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Circulating tumor cells count as a predictor of survival in lung cancer

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ABSTRACT

The presence of circulating tumor cells (CTCs) in the peripheral blood of cancer patients was first described in the second half of the 19th century, but research interest in their potential clinical utility has intensified and greatly expanded only in recent years. Herein, we summarize and critically discuss current knowledge on CTC count as a predictor of survival in lung cancer, and comment on the existing challenges and future perspectives in this field. The majority of data published to date, including the results of almost all large cohorts, are strongly supportive of the value of CTC enumeration as a predictor of survival, mainly in advanced/metastatic non-small and small cell lung cancer (NSCLC and SCLC, respectively). Nonetheless, additional research is warranted to establish the prognostic relevance of CTC count in other clinical settings, mainly encompassing earlier-stage disease as well as specific molecular subtypes of NSCLC (e.g. EGFR mutation-positive or ALK-positive cases).

1. Introduction

Lung cancer is the first cause of tumor-related death worldwide accounting for 1,6 million cancer deaths annually or 1 of every 4 cancer deaths (Ferlay et al., 2015; Siegel et al., 2016). It is currently estimated that 80% among all newly diagnosed lung cancer cases annually will ultimately succumb to their disease, although it is hoped that recent introduction of novel targeted and immunotherapeutic agents in routine oncology practice may, hopefully, alter this dire picture in the near future (Allemani et al., 2015; Herzberg et al., 2017). Considering the advanced disease stage at initial presentation and diagnosis in more than 50% of patients (Ramalingam et al., 2011), the profound clinical, histological and genomic heterogeneity of lung cancer, the challenge of obtaining adequate tissue for pathological confirmation of disease and performance of adjuvant molecular testing and the rapid and, almost universal, development of therapy resistance in the advanced/metastatic setting (Hirsch et al., 2010; Chang, 2011), these dismal survival statistics are hardly surprising, highlighting not only the aggressive nature of this malignancy but also the need to improve current treatment options.

Numerous factors may influence treatment, the most critical of which are disease stage, histological subtype of tumor and performance status of patients. Surgery (segmentectomy, lobectomy, pneumonectomy), alone or followed by chemotherapy and/or radiation therapy, offers the best chance of cure and long-term survival for localized lung cancer, while treatment options for advanced and metastatic disease include variable combinations of cytotoxic chemotherapy, radiotherapy and targeted biologic agents, often resulting in significant and additive toxicity (Wu et al., 2017). Thus, improved prediction of probability of disease recurrence and survival and treatment response are needed for a more accurate selection of lung cancer patients who might benefit the most from available treatments, with the ultimate aim to increase treatment efficacy without a parallel increase of unnecessary treatment-related side effects (Gazdar and Schiller, 2011).

Although the presence of circulating tumor cells (CTCs) in the peripheral blood of cancer patients was first described in 1869 by Ashworth (1869), followed by Stephen Paget's "seed and soil" hypothesis in 1889 (Paget, 1889), research interest in their clinical utility has expanded only in recent years, in parallel with the development of novel technology platforms for their isolation and subsequent analysis. CTCs are a subset of tumor cells with the ability to escape from the primary site, intravasate into nearby blood and/or lymphatic vessels, survive into the challenging microenvironment of bloodstream, extravasate from the vascular system into the surrounding tissue and form micrometastases in secondary organs with the potential of growth into macroscopic tumors (Joosse et al., 2015; Kang and Pantel, 2013).

The detection and subsequent enumeration of CTCs in the peripheral blood of patients with non-small cell or small cell lung cancer (NSCLC or SCLC, respectively) is increasingly investigated as a novel biomarker of tumor's growth dynamics, with the potential to offer independent prognostic information and/or predict response to treatment. The results of previously published studies on the prognostic value of CTCs in the above clinical settings are generally supportive of its role as a predictor of disease relapse and/or survival, but controversy

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remains regarding its exact clinical relevance in routine practice (Pantel and Alix-Panabières, 2010; Lianidou et al., 2015; Normanno et al., 2016).

Herein, we provide a narrative review of the existing data on the prognostic role of peripheral CTCs in lung cancer and briefly comment on current challenges and future perspectives in this field. PubMed database was searched using the terms: "NSCLC" or "SCLC" AND "circulating tumor cells". Eligible studies for inclusion were those which: a) correlated CTC count with survival endpoints, e.g. overall survival (OS), disease free survival (DFS) and progression free survival (PFS), in patients with NSCLC or SCLC, and b)were written in English; studies with heterogeneous patient populations including not only NSCLC or SCLC cases but also patients with other solid tumors were excluded from our analysis. Studies investigating the prognostic value of CTCs in the central circulation only (e.g. pulmonary vein) and not in peripheral venous blood were also excluded.

2. CTC detection methods

In order to detect the infrequent isolated tumor cells among millions of normal hematopoietic cells (it is estimated that 1–10 CTCs can be found per 1 ml of whole blood, at a background of one billion normal blood cells) (Joosse et al., 2015; Nagrath et al., 2007), an enrichment step is first required. Methods for CTC enrichment are generally classified into label-dependent and label-independent techniques. The former (label-dependent) techniques are based on the biological features of tumor cells (e.g. expression of cell surface markers), while the latter (label-independent) are based on their intrinsic physical properties (most commonly cell size and density) (Joosse et al., 2015; Alix-Panabières and Pantel, 2013; Hanssen et al., 2015; Krebs et al., 2010).

