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Circulating tumor cells in small-cell lung cancer: a predictive and prognostic factor

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Background: Initial response of small-cell lung cancer (SCLC) to chemotherapy is high, and recurrences occur frequently, leading to early death. This study investigated the prognostic value of circulating tumor cells (CTCs) in patients with SCLC and whether changes in CTCs can predict response to chemotherapy.

Patients and methods: In this multicenter prospective study, blood samples for CTC analysis were obtained from 59 patients with SCLC before, after one cycle, and at the end of chemotherapy. CTCs were measured using CellSearch® systems.

Results: At baseline, lower numbers of CTCs were observed for 21 patients with limited SCLC (median = 6, range 0–220) compared with 38 patients with extensive stage (median = 63, range 0–14 040). Lack of measurable CTCs (27% of patients) was associated with prolonged survival (HR 3.4; $P \leq 0.001$). CTCs decreased after one cycle of chemotherapy; this decrease was not associated with tumor response after four cycles of chemotherapy. CTC count after the first cycle of chemotherapy was the strongest predictor for overall survival (HR 5.7; 95% CI 1.7–18.9; $P = 0.004$).

Conclusion: Absolute CTCs after one cycle of chemotherapy in patients with SCLC is the strongest predictor for response on chemotherapy and survival. Patients with low initial CTC numbers lived longer than those with higher CTCs.

Key words: circulating tumor cells, prognosis, small-cell lung cancer, treatment prediction

introduction

Small-cell lung cancer (SCLC) is a disease with high propensity for hematogenously spread metastases, often already present in early-stage disease. Classically, SCLC is divided into limited disease stage (LD, localized disease) and extensive disease stage (ED, metastasized disease). Mortality of SCLC remains high; even in patients with LD, 5-year survival is only ∼10% [1] (maximum 26%) [2]. This is due to metastases in many organs and perhaps to circulating tumor cells (CTCs) that originate from detachment of the primary tumor mass and migration of tumor cells to secondary sites via the lymphatic and blood system. The presence of CTCs has been demonstrated in the blood of patients with various solid tumors [3]. Their presence has been associated with poor outcome in metastatic breast, colorectal, prostate, gastric, and non-SCLC [4–8]. In SCLC, the presence of ≥2 CTCs/7.5 ml of peripheral venous blood was found in 75% of patients with both LD and ED [9, 10].

A problem in the analysis of CTCs may be the low number of CTCs encountered in whole blood, potentially affecting the reproducibility of counting these tumor cells. An additional aim of this study was to assess the repeatability of two independent measurements at each time point.

The presence of CTCs may rather be a reflection of the metastatic potential of the tumor and therefore may correlate better with survival than the bulk of disease as reflected by tumor imaged with computed tomography (CT) [11, 12]. In this study, the predictive value of CTCs for progression-free survival (PFS) and overall survival (OS) was studied. Furthermore, we hypothesize that the resistant CTCs present after one cycle of chemotherapy are those that determine the fate of SCLC patients in terms of OS.

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patients and methods

study design

This is a prospective study from four medical centers in consecutive patients with SCLC. Patients were evaluated with laboratory tests, CT of the chest and upper abdomen and when indicated with magnetic resonance imaging of the brain and radionuclide bone scan or positron emission tomography (PET) with [^{18}F]2-fluoro-2-deoxy-D-glucose (FDG). Patients were staged according to the new Tumor, Node, Metastasis classification (TNM 7th edition), and for comparison with older studies, they were reclassified them into LD and ED [13].

CTs were made after two and four cycles of chemotherapy for tumor response assessments and thereafter imaging was carried out every 12 weeks. Tumor size and response to therapy were independently re-evaluated according to RECIST version 1.1 [14]. If a patient progressed before a second CT scan had been made, this patient was denoted as progressive. PFS was determined from the start of chemotherapy until tumor progression or loss of follow-up. OS was measured from the start of the first chemotherapy until death or loss of follow-up. The study was approved by all medical ethical committees.

patients

Inclusion criteria were cytological or histological confirmation of SCLC, >18 years of age, performance score (PS) ≤3 and able to receive first-line chemotherapy. All patients were treated with four cycles of platinum-based doublets followed by prophylactic cranial irradiation. Those with cT1-4N0-3M0 (LD stage) received concomitant thoracic radiotherapy during their second and third chemotherapy cycles.

