, ScreenCell systems – ScreenCell, France, MetaCell – MetaCell, Czech Republic). The advantage is that the captured cells remain intact allowing their subsequent morphological and molecular analysis [16-19]. This approach could be performed also in a microfluidic setting, where the separation results in a precisely defined topography of microstructures and the laminar flow in microchannels [20]. Advanced technology, such as CTChip by Clearbridge Biomedics, enables to isolate single CTCs with automatic vision-based enumeration and analysis. Methods using size-dependent hydrodynamic forces as formation of microscale vortices or Dean-coupled inertial migration has been also published [21,22], as well as active acoustophoresis technique that practices an external acoustic force to separate different cells in the microchannel [23]. The downside of this method is false-positive result in case of leucocytes capture, false negativity in case the cells become more plastic during EMT and altered functions of isolated CTCs due to mechanical stress during isolation [24-26].

b. Electrokinetic isolation of CTCs

Cells are electrically neutral, but in the electric field polarisable and electric dipoles moments are induced in them [27]. The magnitude and direction of these dipole moments depend on the polarity and conductivity of cell membrane and cytoplasm, cells phenotype, physiological state and morphology [28]. Factors affecting this method is the gradual change of dielectric characteristics due to ion leakage, thus the isolation should be completed as fast as possible. Unfortunately, the process is still relatively slow [29].

3. Antibody-based methods for CTC detection and isolation

The most common method for detection as well as isolation of CTCs. The principle is the antibody-antigen specific binding, mainly done by immunochemistry, but other techniques like Raman spectroscopy, photoacoustic flowmetry and nuclear magnetic resonance have been investigated [30-32]. CTCs are captured to the antibody-mediated matrix most often in a form of magnetic particles or microchannels. The performance of this method depends on the antigen it represents. For detection of CTCs most widely EpCAM and different subtypes of CK are used, while more organ- and tumour-specific markers, such as CEA, EGFR, PSA, HER2, MUC1 could be applied. Up to this date, no marker met the high specificity required for the ideal detection and isolation of CTCs.

a. Immunochemistry methods for CTC detection

Although still not achieving ideal performance in practice, it is considered the most reliable and specific method of CTC detection. CTCs are often referred as CK positive /DAPI positive/ CD45 negative cells [33]. While CD-45 negativity rules out white blood cells, DAPI excludes cell fragments and debris. Flow cytometry, including fluorescence-activated cell sorting (FACS) and the more popular image cytometry mainly referring immunofluorescence microscopy is used in this method. The lat-

ter could incorporate several markers and different molecular (FISH) or cytomorphological (N/C ratio) assays which improve the specificity of detection and integrate automated digital microscopy and computerized post-processing for better practical use [34]. CellSearch system (Menarini, Italy) is the only FDA approved assay for CTC detection. About 99% detection sensitivity was reached by the HD-CTC array, which without the enrichment process, could detect CTC aggregates with high clinical significance in micrometastasis development as well [35-36]. Living CTCs for prognostic significance for a variety of carcinomas could be detected by a novel approach called EPISPOT [37].

b. Immunomagnetic methods of CTC isolation

Magnetic field can be successfully used to isolate CTCs if their magnetic characteristics are selectively modified. Cancer cells can be tagged by antibody-conjugated magnetic microbeads or nanoparticles that bind to a specific surface antigen [38]. In a non-uniform magnetic field the tagged cells migrate towards areas of higher magnetic flux density where they are captured [39].

c. Adhesion-based methods for CTC isolation

This method focuses on an adhesion surface, whose biochemical and topographical properties have been modified to attract and capture cancer cells. This can be performed in static or in continuous-flow microfluidic modes [40]. In the first mentioned, the sample is left incubated on a collagen-coated surface. During incubation CTCs with invasive characteristics tend to invade the surface and are captured, while the rest non-target cells are washed off [41]. The second is achieved by flowing the sample through a straight microchannel coated with antibody against CTCs so the target cells can effectively interact with the capture surface [42].

Endometrial cancer

Cancer of the corpus uteri (EC) is the 7th most commonly diagnosed cancer in female population worldwide with 382,100 estimated new cases and 89,900 deaths in 2018 [43]. In the developed countries it represents the fourth leading cancer in women and the most common malignancy of the female genital tract. In the United States 63,230 new cases and 11,350 deaths were estimated in 2017 [44]. In Europe, the number of new cases was about 100,000 with an incidence of 13.6 per 100,000 in 2012 [45].

Despite the absence of a reliable screening tool, EC is most often diagnosed in early stage because of symptomatic postmenopausal uterine bleeding. Hematogeneous spread is in correlation with deep myometrial invasion [46]. Surgery is the primary treatment method, in addition with adjuvant radiotherapy and chemotherapy in advanced and high-risk cases.