Label-dependent CTC enrichment mainly employs immunomagnetic separation techniques, leading to selection of CTCs with the use of ferrofluids or magnetic beads coated with antibodies against epithelial antigens expressed on the surface of CTCs and not by the surrounding blood components (positive CTC selection) or against antigens expressed by blood cells only (negative CTC selection/depletion) (Joosse et al., 2015; Nagrath et al., 2007; Alix-Panabières and Pantel, 2013; Hanssen et al., 2015; Krebs et al., 2010; Toss et al., 2014; Mostert et al., 2009). The epithelial cell adhesion molecule (EpCAM) and leukocyte common antigen (CD45) are the most commonly used antigens for positive and negative selection of CTCs, respectively (Alix-Panabières and Pantel, 2013). In addition to the above methods, novel sophisticated diagnostic platforms for CTC enrichment have been developed, including the use of microfluidic assays (e.g. CTC-chip) or in vivo isolation techniques (e.g. GILUPI CellCollector™) (Nagrath et al., 2007; Sequist et al., 2009; Thege et al., 2014; Saucedo-Zeni et al., 2012; Gorges et al., 2016a).

With the use of label-independent techniques, CTCs are isolated according to cell size (e.g. ISET® filtration), density (e.g. Ficoll–hypaque density gradient separation, OncoQuick), deformability (e.g. atomic force microscopy), dielectric properties (e.g dielectrophoresis) or a combination of physical features (e.g. label-free microfluidic techniques) (Harouaka et al., 2013; Vona et al., 2000; Müller et al., 2005; Gertler et al., 2003; Kallergi et al., 2016; Shim et al., 2013a; Jen and Chang, 2011; Shim et al., 2013b; Moon et al., 2011).

Yet, even after an efficient enrichment step, CTCs need to be further identified and isolated from a substantial number of remaining blood cells (Joosse et al., 2015). A large variety of immunological, molecular and functional-based strategies may be used for this purpose, and are further classified into cytometric (whole cell-based) or nucleic acid-based techniques (Joosse et al., 2015; Krebs et al., 2010; Toss et al., 2014). Conventional or automated scanning microscopes and cytometers, in combination with immunocytochemistry (ICC) or immunofluorescence for the expression of various epithelial (e.g. cytokeratins), mesenchymal or tissue-specific markers − along with staining for the nuclear dye 4′, 6-doamidino-2-phenylindole (DAPI)- allow the detection and enumeration of CTCs (Joosse et al., 2015; Krebs et al., 2010; Toss et al., 2014; Millner et al., 2013). Nucleic acid-based assays target gene alterations or tumor-specific mRNA transcripts, mainly employing qualitative or quantitative reverse transcriptase-PCR (RT-PCR or qRT-PCR), while functional assays, like the EPithelial Immuno SPOT technology (EPISPOT) detect cell-secreted proteins, thus leading to isolation of only viable CTCs (Toss et al., 2014; Smith et al., 1991; Stathopoulou et al., 2003; Alix-Panabières and Pantel, 2015; Soler et al., 2017).

Assays for CTC isolation and analysis, combining enrichment and detection steps (e.g. CellSearch, AdnaTest, and the above described techniques ISET and EPISPOT), are also commercially available (Vona et al., 2000; Soler et al., 2017; Allard et al., 2004; Allard and Terstappen, 2015; Andreopoulou et al., 2012). The CellSearch® system is the only U.S. Food and Drug Administration (FDA) approved test for the enumeration of CTCs of epithelial origin in patients with metastatic breast, prostate and colorectal cancer (Gorges et al., 2016b). This widely used cytometric platform employs immunomagnetic separation for CTC enrichment using EpCAM-coated ferrofluids, followed by immunofluorescent staining for cytokeratins (CK8, 18 and 19), CD45 and DAPI (Allard et al., 2004).

Although a comparative analysis of the pros and cons of CTC enrichment and detection methods is outside the scope of the present review, a main limitation of label-dependent techniques must be emphasized, i.e. their inability to efficiently capture CTCs which have switched to a mesenchymal phenotype via epithelial to mesenchymal transition (EMT). (Joosse et al., 2015; Alix-Panabières and Pantel, 2013; Grover et al., 2014). EMT is a key process for the formation of metastases, aiming to confer improved invasive and survival traits to tumor cells and thus facilitate their detachment from the primary site and metastatic spread to secondary locations; furthermore, it may be partial or complete, leading to highly heterogeneous subpopulations of CTCs with hybrid/mixed epithelial-mesenchymal or purely mesenchymal phenotypes, respectively, (Garg, 2017; Jolly et al., 2016; Joosse and Pantel, 2013; Lecharpentier et al., 2011). CTCs that have undergone any degree of EMT may be difficult to discern from normal hematopoietic cells on the basis of their immunological properties alone, while their increased levels in the bloodstream may carry significant prognostic implications as an indicator of a more aggressive disease course, higher metastatic potential and drug resistance (Garg, 2017; Jolly et al., 2016; Joosse and Pantel, 2013; Lecharpentier et al., 2011).

3. Prognostic significance of CTCs in lung cancer

The potential association between CTC count and prognosis has been previously investigated in several observational studies or exploratory analyses of clinical trial data. A summary of these studies is shown in Tables 1 and 2 (including NSCLC and SCLC cohorts, respectively) (Hofman et al., 2011a; Hofman et al., 2011b; Krebs et al., 2011; Nieva et al., 2012; Isobe et al., 2012; Punnoose et al., 2012; Hirose et al., 2012; Muinelo-Romay et al., 2014; Juan et al., 2014; Zhang et al., 2016; Bayarri-Lara et al., 2016; Crosbie et al., 2016; He et al., 2016; Qi and Wang, 2017; Chudasama et al., 2017; Zhou et al., 2017; Yang et al., 2017; Coco et al., 2017; Lindsay et al., 2017; Li et al., 2017; Hou et al., 2009; Hou et al., 2012; Naito et al., 2012; Hiltermann et al., 2012; Igawa et al., 2014; Normanno et al., 2014; Huang et al., 2014; Cheng et al., 2016; Messaritakis et al., 2017a; Shen et al., 2017; Salgia et al., 2017; Messaritakis et al., 2017b), while their results are analyzed in detail below.

