

# Circulating tumor cells as trigger to hematogenous spreads and potential biomarkers to predict the prognosis in ovarian cancer

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**Abstract** Despite several improvements in the surgical field and in the systemic treatment, ovarian cancer (OC) is still characterized by high recurrence rates and consequently poor survival. In OC, there is still a great lack of knowledge with regard to cancer behavior and mechanisms of recurrence, progression, and drug resistance. The OC metastatization process mostly occurs via intracoelomatic spread. Recent evidences show that tumor cells generate a favorable microenvironment consisting in T regulatory cells, T infiltrating lymphocytes, and cytokines which are able to establish an “immuno-tolerance mileau” in which a tumor cell can become a resistant clone. When the disease responds to treatment, immunoediting processes and cancer progression have been stopped. A similar inhibition of the immunosuppressive microenvironment has been observed after optimal cytoreductive surgery as well. In this scenario, the early identification of circulating tumor cells could represent a precocious signal of loss of the immune balance that precedes cancer immunoediting and relapse. Supporting this hypothesis, circulating tumor cells have been demonstrated to be a prognostic factor in several solid tumors such as colorectal, pancreatic, gastric, breast, and genitourinary cancer. In OC, the role of circulating tumor cells is still to be defined. However, as opposed to healthy women, circulating tumor cells have been

demonstrated in peripheral blood of OC patients, opening a new research field in OC diagnosis, treatment monitoring, and follow-up.

**Keywords** Circulating tumor cells · Immunoediting · Ovarian cancer · Prognostic markers · Tumor immunology

## Introduction

In developed countries, ovarian cancer (OC) is the second most common malignant tumor of the female reproductive system, reaching the eight world position for morbidity and mortality rates [1]. Optimal cytoreduction followed by platinum- and taxane-based chemotherapy represents the cornerstone treatment of OC (<http://www.cancer.gov/types/ovarian/hp/ovarian-epithelial-treatment-pdq>). Approximately 75 % of all OC patients are diagnosed at stage III–IV when transperitoneal, hematogenous, and lymphatic dissemination have already occurred; in this scenario, surgery is often multivisceral and highly complex and survival chances limited. Despite surgical improvement [2], recurrences remain the most challenging obstacle to overcome. In the past decades, only the intraperitoneal administration of chemotherapy in optimally debulked OC patients and the adoption of bevacizumab as maintenance therapy have been associated with an improved survival [3, 4]. At present, we are still unable to identify patients who will have different oncologic outcomes at the time of diagnosis. In other words, pathologic and molecular prognostic factors are still lacking. As a matter of fact, OC patients are subdivided into platinum refractory, resistant, and sensitive based on their progression-free survival.

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In an attempt to identify molecular markers that may serve as prognostic indicator and as target for new molecular chemotherapeutic agents, a number of studies have investigated the prognostic value of oncogenes and tumor suppressor genes presumed to be involved in the development and progression of OC, such as overexpression of protein p185 and amplification of the encoding oncogene HER-2/neu [5]. Unfortunately, data regarding overexpression and clinical significance of molecular targets are still conflicting and far from clinical applicability.

Recently, it has been found that host's cellular adaptive immune system mounts a response to many solid tumors, including OC, mediated by tumor-infiltrating T lymphocytes (TILs) [6]. In this view, biomarkers to capture the TIL immunosurveillance for cancer prognosis and prediction of therapeutic response could be developed [7]. Similarly, the microRNA-200 family and circulating tumor DNA (ctDNA) were found to be useful OC biomarkers [8–10]. Finally, circulating tumor cells (CTCs) recruited in peripheral blood were identified as a marker of hematogenous spread in various solid tumors [11]. So far, their predictive and prognostic value has been proven in breast [12], colorectal [13], lung [14], esophageal [15], liver [16], pancreatic [17], and prostate cancers [18]. Recently, the presence of positive CTCs was associated with deep myometrial invasion and lymph node positivity in endometrial cancer [19].

The role of CTCs in OC is still to be defined. However, conversely to healthy women, CTCs have been demonstrated in peripheral blood of OC patients [20]. This finding could open new scenarios in OC diagnosis and treatment monitoring.