CTC enumeration

Two 10-ml Cellsave (Veridex, Raritan) preservative tubes were drawn before the initiation of therapy, before cycle 2, and after cycle 4. Blood tubes were stored at room temperature and centrally processed within 96 h of collection. Two 7.5 ml of aliquots were processed on the CellTracks® Autoprep, using the Cellsearch® epithelial cell kit (Veridex) for CTC enrichment and staining. Image acquisition and CTC enumeration was carried out on the Celltracks™ analyzer II as described previously [3]. CTC counting was carried out without knowledge about clinical characteristics, and CTCs were blinded to the treating physician and independently merged with clinical data.

statistical analysis

Test characteristics (sensitivity and specificity) of the highest quartile (215 cells/7.5 ml), median (16 cells/7.5 ml), cutoffs at 5 cells/7.5 ml and lowest quartile (2 cells/7.5 ml) CTCs were determined with ROC curves based on median PFS and OS. Repeatability of CTC counts between the two different 7.5-ml blood tubes was expressed as intraclass correlation coefficient [Ri = between-subject variance/(within + between-subject variance)] and as coefficient of repeatability (CR = 2 SD of the mean difference of repeated measurements) [15, 16]. Differences between groups were tested using Fisher's exact test when data were binary, or with the independent-samples Mann–Whitney *U* test in the case of continuous variables. Correlations between tumor response and CTC at different time points were tested with non-parametric Spearman's rho bivariate correlation with a two-tailed test for significance. Kaplan–Meier estimates of survival were based on the number of CTCs at baseline, after one cycle, and at the end of treatment. Survival curves were compared using the log-rank test (Mantel–Cox). Cox proportional hazards regression analysis was used to estimate multivariate hazard ratios for PFS and OS for the following covariates: age, disease stage, ECOG PS, tumor response, and CTCs before therapy, after the first and last cycle of chemotherapy. For CTC before therapy, tumor response was kept out of the model. By stepwise elimination of covariates with $P > 0.05$, the best model was identified. Statistical analysis was carried out using SPSS (release 18.0.3, IBM, NY). *P*-values of ≤0.05 were considered significant.

results

patient characteristics

Fifty-nine patients from four hospitals were included in this study. The median age of the patients was 64 (range 47–84) years, 35 patients were male, 3 had recurrent disease; 21 patients had cT1a-4N0-3M0 disease (LD) and 38 had cT1a-4N0-3M1 disease (ED) upon diagnosis. The median follow-up was 280 days (range 5–1424); 12 patients died within 9 weeks. All patients had a CT scan of the chest and upper abdomen before treatment, and 49 patients had an evaluation CT scan after completing therapy. The reason for not having serial imaging studies available for review was death before the first follow-up imaging (10 patients). Patient characteristics are listed in Table 1. Significant differences between LD and ED patients were observed for TNM stage, response to chemotherapy, total number of CTCs at baseline, tumor response after four cycles and OS. No significant differences in CTCs were observed after one and after four cycles of chemotherapy.

CTC test characteristics

The repeatability of CTC measurements at the three time points was high with an intraclass correlation coefficient Ri = 0.997 and a high CR ($r^2 = 0.990$) (supplementary Figure S1, available at *Annals of Oncology* online). With ROC analysis (AUC = 0.73; $P = 0.003$), the highest specificity (93%) for OS was observed at 215 CTC in 7.5 ml of blood (the highest quartile), but with poor sensitivity (60%). At 16 CTCs (the median quartile), specificity decreased to 65% and sensitivity improved to 67%; at 5 CTCs, specificity was 41% and sensitivity was 80%. At the lowest quartile, i.e. 2 CTCs, specificity was 41% and sensitivity was 87%. In this study, 2 CTCs in 7.5 ml of blood could be detected reliably at the lowest level with highest sensitivity; therefore, this cut-off was chosen for further analysis.