3.1. Non-small cell lung cancer (NSCLC)

3.1.1. Resectable or locally advanced NSCLC (stages I–III)

Bayarri-Lara et al. (2016) prospectively assessed the prognostic value of CTCs in 56 patients with resectable (stage I-IIIA) NSCLC, using immunocytochemistry methods for CTC detection. Positive

Table 1
Prospective studies investigating the prognostic value of CTC count in patients with NSCLC (in chronological order).

Authors (year) [Ref]	Number of evaluable patients	Disease Stage	CTC Detection Method	CTC detection rate (baseline)	Cut-off point for survival analysis	Main Results
Hofman et al. (2011a)	208	I-IV	ISET	49%	50 CTCs/10 ml	The presence of ≥50 CTCs at baseline (preoperatively) was independently associated with shorter DFS and OS (HR: 2.631; 95% CI: 1.557–4.651; p = 0.003 and HR: 2.096; 95% CI: 1.331–3.300; p = 0.001, respectively).
Hofman et al. (2011b)	210	I-IV	CellSearch and ISET	69% (using CellSearch and/or ISET)	1 CTC/7 ml	The presence of CTCs, detected by CellSearch and/or ISET, was independently associated with shorter DFS (HR: 1.235; 95% CI: 1.056–1.482; p < 0.001).
Krebs et al. (2011)	101	III-IV	CellSearch	21%	5 CTCs/7.5 ml	Baseline CTC count was independently associated with PFS (HR: 4.85; 95% CI: 1.93–12.17; p = 0.001) and OS (HR, 7.92;95% CI: 2.85–22.01; p < 0.001). CTC count evaluation at two time points (pre- and post-treatment), was an independent predictor of PFS (HR, 12.06; 95% CI, 3.77–38.65; p < 0.001) and OS (HR: 15.65; 95% CI: 3.63–67.53; p < 0.001).
Nieva et al. (2012)	28	IV	ICC (without enrichment)	68%	5 CTCs/ml (mean CTC count over multiple blood draws)	Increased overall CTC count was associated with shorter OS (p = 0.0035).
Isobe et al. (2012)	24	IV	CellSearch	33.3%	1 CTC/7.5 ml	Increased CTC count at baseline was significantly associated with shorter OS (HR: 2.9; 95% CI: 1.6–54.1; p = 0.012].
Punnoose et al. (2012)	37	IV	CellSearch	76%	5 CTCs/7.5 ml	Baseline CTC count showed no significant association with PFS. Decrease in CTC count (at the 56th day of treatment) was associated with longer PFS (p = 0.006).
Hirose et al. (2012)	33	IV	CellSearch	36.4%	1 CTC/7.5 ml	Baseline CTC count was not significantly associated with survival (PFS/OS).
Muinelo-Romay et al. (2014)	43	IIIB-IV	CellSearch	41.9%	5 CTCs/7.5 ml (baseline)	Increased CTC count at baseline was significantly associated with worse PFS and OS (p = 0.034 and p = 0.008, respectively) at univariate analysis, and with worse PFS (HR: 4.3; 95% CI: 1.3–14.4; p = 0.016) at multivariate analysis.
					2 CTCs/7.5 ml (post-treatment)	Increase in CTC count from baseline to the 5th chemotherapy cycle was significantly associated with shorter PFS and OS (p = 0.003 and p = 0.019, respectively).
Juan et al. (2014)	37	III-IV	CellSearch	24%	2 CTCs/7.5 ml	Neither baseline CTC count nor CTC count change from baseline to completion of the second chemotherapy cycle were found to significantly correlate with PFS and OS.
Zhang et al. (2016)	46	IIIB-IV	Cyttel	87%	8 CTCs/3 ml	Increased CTC count at baseline was an independent predictor of shorter PFS and OS (HR: 0.360; 95% CI: 0.151–0.862; p = 0.022 and HR: 0.316; 95% CI: 0.126–0.792; p = 0.014, respectively).
Bayarri-Lara et al. (2016)	56	I-IIIA	ICC	51.8%	1 CTC/10 ml	CTC presence after surgery was an independent predictor of DFS (HR: 5.75; 95% CI: 1.50–21.95; p = 0.01).
Crosbie et al. (2016)	27	I-IIIA	CellSearch	22%	1 CTC/7.5 ml	CTC detection at baseline (preoperatively) was independently associated with reduced DFS and 3-year survival (HR: 5.26; 95% CI: 1.48–18.68; p = 0.01 and HR: 3.73; 95% CI: 1.17–11.93; p = 0.026, respectively).
He et al. (2016)	66	IIIB-IV	Flow Cytometry	NR	68.5 CTCs/ml	Increased CTC count at baseline was associated with shorter 3-year survival and PFS (p = 0.01 and p = 0.005, respectively).
Qi and Wang (2017)	100 SCC	III	CellSearch	29%	5 CTCs/7.5 ml	Increased CTC count, both before and after one cycle of chemotherapy, was an independent predictor of PFS and OS (p values not available).
Chudasama et al. (2017)	23	I-IV	ScreenCell	76.1%	1 CTC/3 ml	CTC detection at baseline (preoperatively) was associated with improved OS (HR: 0.04453; 95% CI: 0.007131–0.2781; p < 0.0009).
Zhou et al. (2017)	59	III-IV	CellSearch	40.68%	2 CTCs/7.5 ml	Increased CTC count at baseline, was an independent predictor of OS and PFS (HR: 5.32; 95% CI: 1.62–17.50; p < 0.05 and HR: 3.93; 95% CI: 1.38–11.15; p < 0.05, respectively). Increased CTC count after two chemotherapy cycles was an independent predictor of OS (HR: 3.07; 95% CI; 1.33–7.05; p < 0.05).
Yang et al. (2017)	107	IIIB-IV	CellSearch	44%	5 CTCs/7.5 ml	Increased CTC count at baseline was an independent predictor of both PFS and TTF in multivariate analysis (HR: 6.833; 95% CI: 3.947–13.572, p < 0.001 and HR: 8.635; 95% CI: 2.341–15.613; p < 0.001). Increased CTC count on the 28th day of chemotherapy was the strongest predictor of PFS in multivariate analysis (HR: 8.017; 95% CI: 4.421–13.733, p < 0.001).
Coco et al. (2017)	73	IIIB-IV	ScreenCell	NA	6 CTCs/3 ml	Baseline CTC count had no statistically significant association with OS or PFS.
Lindsay et al. (2017)	125	IIIB-IV	CellSearch	40.8%	5 CTCs/7.5 ml	Increased CTC count at baseline was independently associated with OS (HR: 0.55; 95%CI: 0.33–0.92; p = 0.022).