### Disseminated tumor cells and circulating tumor cells

Disseminated tumor cells (DTCs) and CTCs have first been identified and considered the potential precursors of metastatic disease in the 1990s [21]. Fidler et al. highlighted that CTCs reach distant organs developing metastases by three processes: endosmosis (invasion of surrounding tissue, blood, and lymph circulation), exosmosis (exudation from microvascular architecture), and oecesis (germination into remote organs where visible tumor lesions are developed) [22].

It is unclear whether DTCs and CTCs represent identical cell populations observed at a different anatomic location (bone marrow and bloodstream, respectively) and distinct stage of tumor progression. Overall, the existence of CTCs in the bloodstream and the settlement of these cells in secondary organs such as liver, lungs, and mostly bone as DTCs is generally accepted. In this scenario, bone marrow sampling (such as sampling from other organs) is a rather invasive procedure, which is not widely accepted in the clinical management. Surely, detection of CTCs seems more practical than

DTCs due to a systematically feasible evaluation of CTCs in peripheral bloodstream, with respect to bone biopsy. Furthermore, CTCs seems to be more sensitive than DTCs in evaluating tumor progression [23]. For these reasons, focus on DTCs has been shifted to the detection of tumor cells in peripheral blood.

### Identification of circulating tumors cells in ovarian cancer

CTCs are tumor cells that spread into the bloodstream from the primary tumors, recurrences, or metastases and possess antigenic and genetic tumor-specific characteristics. CTCs have been identified in epithelial cancer patients, while they were absent in healthy subjects [19]. Particularly, six genes appeared very highly expressed in the cancer cell lines and absent in healthy women; this identification of tumor cells may demonstrate the potential utility for early detection, clinical monitoring, and treatment control of gynecological malignancies [20, 24].

Detection methods consist in immunocytochemistry (IHC) and reverse transcription-polymerase chain reaction (RT-PCR). Compared with IHC, RT-PCR seems to be more sensitive (HR 3.49 vs 1.70) [25], suggesting RT-PCR as the promising methods in identifying CTCs in OC patients. However, so far, the US Food and Drug Administration (FDA) has approved only IHC as the method of choice for detecting CTCs of epithelial origin in clinical practice.

Unfortunately, CTCs in OC are present in low concentration (1/109 blood cells or 1/106 nucleated blood cell); hence, pre-enrichment methods to highlight their presence are not only needed but mandatory. Methods to enrich and detect these clusters of cells are size-based, density-based, immunomagnetic separation, microfluidic-based [26].

### Association of CTCs and clinical outcome

In accordance with other studies, Obermayr et al. [20] has detected CTCs at baseline in 24 % of the patients with primary [27–29]. Studies of CTCs in OC patients demonstrated that CTCs are associated with poor clinical outcome [30–34].

Pearl et al. showed significant differences in CTCs' detection rates in OC patients with regard to International Federation of Gynecology and Obstetrics (FIGO) tumor stage (90.7 % in stage III–IV patients vs 46.4 % in stage I–II patients,  $p < 0.00001$ ), PFS (4 vs 30 months  $p = 0.024$ ), and OS (5 vs 41 months,  $p = 0.0219$ ) [30]. CTC detection rates did not differ based on age, tumor grade, histology, amount of residual disease, and platinum sensitivity [30]. Considering that in OC patients, progression from intraperitoneal tumor residues generally occurs much earlier than the development

of distant metastasis (median lead time of 23 to 56 months) [35], CTC detection in OC could be associated with adverse clinicopathological features and a worse clinical outcome.

A significant decrease in OS was found in OC patients with detectable CTCs (35 vs 15 months of median survival, respectively  $p=0.042$ ) [36]. Another study showed that patients with complete resection of the primary tumor had a significantly lower CTC detection rate than patients with macroscopic residual disease after surgery, thus suggesting a correlation with prognosis [29]. A similar correlation had been previously shown with the Treg cell population [37, 38]. These data have been later confirmed assuming that a persistence of CTCs after chemotherapy could be a strong indicator of poor therapy response as well [39].

On the contrary, some evidences have shown a negative association in terms of progression-free survival and overall survival and CTCs [29, 36]. Thus, the prognostic value of CTCs in OC remains controversial. In order to solve this unanswered question, three meta-analyses interrogating on the prognostic value of CTCs in OC have been performed in 2015 [23, 40, 41]. These meta-analyses have found a strong relationship of CTCs with advanced FIGO stage and treatment response in patients with OC. No significant association was observed between CTCs and histological subtypes, macroscopic residual disease, lymph nodes metastasis [23], and oncologic outcome [40]. Furthermore, the presence of CTCs was closely associated with elevated CA-125 blood values [40].