CTC and patient characteristics

Out of 59 patients, 43 (73%) had two or more CTCs in 7.5 ml of blood at baseline (range 0–14 040). Of the remaining 16 patients, 9 had 0 CTCs (7 LD, 2 ED) and 7 patients had 1 CTC in 7.5 ml of blood (3 LD, 4 ED). Of 38 patients with ED, 32 (84%) had ≥2 CTCs in 7.5 ml of blood (range 0–14 040). This contrasted with patients with LD, where 11 of 21 patients (52%) had ≥2 CTCs in 7.5 ml of blood (range 0–220). Changes in CTCs for all 44 patients for whom a post-treatment blood sample was obtained are shown in Figure 1. CTCs dropped dramatically following the first cycle of chemotherapy in most patients. Twelve patients died within 9 weeks; these patients had high CTCs at baseline. The decrease in CTCs from baseline to those after one cycle of chemotherapy and the absolute CTCs after one cycle of chemotherapy did not

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Table 1. Clinical characteristics of 59 patients with small-cell lung cancer

Characteristic	All patients	LD	ED	*P*-value
Age, years (minimum–maximum)	64 (47–84)	67 (47–84)	62 (47–81)	0.39#
Male/female	35/24	12/9	23/15	1.0*
ECOG performance status, *n* (%)				
0	27 (46)	12 (57)	15 (40)	0.09#
1	22 (37)	8 (38)	14 (37)	
2	4 (7)	0 (0)	4 (10)	
3	6 (10)	1 (5)	5 (13)	
Stage, *n* (%)		21 (36)	38 (64)	
Stage TNM 7th edition, *n* (%)				
2a	2 (3)	2 (10)		
3a	12 (20)	12 (57)		
3b	7 (12)	7 (33)		
4	38 (65)		38 (100)	
Primary disease[a], *n* (%)	56 (95)	21 (100)	35 (92)	0.55*
Response to chemotherapy, *n* (%)				
Complete response	11 (19)	7 (33)	4 (11)	0.009#
Partial response	30 (51)	11 (52)	19 (50)	
Stable disease	7 (12)	2 (10)	5 (13)	
Disease progression	11 (19)	1 (5)	10 (26)	
Response CT after four cycles[b], *n* (%)	47 (80)	20 (95)	27 (71)	0.04*
CTCs				
Baseline, *n* (median; minimum–maximum)	59 (16; 0–14 040)	21 (6; 0–220)	38 (63; 0–14 040)	≤0.001#
After one cycle[b], *n* (median; minimum–maximum)	37 (0; 0–1681)	18 (0; 0–6)	19 (1; 0–1681)	0.10#
After four cycles[b], *n* (median; minimum–maximum)	34 (1; 0–117)	16 (0; 0–3)	18 (1; 0–117)	0.11#
Percentage remaining CTC at visit 2[c], *n* median (minimum–maximum)	37 (0; 0–300)	18 (0; 0–300)	19 (0; 0–52)	0.46#
Percentage change in tumor volume according to RECIST[d], *n*, median (minimum–maximum)	49 (61; −58 to 100)	20 (67; 23–100)	29 (58; −58 to 86)	0.05#
Overall survival days, *n*, (median; minimum–maximum)	59 (280; 5–1424)	21 (356; 9–1424)	38 (213; 5–818)	0.001#

CTC, circulating tumor cell; ED, extensive disease stage; LD, limited disease stage; CT, computed tomography; TMN, Tumor, Node, Metastasis classification.
[a]Primary disease: small-cell lung cancer not treated for the disease with chemotherapy before (e.g. first-line treatment).
[b]Cycle: cycle of chemotherapy, one cycle corresponds with 3 weeks.
[c]Percentage remaining CTC = (CTC visit 2/CTC baseline) × 100.
[d]Two CT scans after two cycles included.
**P*-value Fisher's exact test.
#*P*-value Mann–Whitney *U* test.

correlate with tumor response (respectively $r_s = 0.18$, $P = 0.24$ and $r_s = -0.27$, $P = 0.08$, for both $n = 44$).

prognostic significance of CTCs at baseline

The median survival for patients with CTCs > 215 (highest quartile) was 157 days compared with 729 days for patients with CTCs < 2 (lowest quartile) (log-rank test, $P \leq 0.001$). After disease stage, the strongest prognostic factor for survival was CTC < 2 at baseline [HR 3.0 (95% CI 1.4–6.6) for PFS and 3.1 (95% CI 1.3–7.1) for OS; supplementary Figure S1, available at *Annals of Oncology* online].

univariate survival analyses and multivariate cox proportional hazards regression analyses

CTC analysis after one cycle of chemotherapy at a median of 3 weeks (range 2–5 weeks) from baseline was available for 37 patients. Of these patients, those with CTC < 2 ($n = 29$) at the second time point lived longer, both measured by PFS (10.7 versus 2.9 months; $P \leq 0.001$) and OS (12.3 versus 8.1 months; $P \leq 0.001$). A third blood sample after the completion of therapy was obtained in 34 patients at a median of 14 weeks (range 10–19 weeks). Patients with CTC < 2 ($n = 27$) after four cycles of chemotherapy lived longer as measured by PFS (7.9 versus 3.9 months; $P = 0.007$) and OS (12.3 versus 8.1 months; $P = 0.05$).