The largest study was published by Kiss et al, in which blood from 92 patients with various grades and stages of EC was isolated for CTCs. Positivity reached 75% of patients and a method described a successful size-based separation method with high detection rate of viable CTCs with proliferation potential (Metacell®). In addition, there was no significant difference between CTC presence and differentiation level (grade), stage of disease and lymph node involvement [47].

Other studies involved rather a smaller number of high-risk EC patients with EpCAM-positive CTCs isolated by CellSearch. Bogani et al isolated CTCs in 2 EC patients from 28 (7% positivity). Both patients were in stage IIIC and CTCs presence was significantly correlated with myometrial invasion and lymph node positivity [48]. Association in CTCs and cervical involvement was published by Ni et al. From 40 EC patients 6 were positive for CTCs (15% positivity), whereas 3 patients had FIGO stage I and 3 patients had stage III with no significant difference in the quantity of cells. Also, no significant correlation was found between CTCs and serum CA125/human epididymis protein 4 (HE4) levels. One patient with type II stage I had repeated CTC examination after the first dose of adjuvant therapy [49]. Another study was provided by Lemech et al in which demonstrated 18 CTC positive EC patients from 30 (60% positivity). CTC correlated with higher stage disease, worse survival, non-endometroid histology over endometroid and tumour size bigger than 5 cm. In addition, CTCs and FFPE tissue blocks were placed for immunohistochemistry staining of EpCAM and stathmin primary antibodies and put in correlation with CTC status. Stathmin was overexpressed in all CTC-positive patients whose tissue was stained (7 patients). This could mean that stathmin has potential as a marker of PIK3K pathway activity which is one of the most studied pathways in EC with aberrations including oncogenic PIK3CA mutations and PTEN loss of function [50].

Further studies observed the presenting genes in CTCs in patients with high-risk EC. Due to the high expression in the investigated cell lines, Cytokeratin 19 and claudin 4 were identified as a suitable gene marker for CTCs in endometrial adenocarcinoma [51]. Obermayr et al conducted a multimarker analysis of six genes (CCNE2, DKFZp762E1312, EMP2, MAL2, PPIC and SLC6A8)

which were positively identified in 64% in a group of 25 EC patients [52]. The expression of thyroid transcription factor (TTF-1) in CTCs was strongly correlated with TNM staging, vascular infiltration and lymphatic spread. Progression-free survival rate and median survival time decreased in the TTF-1 positive cohort, while recurrence rate was significantly lower in the negative group [53]. Finally, Alonso-Alconada et al described the association of molecular CTC-phenotype with plasticity, stemness and epithelial-to-mesenchymal transition (EMT) features which promotes CTC dissemination. Markers of EMT show higher expression in ETV5, NOTCH1, SNAI1, TGFB1, ZEB1 and ZEB2. Expression of ALDH and CD44 pointed to an association with stemness, while the expression of CTNNB1, STS, GDF15, RELA, RUNX1, BRAF and PIK3CA suggests potential therapeutic targets. The significance to clinical practice could be the identification of patients in need of additional systemic therapies after primary surgery to avoid metastasis and to eliminate the risk of recurrence in the future [54].

Cervical cancer

According to a recently published study by the GLOBOCAN, cervical cancer (CxCa) is the third most common cancer after breast and lung cancer worldwide and is also third in cancer-related deaths in female population [43]. Cervical cancer is the most frequently diagnosed cancer in more than half of the countries in Africa and accounting for about 30% of total cancer cases and deaths in the region [55]. In the USA, an estimated 13,240 cases of invasive cervical cancer are expected to be diagnosed with 4,170 deaths in 2018 [44]. In the European Union, there were about 34,000 new cases of cervical cancer and more than 13,000 deaths in 2012 [56].

The etiology of cervical cancer is the infection of cell by Human Papilloma Virus (HPV) and belongs to the so-called virus-induced cancers [57]. The cancers express viral oncogene transcripts specific for infected cells [58]. Over 99% of CxCa are high-risk HPV positive, while the oncogenic properties are mediated by the viral oncogenes E6 and E7 which are responsible for the inactivation of p53 and pRb tumour suppressor proteins [59,60]. The tumour is active only if E6 and E7 are expressed, otherwise cancer cells apoptosis is initiated by the restored p53 and pRb proteins [60]. Therefore, viral oncogene transcripts E6/E7 are the ideal markers for the detection of tumour cells in cancer patients. On this basis it was established a method by Pfitzner et al for detection and quantification of CTCs by digital RT-PCR [61]. She describes a CTC detection rate of 66% in patients witch systemic spread and the Digital-Direct-RT-PCR method as a highly sensitive method in separating HPV16/18-E6 expressing cells from a large number of HPV negative cells. This method could be applied in other tumour types where tumour specific transcripts are already discovered.