Interestingly, the meta-analytic data did not support a significant association with residual disease after surgery [23, 40]. Such an association has only been demonstrated only in few studies [20, 29].

A limitation of these meta-analyses is represented by the heterogeneity of the methods adopted in the different studies such as method used to identify CTCs and the cutoff used to predict clinical outcome [39, 42, 43].

Globally, despite the conflicting results reported in the literature (Table 1), all data arising from the recent meta-analyses [23, 40, 41] support a strong correspondence between CTCs detection and oncologic outcome.

### Future directions

The identification of new targets in OC has recently permitted to test new drugs that are now trying to change the biological history of this disease [44–48]. Unfortunately, there is a lack of knowledge in selecting patients and in monitoring the response to these treatments. The immune system could offer a potential environment in which target drugs and immunotherapy could be better monitorized. However, this kind of monitoring represents an indirect method that could be affected by many variables (e.g., immunosuppressive medications with steroids, autoimmune disease, or immune-response exhaustion). A treatment response

**Table 1** Oncologic significance of circulating tumor cells in ovarian cancer

Author	Year	Study type	Population	Pts n	Methods	FIGO/Grade	OS	DFS	Follow-up, months (range)
Marth et al.	2002	Case series	POC	90	Immunomagnetic beads (Dynabeads®)	I-IV/G1-3	NS	NS	25 (16–39)
Judson et al.	2003	Case series	POC/ROC	53	Veridex CellSearch System	I-IV/G1-3	NS	NS	18.7 (12–25)
Fan et al.	2009	Retrospective cohort	POC	25	CAM cell invasion assay	I-IV/G1-4	NS	$p=0.042$	18 (1–80)
Aktas et al.	2011	Prospective	ROC	33	Immunomagnetic selection+ multiplex RT-PCR	Ia-IV/G1-4	$p=0.054-0.047$	NS	28.4 (2–169)
Behbacht et al.	2011	Prospective	ROC	122	CellSearch System	Recurrent/G1-3	NA	NA	>6
Poveda et al.	2011	Prospective	ROC	216	CellSearch System (EpCAM cell isolation)	NA	$p=0.0017$	$p=0.0024$	10 (1–25)
Kühlman et al.	2012	Prospective	POC	63	PCRbased fluorescein microsatellite analysis (QIAamp DNA minikit)	I-IV/G1-4	$p=0.030$	NS	36 (1–70)
Liu et al.	2013	Prospective	POC	20	CellSearch System (EpCAM cell isolation)	III-IV/G1-3	NS	NS	30 (2–60)
Obermayr et al.	2013	Multicenter	POC	48	CellSearch System (EpCAM cell isolation)	II-IV/G1-3	$p=0.001$	$p=0.001$	52 (1–69)
Pearl et al.	2014	Retrospective	Benign POC	216	CAM cell invasion assay	I-IV/G1-3	$p<0.0001$	$p<0.0001$	21.4 (0–140.9)
			Benign POC	41					
			PC	76					
			Fallopian	9					
			POC	3					
Pearl et al.	2015	Prospective	Benign Healthy	123	CAM cell invasion assay	I-IV/NA	NA	$p<0.00001$	NA
			Benign Healthy	49					
			Healthy	64					

POC, primary OC, ROC recurrent OC, CAM cell adhesion matrix, ICC immunocytochemistry, PC peritoneal cancer, NA not available, NS not significant

surveillance model based on clinical response and the effective quantification of tumor cells detected in the bloodstream could be hypothesized in the future. However, an international agreement of the definition of ‘positive’ CTCs in future trial is necessary. Furthermore, more reproducible test to detect and amplify CTCs populations is required. If these goals will be achieved, the detection of CTCs may become a valuable tool to integrate in clinical management in future years.

## Conclusion

Treatment of OC is fortunately evolving into a more individualized approach, with a better understanding of the molecular composition of each patient’s tumor. Data from the literature support a correlation of CTCs not only with advanced stage and poor prognosis in patients with OC but also with treatment response, suggesting that CTCs could be used as an early predictive marker of tumor response in OC patients undergoing conventional or targeted therapy.

## Compliance with ethical standards

**Conflicts of interest** None

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