In the univariate analyses, age, tumor stage, tumor response, and all CTC cut-off levels at baseline and after one and four cycles of chemotherapy were significant predictors of both PFS and OS: sex and PS were not (supplementary Tables S1 and S2, available at *Annals of Oncology* online). In multivariate Cox proportional hazards regression analysis, adjusting for the prognostic covariates, CTCs remained an independent prognostic factor for PFS and OS at all time points. As a predictor, the CTCs < 2 after the first cycle was the only parameter for OS that remained as an independent significant marker in the model, taking into account disease stage, tumor response, PS, sex, and age (Table 2). The decrease in tumor volume as measured by CT after two and four cycles of chemotherapy was not significant in the step-down logistic

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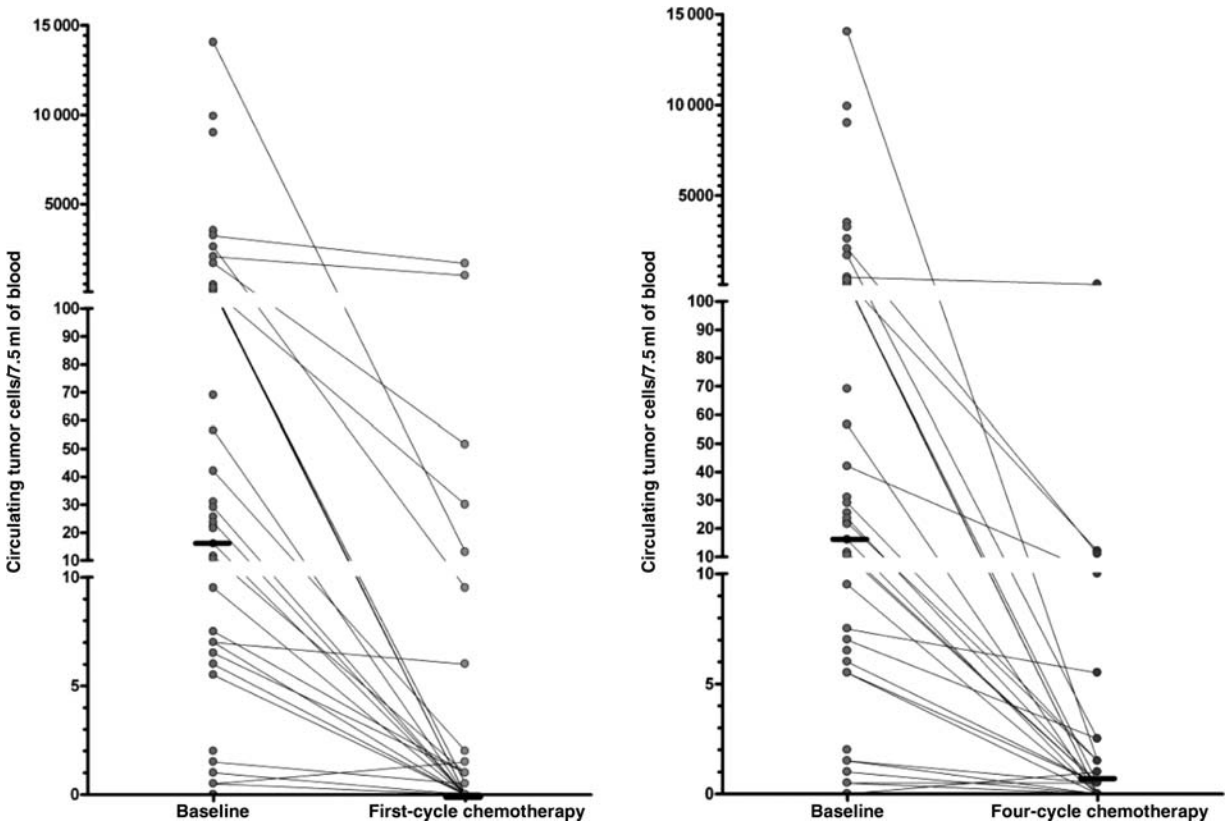