(*continued on next page*)

Table 1 (*continued*)

Authors (year) [Ref]	Number of evaluable patients	Disease Stage	CTC Detection Method	CTC detection rate (baseline)	Cut-off point for survival analysis	Main Results
Li et al. (2017)	23	I-IIIA	MACS + flow cytometry (FAMCell)	91.3%	5 CTCs/15 ml	Increased CTC count at baseline (preoperatively) was associated with shorter TFS (p = 0.001) and OS (p = 0.001).

Abbreviations: CI: confidence interval; CTCs: circulating tumor cells; DFS: disease-free survival; HR: hazard ratio; ICC: immunocytochemistry; ISET: isolation by size of epithelial tumor cells; MACS: magnetic-activated cell sorting; NA: not available; NR: not reported; NSCLC: non-small cell lung cancer; OS: overall survival; PFS: progression free survival; SCC: squamous cell carcinoma; TTF: time to treatment failure; TFS: tumor-free survival

postoperative CTC count (> 1 CTC/10 ml) was associated with disease recurrence and reduced DFS (p = 0.018 and p = 0.008, respectively) in univariate analysis, and with reduced DFS in multivariate analysis (HR: 5.75; 95% CI: 1.50–21.95; p = 0.01), suggesting the potential value of CTC enumeration for stratifying risk of early treatment failure.

In another prospective study by Crosbie et al. (2016), investigating the prognostic value of CTCs in a smaller cohort of operable NSCLC patients, similar conclusions were drawn. After stratifying patients into CTC-positive and CTC-negative groups, using a cut-off of a single CTC per 7.5 ml of blood, an association of baseline (preoperative) CTC count with DFS and (3-year) OS was observed in univariate analysis (p = 0.011 and p = 0.037, respectively) as well as in the multivariate model (HR: 5.26; 95% CI: 1.48–18.68; p = 0.01 and HR: 3.73; 95% CI: 1.17–11.93; p = 0.026, respectively).

Li et al. (2017) evaluated the presence of CTCs, both in peripheral and pulmonary vein blood samples, collected before and during surgical resection, respectively, from 23 patients with stage I-IIIA NSCLC. The method used for enumeration of CTCs was the FAMCell System, combining enrichment of CTCs with EpCAM-dependent immunomagnetic separation and their subsequent detection using immunofluorescence for pan-cytokeratin and CD45 antibodies. Increased count of peripheral vein CTCs at baseline (5 CTCs/15 ml) was found to significantly correlate with reduced tumor-free survival and OS (log-rank p-value = 0.001, for both correlations), thus further supporting the potential prognostic value of this biomarker in operable NSCLC patients. The significance of these data may, nevertheless, be limited by the small number of evaluable patients and the lack of multivariate analysis.

3.1.2. Locally advanced and/or metastatic NSCLC (stages III–IV)

Krebs et al. (2011) evaluated 101 chemotherapy-naive patients with stage III and IV NSCLC for CTCs, before and after 1 cycle of chemotherapy, using the CellSearch platform. Using a cut-off of 5 CTCs/7.5 ml, patients were divided into favorable and unfavorable groups. In multivariate analysis, higher CTC count at baseline was associated with worse PFS (HR: 4.85; 95% CI: 1.93-12.17; p = 0.001) and OS (HR: 7.92; 95% CI: 2.85–22.01; p < 0.001). Furthermore, CTC count evaluation at two time points, before and after the first chemotherapy cycle, was an independent predictor of both PFS (HR: 12.06; 95% CI: 3.77–38.65; p < 0.001) and OS (HR: 15.65; 95% CI, 3.63–67.53; p < 0.001), while reduction of CTC number with chemotherapy was also correlated with increased PFS and OS (p < 0.001 and p = 0.009, respectively). These findings suggest that, aside of CTC enumeration at baseline, monitoring of CTC count status during treatment may also add significant prognostic information.