The presence of the integrated HPV virus in cervical cancer lesions alongside with cancer cell characteristics could be used in additional methods. Telomerase activity is responsible for the restoration of chromosomes length after cell division, which gives the cancer cells their immortality and its expression could be used as a potential biomarker [62]. The expression of hTERT has been identified as a determinant of telomerase activity and is transcriptionally regulated by its promoter [63,64]. Telomerase-specific replication-selective adenoviruses were designed from adenovirus vectors by inserting the hTERT promoter, restricting their proliferation to telomerase activity only, thus could be used in both *in vivo* and *in vitro* cancer cell detection and even in oncolytic virotherapy [65-67]. Takura et al used a modified adenoviral vector OBP-1101 which expresses GFP in infected cells. CTCs were identified in 6 of 23 samples (26% positivity), with no correlation with distant metastasis, overall survival or progression-free survival [68].

On the other hand, Wen et al published that elevated CTCs and SCC-Ag levels were associated with poor disease-free survival. They collected blood samples from 99 patients with locally advanced cervical cancer (FIGO stage IIB-IVA) and CTC were enriched and magnetically separated by anti-CD45 monoclonal antibody coated in magnetic beads and identified by negative enrichment and immune fluorescence *in situ* hybridization (Neim-FISH). The CTC-positive rate was 45.5% and CTC and SCC-Ag alone showed as strong predictors of DFS. The combination of these 2 biomarkers in a new risk model significantly improved their predictive efficiency for survival than CTC or SCC-Ag level alone [69].

Ovarian cancer

Ovarian cancer (OC) is the deadliest gynecological malignancy, with a 5-year survival rate approximately 47% - a number which remained constant over the past two decades. It is the fifth leading cause of cancer death among women in Europe and the United States and the second most common gynecological malignancy [70]. The annual estimates are 295,400 of new ovarian carcinoma cases and 184,800 deaths worldwide [43]. The

highest rates (11.4 per 100,000 and 6.0 per 100,000, respectively) are reported in Eastern and Central Europe [71]. Although China has a relatively low incidence rate of 4.1 per 100,000 due to its large population, the overall estimates are 52,100 new cases and 22,500 related deaths in 2015 [72]. The same year 21,290 new cases and 14,180 were estimated in the USA [73].

Early diagnosis improves survival, but unfortunately only 15% of ovarian cancers are diagnosed at an early localized stage. Most ovarian cancers are epithelial in origin and treatment prioritizes cytoreductive surgery followed by cytotoxic platinum and taxane chemotherapy. While most tumours initially respond to treatment, unfortunately recurrence is likely to occur within a median of 16 months in advanced-stage disease [74]. Postoperative residual tumour is one of the most important prognostic factors in advanced ovarian cancer [75]. Despite new therapeutic concepts are being used as antiangiogenic therapy or PARP inhibitors, more than half of all patients experience recurrence resulting in poor overall prognosis [76].

There are many studies evaluating the possible prognostic significance of CTCs in OC. Despite the early studies in which detection of tumour cells in the bone marrow and/or blood was not associated with poor prognosis [77], just CTC-positive patients had statistically more grade 3 tumours [78], and later studies proved their profitable use. In a large systematic review conducted by Cui et al on 10 relevant studies with 1164 patients showed a strong association of CTCs (disseminated tumour cells as well) with advanced staging (stage III-IV), poor prognosis (low OS, shortened PFS, DFS), and treatment response (platinum resistance). On the other hand, no association was found with tumor histology, lymph node metastasis and optimal or suboptimal surgery [79]. In a novel electronically conductive and nanoroughened microfluidic platform-based chip was introduced by Lee et al with 98.1% detection rate of CTCs in 54 OC participants. Additionally, reduced OS in patients with recurrent disease and chemoresistance correlated with CTC-cluster positive samples [80]. High detection rate of CTCs (90%) was published by Zhang et al, when from 109 newly diagnosed OC 98 were CTC-positive. The number of CTCs was significantly lower in stage I patients than in advanced stages. High diagnostic significance could be a 100% detection rate in 7 "occult" patients without epithelial ovarian carcinoma symptoms, while CA-125 was elevated only in 4 patients (57%). Elevated expression of EpCAM and HER2 in CTCs were associated with chemoresistance and shorter overall survival [81].