Figure 1. Change in the number of circulating tumor cells (CTCs) per 7.5 ml of blood at baseline, after one cycle of chemotherapy and after four cycles of chemotherapy in small-cell lung cancer patients. The long thick dash denotes the median CTC number; in 17 patients (39%), CTCs dropped to zero cells; in 9 patients (20%), CTCs remained at zero cells. In one patient (2%), CTCs increased from 0.5 to 1.5 cells. After four cycles of chemotherapy, 18 patients showed a decrease ($n = 6$), or remained at zero cells ($n = 12$), whereas 9 patients showed an increase compared with the CTC values after one chemotherapy cycle (data not shown).

model (supplementary Figure S3, available at *Annals of Oncology* online).

discussion

In this multicenter study, CTCs were present in 73% of patients with SCLC (both ED and LD) as shown previously [9, 10]. The absolute numbers of CTCs after one cycle of chemotherapy was the strongest predictive factor for survival in a multivariate Cox regression analysis. Remarkably, CTC count was not associated with tumor response. Therefore, CTCs appear to reflect a more active tumor compartment than the bulky tumor estimated by imaging. This implies that disseminated tumor cells in blood are more important for predicting the fate of these patients than the volume of the remaining bulk of the tumor mass following treatment.

This is the first study to demonstrate that CTCs in SCLC are a better predictor of survival than both disease stage and tumor response determined by CT imaging. In line with our study, a previous study demonstrated that low CTC numbers after chemotherapy in SCLC is associated with a prolonged survival with an HR of 2.76. In the study by Naito et al. [10], no differences between radiologic responses and post-treatment CTC levels could be demonstrated. Our study differed in the timing of CTC measurement [after one cycle (in this study) versus after treatment], in the number of progressive disease patients (1 versus 12 patients) and stable disease (7 versus 5 patients) after therapy, and the number of LD being 21 versus 27 and ED being 38 versus 24 patients, and finally a lower cut-of number was chosen in the current study (2 versus 8 CTC/7.5 ml of blood) compared with the study by Naito et al. [10]. All of these may explain this difference in results. The unusually small difference in survival times for LD and ED (12.6 versus 10.1 month) in this study will have influenced our regression model, thereby rendering disease stage a less strong predictor than CTCs.

For CTC enumeration, we used the CellSearch system that has been extensively validated in patients with metastatic carcinomas [3–5, 8]. The system enriches cells from 7.5 ml of blood expressing the epithelial cell adhesion membrane (EpCAM) antigen and identifies CTCs as nucleated cells expressing cytokeratin 8/18 or 19 and lacking the leukocyte antigen CD45. Several reports suggest that CTCs can be effectively detected with this test system, also in SCLC [9, 17, 18]. In neuroendocrine tumors, EPCAM expression has been detected [19]. In addition, metastasis in SCLC patients were found to express EPCAM [18].

In the current study, we also addressed the issue of repeatability of measuring CTCs in SCLC, which is important when measuring low numbers of CTCs. As demonstrated by the Bland–Altman plot, repeatability was also robust with an

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Table 2. Uni- and multivariate analyses of predictive factors for overall survival in patients with small-cell lung cancer

Variables	Univariate[a]			Multivariate[b]		
	Median OS		*P*-value	HR	95% CI	*P*-value
Age (median 64 years)	10.1	12.6	0.05	1.3	0.5–3.4	0.54
Sex (male versus female)	11.0	12.3	0.36	1.3	0.5–3.3	0.66
Performance score (2.3 versus 0.1)	3.7	11.8	0.19	5.0	1.2–20.7	0.03
Tumor stage (ED versus LD)	10.1	12.6	0.03	1.6	0.6–4.2	0.34
Tumor response	5.2	12.2	0.003	1.8	0.5–6.8	0.37
CTC baseline cut-off 2 cells	10.9	24.0	0.03	1.9	0.7–5.3	0.23
CTC after one chemo-cycle cut-off 2 cells	8.1	12.3	≤0.001	3.5	0.8–15.3	0.09
Same model as above for various CTCs in stepdown Cox regression analysis						
Performance score (2.3 versus 0.1)	3.7	11.8	0.19	4.6	1.2–18.0	0.03
Tumor stage (ED versus LD)[c]	10.1	12.6	0.03	1.5	0.6–3.9	0.39
CTC baseline cut-off 2 cells	10.9	24.0	0.03	2.3	0.8–6.3	0.11
CTC after one chemo-cycle CTC cut-off 2 cells	8.1	12.3	≤0.001	5.7[d]	1.7–19.0	0.004