Nieva et al. (2012) investigated the prognostic significance of CTC count in multiple blood samples −drawn at consecutive time points within a period of 12 months in total- from 28 patients with metastatic NSCLC. Using an enrichment-free method for CTC enumeration and a cut-off of 5 CTCs/ml for survival analysis, reflecting the mean CTC count of all available measurements, the authors found a statistically significant correlation between higher levels of the above variable and shorter OS (p = 0.0035).

Isobe et al. (2012) investigated the prognostic value of CTC count, using the CellSearch platform, in a small cohort comprising 24 metastatic NSCLC patients with EGFR-positive primary tumors, after development of resistance to EGFR tyrosine kinase inhibitor treatment. Increased CTC count at baseline (≥1 CTC/7.5 ml) was significantly associated with shorter OS (HR: 2.9; 95% CI: 1.6–54.1; p = 0.012) in univariate analysis. In another series of 37 relapsed, stage IV NSCLC patients, by Punnoose et al. (2012), performed within the context of a phase II clinical trial of erlotinib and pertuzumab, peripheral blood samples were obtained at several time points, before and during chemotherapy treatment, and were analyzed for CTCs using the CellSearch platform. Although baseline CTC count −using a cut-off of 5 CTCs/7.5 ml- was not predictive of survival, a decrease in CTC count, between baseline values and those obtained at the 56th day of treatment, was

Table 2
Prospective studies investigating the prognostic value of CTC count in patients with SCLC (in chronological order).

Authors (year) [Ref]	Number of evaluable patients	Disease Stage	CTC Detection Method	CTC detection rate (baseline)	Cut-off point for survival analysis	Main Results
Hou et al. (2009)	50	LD and ED	CellSearch	86%	300 CTCs/7.5 ml	Increased CTC counts at baseline and on the 22nd day of treatment were associated with shorter OS (HR: 1.1; 95% CI: 1.02–1.18; p = 0.015 and HR: 1.43; 95% CI: 1.09–1.86; p = 0.01, respectively).
Hou et al. (2012)	97	LD and ED	CellSearch	85%	50 CTCs/7.5 ml	Increased CTC count at baseline (before treatment) was an independent predictor of PFS and OS (HR: 2.01; 95% CI: 1.17–3.46; p = 0.011) and OS (HR: 2.45; 95% CI: 1.39–4.30, p = 0.002). Change in CTC count after one chemotherapy cycle was an independent predictor of OS (HR: 4.1; 95% CI: 1.1–15.1, p = 0.03)
Naito et al. (2012)	51	LD and ED	CellSearch	68.6%	8 CTCs/7.5 ml	Increased CTC count at baseline, was significantly associated with shorter 1-year survival (p = 0.0014). Increased CTC count after completion of first-line chemotherapy and at relapse was associated with shorter post-treatment and post-relapse survival, respectively (p = 0.0096 and p < 0.0001, respectively)
Hiltermann et al. (2012)	59	LD and ED	CellSearch	73%	215 cells/7.5 ml	CTC count at baseline and after one and four chemotherapy cycles, respectively, were significantly associated both with PFS and OS in univariate analysis (all cut-offs).
					16 cells/7.5 ml	Increased CTC count (2 CTCs cut-ff) after one chemotherapy cycle was the strongest independent predictor of OS (HR, 5.7; 95% CI: 1.7–18.9; p = 0.004)
					5 cells/7.5 ml	
					2 CTCs/7.5 ml	
Igawa et al. (2014)	30	LD and ED	TelomeScan	96%	2 CTCs/7.5 ml	Increased CTC count at baseline was independently associated with OS (HR, 3.91; 95% CI: 1.19–12.87; p = 0.026)
Normanno et al. (2014)	60	ED	CellSearch	90%	Variable cut-offs for baseline CTC count (2–282 CTCs/7.5 ml) 89% reduction was used as cut-off for CTC count change after one chemotherapy cycle	CTC count reduction > 89% after chemotherapy was correlated to lower risk of death (HR: 0.24; 95% CI: 0.09–0.61). Addition of CTC count change to the prognostic model significantly increased its accuracy (bootstrap *p*-value = 0.009).
Huang et al. (2014)	24	ED	CellSearch	NR	5 CTCs/7.5 ml	Neither CTC count at baseline nor CTC count change from baseline to post-treatment were significantly associated with OS.
Cheng et al. (2016)	89	ED	CellSearch	87.6%	10 CTCs/7.5 ml	CTC count at baseline was independently associated with OS (HR: 0.304; p < 0.0001). CTC count after the second chemotherapy cycle was independently associated with PFS (HR: 0.467; p = 0.0211) and OS (HR: 0.295; p = 0.0002). CTC L-D-H algorithm (< 10 CTCs before and after treatment or CTC count change after treatment of > 150 CTCs) was independently associated with PFS and OS (HR: 0.484; p = 0.0149 and HR: 0.307; p < 0.0001, respectively).
Messaritakis et al. (2017a)	56	LD and ED	CellSearch	50%	5 CTCs/7.5 ml	Increased CTC count at baseline was significantly associated with shorter PFS and OS (HR: 0.3; 95% CI: 0.2–0.6; p < 0.001 and HR:0.3; 95% CI: 0.2–0.6; p = 0.001 respectively). Increased CTC count after one treatment cycle was independently associated with decreased OS (HR: 0.209; 95% CI: 0.1–0.6; p = 0.005).
Shen et al. (2017)	80	LD and ED	LT-PCR	83.8%	14.0 FU/3 ml	Increased CTC count at baseline showed an independent association with reduced PFS (HR: 0.656; 95% CI: 0.473–1.000; p = 0.049) and a significant (albeit marginal) association with reduced OS (HR: 0.59; 95% CI: 0.31–1.01; p = 0.056) in univariate analysis only.
Salgia et al. (2017)	42	ED	CellSearch	83%	6 CTCs/7.5 ml	Increased CTC count at baseline was associated with shorter PFS and OS (p = 0.024 and p = 0.017, respectively). Increased CTC at day one of the second chemotherapy cycle was significantly associated with PFS and OS (p = 0.001 in both instances)
Messaritakis et al. (2017b)	83	LD and ED	CellSearch	60.2%	5 CTCs/7.5 ml	Increased CTC count at baseline and on disease progression was independently associated with reduced PFS and OS, respectively (HR: 1.9; 95%; CI: 0.7–3.6; p = 0.032 and HR: 2.1, 95%CI: 1.0–4.5; p = 0.043, respectively)