Not only OC is often diagnosed in later stages, but preoperative differential diagnosis of existing adnexal masses is also a challenge. Many studies have examined various modalities (biomarkers like CA-125 and HE4 levels, imaging studies like ultrasound, CT, MRI, PET and their combinations), while Suh et al studied CTCs as a new platform in the evaluation of findings on the ovaries [82]. From a total number of 87 patients, at least one CTC was found preoperatively in 49 (56.3%): 19/43 (44.2%) were benign, 10/10 (100%) early-stage and 14/21 (66.7%) advanced-stage cancer. Only 1 healthy control from 22 (4.5%) was positive for CTCs. In further analysis, preoperative CTC detection was more sensitive in benign vs. early stage (stage I and II) cancer compared with benign vs all-stage cancer and remained even in benign vs stage I cancer. Other diagnostic modalities showed a reversed pattern: modest performance in early-stage cancer and significant in all-stage cancer including borderline tumours. CTCs showed no association with CA-125 levels or ROMA index and could reflect early hematogeneous metastasis before even peritoneal spread. Another study assessed CTCs in 49 women with newly diagnosed complex pelvic masses. No CTCs were found in benign histology cases (0/14) while malignancy was associated with CTCs in 9/35 (25.7%). CTCs were detected only in 5/29 (17.2%) patients diagnosed with OC (all 5 patients had stage III or IV), and of the rest 5 patients 4 were CTC-positive (80%) and diagnosed with non-ovarian origin tumor that metastasized to the ovaries (2 Krukenberg tumour, 1 metastatic endometrial cancer, 1 abdominal soft-tissue sarcoma with peritoneal carcinomatosis) [83].

Another potential benefit of CTCs is that they could have a role in indicating invisible cancer lesions after complete or minimal-residual cytoreductive operations. CTCs present before surgery or neoadjuvant chemotherapy indicate a higher risk of death even after optimal debulking surgery (R0) [84].

In the majority of human malignancies PIK3K/AKT/mTOR signalling pathway is aberrantly activated stimulating proliferation and cell survival [85]. This pathway has also been reported in OC, while EMT is responsible for chemoresistance [86]. Chebouti et al analysed the incidence of epithelial (EpCAM, MUC-1) and EMT-like (PI3Ka, AKT-2, Twist) CTC at primary diagnosis of ovarian cancer (91 patients) and how their detection was altered by platinum-based chemotherapy. Higher number of EMT-like CTCs (30%) were detected than epithelial subtype of CTCs (18%) prior to surgery, which further increased in EMT-like CTCs even after chemotherapy (52%), but decreased in the epithelial subtype of CTCs (14%). Epithelial and EMT-like

CTCs exhibit a low phenotypic overlap as only a minor fraction of CTC-positive patients showed dual positivity for both phenotypes (18% before surgery and 12% after surgery). After chemotherapy a shift towards PIK3Ka and Twist expression was found, which could have a clinical interest as these signalling pathways could be responsible for the recurrence of OC [87].

Further studies of CTC characteristics showed that the presence of ERCC1-positive CTCs at primary diagnosis is and independent predictor of platinum resistance [88]. Auxiliary assessment of ERCC1 transcripts increase the CTC detection rate and presence of ERCC1-positive CTCs reduce progression-free survival and overall survival, while their persistence indicates poor post-therapeutic outcome [89].

Many authors published articles on molecular characterization of CTCs in OC patients. One of the largest studies was conducted by Obermayr et al, in which 11 gene markers (PPIC, GPX8, CDH3, TUSC3, COL3A1, LAMB1, MAM, ESRP2, AGR2, BAIAP2L1, TFF1, EPCAM) were studied in a cohort of 200 patients before therapy (surgery or neoadjuvant chemotherapy) and during follow-up. PPIC gene (Cyclophilin C) was overexpressed in 34 cases (17%) and PPIC positivity during follow up period (13 cases 14% positivity) showed significantly shorter disease-free survival, overall survival and platinum resistance [90]. Another large study of 118 OC patients was conducted by Kolostova et al, successful isolation of CTCs in 77 patients showed 65.2% positivity, from which further 20 patients were tested for gene expression. CTCs overexpressed MUC1 and EPCAM in more than 90% cases, KRT18 and KRT19 was also elevated, while MUC16 (CA125) was detected only in 30% [91]. In another study from the same authors 40 patients with OC were enrolled in a gene expression study. Statistically significant difference was confirmed for the following genes ($p<0.02$): KRT7, WT1, EPCAM, MUC16, MUC1, KRT18 and KRT19. The results suggest that the combination of the above listed genes could confirm CTCs presence in OC patients with higher specificity than when gene analysis tests are performed for one marker only [92].