LD, limited disease stage; ED, extensive disease stage; CTC, circulating tumor cell; OS, overall survival.

[a]Kaplan–Meier estimate.

[b]Cox regression analysis.

[c]Disease stage not significant in the model; when left out of the model, CTC became significant (data not shown).

[d]Tumor response not in the final model (not significant); both disease stage and PS not significant in the final model—CTC as the only remaining parameter after one cycle of chemotherapy, HR 7.9 (95% CI 2.6 to 24.2 $P \leq 0.001$).

excellent intraclass correlation coefficient (Ri = 0.997). These results imply that the determination of CTCs at very low numbers is a reliable, minimally invasive method, and may be useful for monitoring SCLC patients. The sensitivity for OS of the highest quartile of CTCs (≥215 cells/7.5 ml of blood) is 60% in this study, but increases to 87% in the lowest quartile (<2 cells/7.5 ml of blood). Thus it may be justified to use a threshold for unfavorable CTC counts in SCLC of ≥2 CTC/7.5 ml, which is lower than the ≥3 CTC/7.5 ml used for colorectal cancer and ≥5 CTC/7.5 ml for breast and prostate cancer [3–5, 8]. The robustness remains when considering the extreme low-CTC background in patients with benign disease and healthy controls [3–5, 8].

The median survival for all LD-stage patients (12 months) is rather short in our population due to comorbidities. Staging procedures were according national guidelines and included CT scans, FDG-PET, and sometimes bone scans. In selected studies, the median OS for LD in SCLC is reported to be around 20 months [20]. After chemotherapy, the phenotype of SCLC is often changed into a more complex, secondary chemo-resistant type [21]. In SCLC patients, even very aggressive treatment regimens do not have a significant effect on tumor relapse [22]. Apparently, the tumor is able to transform into a state, whereby regular chemotherapy is ineffective. Changes in histology may reflect a differentiation toward a more chemo-resistant profile [21, 23]. In metastatic breast cancer, CTCs have been shown to be a biomarker reflecting the intrinsic biology of the tumor [12]. In SCLC, the CTCs present at baseline may thus not all represent the most malignant subpopulation as suggested before [24]. Instead, the CTCs surviving the first cycle of chemotherapy may be regarded as a marker of chemotherapy resistance. This is consistent with our results showing that baseline CTC counts were not correlated with tumor response, and that tumor response was a worse predictor of prognosis than the CTCs after the first cycle of chemotherapy.

SCLC is often regarded as a systemic disease, and survival even in patients with LD is poor [21]. Even the recently introduced TNM system for SCLC shows variation in survival point estimates, leading to a rather poor discrimination in 5-year survival, ranging from 38% for cT1N0M0 to 1% for M1b disease [25]. Prognostic models for SCLC using PS and laboratory tests provide a similar estimation of survival as the combination of PS and disease stage assessed by imaging tests [26]. There is a need for easy available (blood) tests for better prognostic and predictive systems in cancer in general, but especially in SCLC [27]. In breast cancer, CTCs after 1 month of treatment have been shown to be a better predictor of OS than radiologic follow-up by CT at 12 weeks [11]. In advanced gastric cancer, CTCs after the first cycle of chemotherapy were the strongest predictor of OS as well [6]. Therefore, assessment of CTCs is an early reproducible indication of disease status.

In conclusion, CTCs are a highly reproducible predictive end point for survival and may determine therapeutic tumor changes better than imaging. CTCs should be validated in large studies.

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disclosures

HT and AGJT were employees at Immunicon Corporation at the start of the study. LWMMT is a consultant for Veridex LLC and receives research funding from Veridex LLC. All remaining authors have declared no conflicts of interest.

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