Abbreviations: CI: confidence interval; CTCs: circulating tumor cells; ED: extensive disease; HR: hazard ratio; LD: Limited disease; L-D-H: low-drop-high; LT-PCR: ligand targeted-polymerase chain reaction; NR: not reported; OS: overall survival; PFS: progression free survival; SCLC: small cell lung cancer.

significantly correlated with longer PFS (p = 0.006). CTC count reduction at the latter time point (56th day) as compared to baseline among non-responders −patients with stable or progressive disease by RECIST criteria- was also associated with worse PFS (p = 0.0250).

Muinelo-Romay et al. (2014) investigated the prognostic significance of CTC count in 43 advanced NSCLC patients, using blood samples obtained before the first, second and fifth cycle of platinum-based chemotherapy and different cut-offs for baseline and subsequent survival analyses (5 versus 2 CTCs/7.5 ml, respectively). Increased CTC count at baseline was found to correlate with shorter PFS and OS (p = 0.034 and p = 0.008, respectively) in univariate analysis, while an independent association with PFS was retained in the multivariate model (HR: 4.3; 95% CI: 1.3–14.4; p = 0.016). Additionally, an increase of CTC count from baseline to the 5th chemotherapy cycle was significantly associated with worse PFS and OS (p = 0.003 and p = 0.019, respectively).

Four other recent studies on this topic (Qi and Wang, 2017; Zhou et al., 2017; Yang et al., 2017; Lindsay et al., 2017), including two cohorts including more than 100 participants each (Yang et al., 2017; Lindsay et al., 2017), present additional evidence supporting the prognostic value of CTC enumeration in advanced NSCLC. Yang et al. (2017) and Lindsay et al. (2017) investigated the prognostic value of CTC count in 107 and 127 patients with stage IIIB-IV NSCLC, respectively, using the same diagnostic platform (CellSearch) and the same cut-off value (5 CTCs/7.5 ml) for survival analyses. Increased CTC counts, measured at baseline and on the 28th day of chemotherapy, were the strongest predictors of TTF and PFS in multivariate analysis, respectively (HR: 8.635; 95% CI: 2.341–15.613; p < 0.001 and HR: 8.017; 95% CI: 4.421–13.733, p < 0.001), while baseline CTC count was also independently correlated with PFS (HR: 6.833; 95% CI: 3.947–13.572, p < 0.001) (Yang et al., 2017); furthermore, an independent association between increased CTC count at baseline and OS (HR: 0.55; 95%CI: 0.33–0.92; p = 0.022) but not PFS (HR: 0.68; 95% CI: 0.42–1.1; p = 0.118) was reported by the second research group (Lindsay et al., 2017). Independent associations between baseline CTC count −enumerated using CellSearch- and survival (PFS and OS; HR: 3.93; 95% CI: 1.38–11.15; p < 0.05 and HR: 5.32; 95% CI: 1.62–17.50; p < 0.05, respectively), as well as between CTC count after administration of two chemotherapy cycles and OS (HR: 3.07; 95% CI: 1.33–7.05; p < 0.05) were also observed by Zhou et al. (2017). In another series by Qi and Wang (2017), enrolling 100 patients with locally advanced squamous cell lung cancer, increased CTC count, enumerated by CellSearch before or after one chemotherapy cycle, was an independent predictor of PFS and OS.

Data supporting the prognostic value of CTC count in advanced NSCLC were also obtained in studies employing diagnostic platforms other than CellSearch for CTC isolation and enumeration; He et al. (2016) found significant associations of baseline CTC count (assessed by flow cytometry) with 3-year survival and PFS (p = 0.01 and p = 0.005, respectively), while Zhang et al. (2016) reported that CTC enumeration at baseline −using the antigen-based platform Cytell- may also confer independent prognostic information, with regard to both PFS and OS (HR: 0.360; 95% CI: 0.151–0.862; p = 0.022 and HR: 0.316; 95% CI: 0.126–0.792; p = 0.014, respectively).

In contrast to the above findings, Hirose et al. (2012) failed to observe any statistically significant association between baseline CTC count and PFS or OS, in a cohort of 33 metastatic NSCLC patients, despite a significantly higher rate of progressive disease among CTC-positive cases (p = 0.02), while Coco et al. (2017) reported lack of prognostic relevance of ScreenCell-enumerated CTCs in stage IIIB-IV NSCLC. Juan et al. (2014) failed as well to demonstrate any prognostic value (with regard to PFS or OS) of CTC count at baseline or CTC count change from baseline to completion of the second chemotherapy cycle, in a series of 37 fragile patients with advanced NSCLC, previously enrolled in two multi-institutional phase II trials. Considering the selected features of the study participants (elderly patients > 70 years with PS:0-1 or patients of any age with PS:2), it must nevertheless be noted that the findings of the latter study may not be directly comparable to those derived from different clinical settings.