Concluding remarks

Cancer cells in gynaecological malignancies are present in the circulation of patients and can be isolated and detected by numerous methods. The presence of CTCs seems to be associated by adverse clinicopathological features and worse

clinical outcomes. CTCs have their prognostic value and in times of personal medicine could help in therapy management and its effectiveness control. Recurrences could be detected earlier and reacted more precisely to them.

Conflict of interests

The authors declare no conflict of interests.

References

1. Muinelo-Romay L, Casas-Arozamena C, Abal M. Liquid Biopsy in Endometrial Cancer: New Opportunities for Personalized Oncology. Int J Mol Sci 2018;19:2311.
2. Jia S, Zhang R, Li Z, Li J. Clinical and biological significance of circulating tumor cells, circulating tumor DNA, and exosomes as biomarkers in colorectal cancer. Oncotarget 2017;8:55632-45.
3. Siravegna G, Marsoni S, Siena S, Bardelli A. Integrating liquid biopsies into the management of cancer. Nat Rev Clin Oncol 2017;14:531-48.
4. Cabel L, Proudhon C, Gortais H et al. Circulating tumor cells: clinical validity and utility. Int J Clin Oncol 2017;22:421-30.
5. Brouwer A, De laere B, Peeters D et al. Evaluation and consequences of heterogeneity in the circulating tumor cell compartment. Oncotarget 2016;7:48625-43.
6. Aktas B, Tewes M, Fehm T, Hauch S, Kimmig R, Kasimir-Bauer S. Stem cell and epithelial-mesenchymal transition markers are frequently overexpressed in circulating tumor cells of metastatic breast cancer patients. Breast Cancer Res 2009;11:R46.
7. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest 2009;119:1420-8.
8. Esmaeilsabzali H, Beischlag TV, Cox ME, Parameswaran AM, Park EJ. Detection and isolation of circulating tumor cells: principles and methods. Biotechnol Adv 2013;31:1063-84.
9. Gerges N, Rak J, Jabado N. New technologies for the detection of circulating tumour cells. Br Med Bull 2010;94:49-64.
10. Markou A, Strati A, Malamos N, Georgoulias V, Lianidou ES. Molecular characterization of circulating tumor cells in breast cancer by a liquid bead array hybridization assay. Clin Chem 2011;57:421-30.
11. Andreopoulou E, Yang LY, Rangel KM et al. Comparison of assay methods for detection of circulating tumor

cells in metastatic breast cancer: AdnaGen AdnaTest BreastCancer Select/Detect™ versus Veridex CellSearch™ system. Int J Cancer 2012;130:1590-7.

12. Paterlini-Brechot P, Benali NL. Circulating tumor cells (CTC) detection: clinical impact and future directions. Cancer Lett 2007;253:180-204.
13. Crisan D, Ruark DS, Decker DA, Drevon AM, Dicarlo RG. Detection of circulating epithelial cells after surgery for benign breast disease. Mol Diagn 2000;5:33-8.
14. Kolostova K, Matkowski R, Gürlich R et al. Detection and cultivation of circulating tumor cells in gastric cancer. Cytotechnology 2016;68:1095-102.
15. Kolostova K, Zhang Y, Hoffman RM, Bobek V. In vitro culture and characterization of human lung cancer circulating tumor cells isolated by size exclusion from an orthotopic nude-mouse model expressing fluorescent protein. J Fluoresc 2014;24:1531-6.
16. Desitter I, Guerrouahen BS, Benali-Furet N et al. A new device for rapid isolation by size and characterization of rare circulating tumor cells. Anticancer Res 2011;31:427-41.
17. Hofman VJ, Ilie MI, Bonnetaud C et al. Cytopathologic detection of circulating tumor cells using the isolation by size of epithelial tumor cell method: promises and pitfalls. Am J Clin Pathol 2011;135:146-56.
18. Kolostova K, Spicka J, Matkowski R, Bobek V. Isolation, primary culture, morphological and molecular characterization of circulating tumor cells in gynecological cancers. Am J Transl Res 2015;7:1203-13.
19. Bobek V, Kacprzak G, Rzechonek A, Kolostova K. Detection and cultivation of circulating tumor cells in malignant pleural mesothelioma. Anticancer Res 2014;34:2565-9.
20. Bhagat AA, Hou HW, Li LD, Lim CT, Han J. Pinched flow coupled shear-modulated inertial microfluidics for high-throughput rare blood cell separation. Lab Chip 2011;11:1870-8.
21. Hur SC, Mach AJ, Di Carlo D. High-throughput size-based rare cell enrichment using microscale vortices. Biomicrofluidics 2011;5:22206.
22. Hou HW, Warkiani ME, Khoo BL et al. Isolation and retrieval of circulating tumor cells using centrifugal forces. Sci Rep 2013;3:1259.
23. Augustsson P, Magnusson C, Nordin M, Lilja H, Laurell T. Microfluidic, label-free enrichment of prostate cancer cells in blood based on acoustophoresis. Anal Chem 2012;84:7954-62.
24. Alunni-Fabbroni M, Sandri MT. Circulating tumour cells in clinical practice: Methods of detection and possible characterization. Methods 2010;50:289-97.
25. Cross SE, Jin YS, Rao J, Gimzewski JK. Nanomechanical analysis of cells from cancer patients. Nat Nanotechnol 2007;2:780-3.
26. Kuo JS, Zhao Y, Schiro PG et al. Deformability considerations in filtration of biological cells. Lab Chip 2010;10:837-42.
27. Gonzalez CF, Remcho VT. Harnessing dielectric forces for separations of cells, fine particles and macromolecules. J Chromatogr A 2005;1079:59-68.
28. Gascoyne PR, Vykoukal J. Particle separation by dielectrophoresis. Electrophoresis 2002;23:1973-83.
29. Gascoyne PR, Noshari J, Anderson TJ, Becker FF. Isolation of rare cells from cell mixtures by dielectrophoresis. Electrophoresis 2009;30:1388-98.
30. Ghazani AA, Castro CM, Gorbatov R, Lee H, Weissleder R. Sensitive and direct detection of circulating tumor cells by multimarker μ-nuclear magnetic resonance. Neoplasia 2012;14:388-95.
31. O'Brien CM, Rood KD, Bhattacharyya K et al. Capture of circulating tumor cells using photoacoustic flowmetry and two phase flow. J Biomed Opt 2012;17:061221.
32. Wang X, Qian X, Beitler JJ et al. Detection of circulating tumor cells in human peripheral blood using surface-enhanced Raman scattering nanoparticles. Cancer Res 2011;71:1526-32.
33. Pantel K, Alix-Panabières C, Riethdorf S. Cancer micrometastases. Nat Rev Clin Oncol 2009;6:339-51.
34. Bauer KD, De la Torre-Bueno J, Diel IJ et al. Reliable and sensitive analysis of occult bone marrow metastases using automated cellular imaging. Clin Cancer Res 2000;6:3552-9.
35. Marrinucci D, Bethel K, Kolatkar A et al. Fluid biopsy in patients with metastatic prostate, pancreatic and breast cancers. Phys Biol 2012;9:016003.
36. Cho EH, Wendel M, Luttgen M et al. Characterization of circulating tumor cell aggregates identified in patients with epithelial tumors. Phys Biol 2012;9:016001.
37. Alix-Panabières C. EPISPOT assay: detection of viable DTCs/CTCs in solid tumor patients. Recent Results Cancer Res 2012;195:69-76.
38. Pamme N. On-chip bioanalysis with magnetic particles. Curr Opin Chem Biol 2012;16:436-43.
39. Talasaz AH, Powell AA, Huber DE et al. Isolating highly enriched populations of circulating epithelial cells and other rare cells from blood using a magnetic sweeper device. Proc Natl Acad Sci USA 2009;106:3970-5.
40. Didar TF, Tabrizian M. Adhesion based detection, sorting and enrichment of cells in microfluidic Lab-on-Chip devices. Lab Chip 2010;10:3043-53.
41. Fan T, Zhao Q, Chen JJ, Chen WT, Pearl ML. Clinical significance of circulating tumor cells detected by an invasion assay in peripheral blood of patients with ovarian cancer. Gynecol Oncol 2009;112:185-91.
42. Zheng X, Cheung LS, Schroeder JA, Jiang L, Zohar Y. A high-performance microsystem for isolating circulating tumor cells. Lab Chip 2011;11:3269-76.
43. Ferlay J, Colombet M, Soerjomataram I et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer 2019;144:1941-53.
44. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018;68:7-30.
45. Colombo N, Creutzberg C, Amant F et al. ESMO-ESGO-ESTRO consensus conference on endometrial cancer: Diagnosis, treatment and follow-up. Radiother Oncol 2015;117:559-81.
46. Mariani A, Dowdy SC, Keeney GL, Long HJ, Lesnick TG, Podratz KC. High-risk endometrial cancer subgroups: candidates for target-based adjuvant therapy. Gynecol Oncol 2004;95:120-6.
47. Kiss I, Kolostova K, Matkowski R et al. Correlation Be-

tween Disease Stage and the Presence of Viable Circulating Tumor Cells in Endometrial Cancer. Anticancer Res 2018;38:2983-7.