3.1.3. Early and advanced, operable NSCLC (stages I–IV)

Hofman et al. (2011a) investigated the prognostic value of CTC count, using the ISET assay, in 208 NSCLC patients with operable, stage I–IV disease. The presence of ≥50 CTCs/10 ml of peripheral blood at baseline (preoperatively) was independently associated with a shorter DFS (HR: 2.631; 95% CI: 1.557–4.651; p = 0.003) and OS (HR: 2.096; 95% CI: 1.331–3.300; p = 0.001). Almost concurrently (within the same year), the same research group (Hofman et al., 2011b) published a study evaluating the efficacy of CellSearch and ISET platforms as prognostic tools in 210 patients with resectable NSCLC. Positive CTC count at baseline (> 1 CTC/7 ml) was associated with worse DFS, regardless of the method used (CellSeach only, ISET only, CellSearch and/or ISET), both in univariate and in multivariate analysis. Notably, positive CTC count detected by CellSearch and/or ISET was the strongest indicator of DFS, compared not only to the use of CellSearch or ISET alone, but also to histology and pTNM stage (HR, 1.235; 95% CI, 1.056–1.482; p < 0.001). Conclusively, the authors suggested that CellSearch and ISET can complement one another for the prognostication of patients with operable NSCLC, and that preoperative CTC count detected by both methods may represent a reliable and independent predictor of the clinical outcome in this setting.

Chudasama et al. (2017) investigated the prognostic relevance of another platform for CTC enumeration- i.e. ScreenCell®- in a small cohort of patients with early or late-stage, but operable, NSCLC. In contrast to most previous data, mainly derived from studies using antigen-based diagnostic platforms such as CellSearch, positive preoperative CTC count was found to correlate with an improved, rather than worse, OS (HR: 0.04453; 95% CI: 0.007131–0.2781; p < 0.0009). This seemingly paradoxical finding may, at least partly, be due to the size-based detection method used, leading to isolation of CTCs with little or no prognostic relevance, as commented by the authors themselves, and/or to the small sample size of this study, limiting the statistical power of the results.

3.2. Small cell lung cancer (SCLC)

3.2.1. Limited and extensive disease stage SCLC

The majority of published series on the prognostic value of CTC count in SCLC have included both limited (LD) and extensive disease (ED)-stage patients. Hou et al. (2009) prospectively evaluated the clinical relevance of CTCs in 50 chemo-naive SCLC patients, using the CellSearch platform and a cut-off of 300 CTCs/7.5 ml for survival analysis. Increased CTC counts, both at baseline and on the 22nd day after treatment initiation, were significantly correlated with shorter OS in univariate analysis (HR: 1.1; 95% CI: 1.02–1.18; p = 0.015 and HR: 1.43; 95% CI: 1.09–1.86; p = 0.01, respectively), but not in the multivariate model. Three years later, the same research group performed similar analyses in a larger cohort of chemo-naive SCLC patients, using both the CellSearch and ISET platforms, and a lower cut-off point (50 CTCs/7,5 ml) (Hou et al., 2012). Increased CTC count at baseline (before treatment) was found to independently predict PFS (HR: 2.01; 95% CI: 1.17–3.46; p = 0.011) and OS (HR: 2.45; 95% CI: 1.39–4.30, p = 0.002) after adjusting for stage, performance status, number of metastatic sites, treatment and lactate dehydrogenase levels. Interestingly, reduction in CTC count after one chemotherapy cycle was the strongest predictor of OS in multivariate analysis (HR: 4.1; 95% CI: 1.1–15.1, p = 0.03), independently of baseline CTC count performance status, stage, and number of metastatic sites.

In line with the above findings, Naito et al. (2012) reported statistically significant associations between CTC count at several time points (prior to treatment initiation, after completion of first-line chemotherapy and at relapse) and 1-year survival (p = 0.0014), post-

treatment survival (p = 0.0014) and post-relapse survival (p < 0.0001), respectively. Interestingly, when baseline CTC count values were stratified by disease stage, a statistically significant association with survival was observed only in the ED subset of patients (p = 0.0282) and not in the LD subgroup (p = 0.4387). In another series of LD and ED SCLC patients, by Hiltermann et al. (2012) CTC counts at baseline and after one and four chemotherapy cycles, respectively, were all significantly associated both with PFS and OS in univariate analysis, for all cut-off values evaluated (2, 5, 16 and 215 CTCs per 7.5 ml), retaining their independent significance in the multivariate model after adjusting for all other statistically significant prognostic covariates (age, tumor stage and tumor response). Notably, decreased CTC count (CTCs < 2) after the first chemotherapy cycle was the only variable for OS that retained its independent significance when PS and sex were also included in the model (HR: 5.7; 95% CI: 1.7–19.0; p = 0.004).

Strong, independent associations between CTC count and survival have also been reported by research groups using diagnostic platforms other than CellSearch. An independent association between increased CTC count at baseline and OS (HR, 3.91; 95% CI: 1.19–12.87; p = 0.026) was also reported by Igawa et al. (2014), in a relatively small series of 30 LD and ED SCLC patients, employing a telomerase-specific replication-selective adenovirus OBP-401 assay (TelomeScan®). In a larger study by Shen et al. (2017), enrolling 80 LD/ED SCLC patients and using ligand targeted-polymerase chain reaction for CTC isolation, baseline CTC count had an independent prognostic value with regard to PFS (HR: 0.656; 95% CI: 0.473–1.000; p = 0.049), while a marginally significant association with OS was observed in univariate analysis only (HR: 0.59; 95% CI: 0.31–1.01; p = 0.056 p = 0.056).