48. Bogani G, Liu MC, Dowdy SC et al. Detection of circulating tumor cells in high-risk endometrial cancer. Anticancer Res 2015;35:683-7.
49. Ni T, Sun X, Shan B et al. Detection of circulating tumour cells may add value in endometrial cancer management. Eur J Obstet Gynecol Reprod Biol 2016;207:1-4.
50. Lemech CR, Ensell L, Paterson JC et al. Enumeration and Molecular Characterisation of Circulating Tumour Cells in Endometrial Cancer. Oncology 2016;91:48-54.
51. Kölbl AC, Victor LM, Birk AE, Jeschke U, Andergassen U. Quantitative PCR marker genes for endometrial adenocarcinoma. Mol Med Rep 2016;14:2199-205.
52. Obermayr E, Sanchez-Cabo F, Tea MK et al. Assessment of a six gene panel for the molecular detection of circulating tumor cells in the blood of female cancer patients. BMC Cancer 2010;10:666.
53. Zhang Y, Qu X, Qu PP. Value of circulating tumor cells positive for thyroid transcription factor-1 (TTF-1) to predict recurrence and survival rates for endometrial carcinoma. JBUON 2016;21:1491-5.
54. Alonso-Alconada L, Muinelo-Romay L, Madissoo K et al. Molecular profiling of circulating tumor cells links plasticity to the metastatic process in endometrial cancer. Mol Cancer 2014;13:223.
55. Parkin DM, Ferlay J. Cancer in sub-Saharan Africa. IACR Publ., vol. 167, 2018.
56. Armaroli P, Villain P, Suonio E et al. European Code against Cancer (4th Edition) Cancer screening. Cancer Epidemiol 2015;39 (Suppl 1):S139-52.
57. Dürst M, Glitz D, Schneider A, Zur Hausen H. Human papillomavirus type 16 (HPV 16) gene expression and DNA replication in cervical neoplasia: analysis by in situ hybridization. Virology 1992;189:132-40.
58. Schwarz E, Freese UK, Gissmann L et al. Structure and transcription of human papillomavirus sequences in cervical carcinoma cells. Nature 1985;314:111-4.
59. Walboomers JM, Jacobs MV, Manos MM et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999;189:12-9.
60. Scheffner M, Werness BA, Huibregtse JM, Levine AJ, Howley PM. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. Cell 1990;63:1129-36.
61. Pfitzer C, Schoder I, Schlungraber C et al. Digital-direct-RT-PCR: a sensitive and specific method for quantification of CTC in patients with cervical carcinoma. Sci Rep 2014;4:3970.
62. Counter CM, Avilion AA, Lefeuvre CE et al. Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. EMBO J 1992;11:1921-9.
63. Takakura M, Kyo S, Kanaya T et al. Cloning of human telomerase catalytic subunit (hTERT) gene promoter and identification of proximal core promoter sequences essential for transcriptional activation in immortalized and cancer cells. Cancer Res 1999;59:551-7.
64. Takakura M, Kyo S, Sowa Y et al. Telomerase activation by histone deacetylase inhibitor in normal cells. Nucleic Acids Res 2001;29:3006-11.
65. Kishimoto H, Kojima T, Watanabe Y et al. In vivo imaging of lymph node metastasis with telomerase-specific replication-selective adenovirus. Nat Med 2006;12:1213-9.
66. Takakura M, Nakamura M, Kyo S et al. Intraperitoneal administration of telomerase-specific oncolytic adenovirus sensitizes ovarian cancer cells to cisplatin and affects survival in a xenograft model with peritoneal dissemination. Cancer Gene Ther 2010;17:11-9.
67. Kojima T, Watanabe Y, Hashimoto Y et al. In vivo biological purging for lymph node metastasis of human colorectal cancer by telomerase-specific oncolytic virotherapy. Ann Surg 2010;251:1079-86.
68. Takakura M, Matsumoto T, Nakamura M et al. Detection of circulating tumor cells in cervical cancer using a conditionally replicative adenovirus targeting telomerase-positive cells. Cancer Sci 2018;109:231-40.
69. Wen YF, Cheng TT, Chen XL et al. Elevated circulating tumor cells and squamous cell carcinoma antigen levels predict poor survival for patients with locally advanced cervical cancer treated with radiotherapy. PLoS One 2018;13:e0204334.
70. Goodman MT, Howe HL, Tung KH et al. Incidence of ovarian cancer by race and ethnicity in the United States, 1992-1997. Cancer 2003;97(10 Suppl):2676-85.
71. Reid BM, Permuth JB, Sellers TA. Epidemiology of ovarian cancer: a review. Cancer Biol Med 2017;14:9-32.
72. Chen W, Zheng R, Baade PD et al. Cancer statistics in China, 2015. CA Cancer J Clin 2016;66:115-32.
73. American Cancer Society. Cancer Facts and Figures 2015. Cancer Facts Fig 2015, 2015.
74. Moufarrij S, Dandapani M, Arthofer E et al. Epigenetic therapy for ovarian cancer: promise and progress. Clin Epigenetics 2019;11:7.
75. Du Bois A, Reuss A, Pujade-Lauraine E, Harter P, Ray-Coquard I, Pfisterer J. Role of surgical outcome as prognostic factor in advanced epithelial ovarian cancer: a combined exploratory analysis of 3 prospectively randomized phase 3 multicenter trials: by the Arbeitsgemeinschaft Gynaekologische Onkologie Studiengruppe Ovarialkarzinom (AGO-OVAR) and the Groupe d'Investigateurs Nationaux Pour les Etudes des Cancers de l'Ovaire (GINECO). Cancer 2009;115:1234-44.
76. Martin LP, Schilder RJ. Management of recurrent ovarian carcinoma: current status and future directions. Semin Oncol 2009;36:112-25.
77. Marth C, Kisic J, Kaern J, Tropé C, Fodstad Ø. Circulating tumor cells in the peripheral blood and bone marrow of patients with ovarian carcinoma do not predict prognosis. Cancer 2002;94:707-12.
78. 78. Judson PL, Geller MA, Bliss RL et al. Preoperative detection of peripherally circulating cancer cells and its prognostic significance in ovarian cancer. Gynecol Oncol 2003;91:389-94.
79. Cui L, Kwong J, Wang CC. Prognostic value of circulating tumor cells and disseminated tumor cells in patients with ovarian cancer: a systematic review and meta-analysis. J Ovarian Res 2015;8:38.

80. Lee M, Kim EJ, Cho Y et al. Predictive value of circulating tumor cells (CTCs) captured by microfluidic device in patients with epithelial ovarian cancer. Gynecol Oncol 2017;145:361-5.
81. Zhang X, Li H, Yu X et al. Analysis of Circulating Tumor Cells in Ovarian Cancer and Their Clinical Value as a Biomarker. Cell Physiol Biochem 2018;48:1983-94.
82. Suh DH, Kim M, Choi JY et al. Circulating tumor cells in the differential diagnosis of adnexal masses. Oncotarget 2017;8:77195-206.
83. Lou E, Vogel RI, Teoh D et al. Assessment of Circulating Tumor Cells as a Predictive Biomarker of Histology in Women With Suspected Ovarian Cancer. Lab Med 2018;49:134-9.
84. Obermayr E, Bednarz-Knoll N, Orsetti B et al. Circulating tumor cells: potential markers of minimal residual disease in ovarian cancer? a study of the OVCAD consortium. Oncotarget 2017;8:106415-28.
85. Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat Rev Genet 2006;7:606-19.
86. Ahmed N, Abubaker K, Findlay J, Quinn M. Epithelial mesenchymal transition and cancer stem cell-like phenotypes facilitate chemoresistance in recurrent ovarian cancer. Curr Cancer Drug Targets 2010;10:268-78.
87. Chebouti I, Kasimir-Bauer S, Buderath P et al. EMT-like circulating tumor cells in ovarian cancer patients are enriched by platinum-based chemotherapy. Oncotarget 2017;8:48820-31.
88. Kuhlmann JD, Wimberger P, Bankfalvi A et al. ERCC1-positive circulating tumor cells in the blood of ovarian cancer patients as a predictive biomarker for platinum resistance. Clin Chem 2014;60:1282-9.
89. Chebouti I, Kuhlmann JD, Buderath P et al. ERCC1-expressing circulating tumor cells as a potential diagnostic tool for monitoring response to platinum-based chemotherapy and for predicting post-therapeutic outcome of ovarian cancer. Oncotarget 2017;8:24303-13.
90. Obermayr E, Castillo-Tong DC, Pils D et al. Molecular characterization of circulating tumor cells in patients with ovarian cancer improves their prognostic significance -- a study of the OVCAD consortium. Gynecol Oncol 2013;128:15-21.
91. Kolostova K, Matkowski R, Jędryka M et al. The added value of circulating tumor cells examination in ovarian cancer staging. Am J Cancer Res 2015;5:3363-75.
92. Kolostova K, Pinkas M, Jakabova A et al. Molecular characterization of circulating tumor cells in ovarian cancer. Am J Cancer Res 2016;6:973-80.