The independent prognostic value of CTC enumeration in SCLC is further supported by the results of two recent studies, performed by the same research group (Messaritakis et al., 2017a,b). Increased CTC count, both at baseline and at disease progression was independently associated with reduced PFS and OS, while an independent association between CTC values measured after one chemotherapy cycle and OS was also observed. The diagnostic method used for CTC enumeration was CellSearch, while the cut-off point for survival analysis was 5 CTCs/7.5 ml in both series.

3.2.2. Extensive disease stage SCLC

Normanno et al. (2014) investigated the prognostic value of CTCs in 60 chemotherapy-naive patients with ED SCLC, using CellSearch and several different cut-off points for survival analysis (ranging from 2 to 282 CTCs/7.5 ml). CTC count at baseline and after one chemotherapy cycle and CTC count change between these time points were all significantly associated with OS; however, CTC count reduction > 89% showed the strongest correlation to lower risk of death (HR: 0.24; 95% CI: 0.09–0.61) and addition of this covariate significantly increased the accuracy of the prognostic model (bootstrap p-value = 0.009). These findings suggest that CTC count change during the course of treatment may be a stronger predictor of prognosis than CTC enumeration at a single time point.

In the largest cohort of ED SCLC patients published to date, by Cheng et al. (2016), employing the CellSearch platform for determination of CTC status and using a threshold of 10 CTCs/7.5 ml for survival analysis, CTC count was assessed at various time points (at baseline, after the second cycle of chemotherapy, and on disease progression) and correlated with prognosis. Baseline CTC count was independently associated with OS (HR: 0.304; p < 0.0001), while CTC count after the second chemotherapy cycle was found to independently predict both PFS (HR: 0.467; p = 0.0211) and OS (HR: 0.295;p = 0.0002). Taking into account the high levels of CTC count observed in SCLC patients, and their significant variability, patients were further categorized into subgroups, according to CTC count change from baseline to post-treatment. The first (low-drop-high/L-D-H = 1) subgroup, included patients with less than 10 CTCs/7.5 ml at baseline and after the second chemotherapy cycle, or with CTC count change > 150 CTCs/7.5 ml; patients categorized into the L-D-H = 2 group had a baseline or post-treatment CTC count ≥10 CTCs/7.5 ml or CTC count change ≤150 CTCs/7.5 ml. The L-D-H algorithm was independently associated with PFS and OS (HR: 0.484; p = 0.0149 and HR: 0.307; p < 0.0001, respectively), and was a stronger predictor of survival, as compared to imaging criteria of treatment response/RECIST (HR: 0.321; p < 0.0001 versus HR: 0.611; p = 0.0576). Corroborating these data, Salgia et al. (2017) reported statistically significant associations between increased CTC count at baseline and survival (PFS and OS; p = 0.024 and p = 0.017, respectively) in a smaller series of ED SCLC patients; CTC values measured on the first day of the second chemotherapy cycle were also significantly correlated with PFS and OS (p = 0.001 for each association).

In contrast to all the above findings, Huang et al. (2014) failed to observe any statistically significant associations between CTC count and prognosis in a small cohort of 24 patients with ED SCLC (82); neither baseline CTC count nor CTC count change (from baseline to post-treatment) were correlated with survival, but the authors emphasized the small sample size of the study and its preliminary nature.

4. Conclusions and future perspectives

Despite the presence of significant variability among studies, with regard to patients' populations features, cut-off values for CTC positivity and survival analysis and CTC detection methods used, the majority of published results seem to concur that CTC count may significantly correlate with survival endpoints, thus indicating its potential as a non-invasive biomarker of prognosis, both in SCLC and NSCLC. Notably, almost all large cohorts (involving more than 100 patients), have confirmed the independent prognostic value of baseline and/or post-treatment CTC count and/or CTC count change at variable time points, after adjusting for statistically and clinically significant prognosticators, including disease stage. Nevertheless, while the use of CTCs in lung cancer prognosis is rapidly expanding, some significant obstacles remain to be surmounted.

Development of highly sensitive and specific EpCAM-independent CTC capture platforms, for isolation and subsequent comprehensive molecular analysis of a pure population of a sufficient number of living circulating tumors cells remains an unmet and important goal. Standardization of the assays used to ensure reproducibility and consistency of results, and rigorous testing of the diagnostic accuracy and cost-effectiveness of these techniques in prospective studies are warranted as well. In the clinical arena, large-scale prospective studies with longitudinal follow-up, therapeutically homogeneous patient populations and robust statistical analysis methods and endpoints (e.g. OS) should be conducted for optimal clinical validation and evidence-based implementation of CTCs to routine clinical practice. Most importantly, given the relatively limited amount of longitudinal data on the prognostic value of CTCs in operable, earlier-stage NSCLC, as well as in specific molecular subgroups of patients (e.g. EGFR mutation-positive or ALK-positive cases, eligible for targeted therapies) or in patients who are already receiving or are scheduled to initiate immunotherapy, the need for additional research in these particular settings must also be emphasized. In the era of personalized oncology, and bearing in mind the wide heterogeneity of lung cancer, focus must remain on matching the most suitable biomarkers to each individual patient, with the ultimate aim of improving not only personalized treatment planning but also overall patient care and quality of life.

Conflict of interest

None declared.

Authors' contributions

O.F. performed the initial literature review and wrote the first manuscript draft; D.G. conducted additional literature review and wrote the final manuscript draft; A.C and K.S. revised the manuscript for intellectual content. All authors have approved the final manuscript.

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