Oncology Research, Vol. 25, pp. 1601–1606
Printed in the USA.

0965-0407/17 $90.00 + .00
DOI: https://doi.org/10.3727/096504017X14928634401178
E-ISSN 1555-3906
www.cognizantcommunication.com

Circulating Tumor Cells Predict Prognosis Following Tyrosine Kinase Inhibitor Treatment in EGFR-Mutant Non-Small Cell Lung Cancer Patients

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Epithelial growth factor receptor (EGFR) mutations are present in 10%–26% of non-small cell lung cancer (NSCLC) tumors and are associated with the response to tyrosine kinase inhibitors (TKIs). This study aimed to detect and quantify the presence of circulating tumor cells (CTCs) in EGFR-mutant NSCLC patients and investigate their possible role in providing prognostic information. Enrolled patients received erlotinib (150 mg) or gefitinib (250 mg) orally once daily as the first-line treatment. Serial blood samples were taken at baseline (CTC-d0) and on day 28 (CTC-d28) following the initiation of erlotinib/gefitinib for detection of CTCs using the CellSearch system. CTCs ≥2 were found in 47/107 (44%) and CTCs ≥5 in 17/107 (15%). The CTC measurements were dichotomized as favorable (<5 CTCs) and unfavorable (≥5 CTCs) groups. The median progression-free survival (PFS) interval for patients in the favorable group at baseline was 11.1 months, significantly longer than the median PFS time of 6.8 months achieved by patients in the unfavorable group ($p = 0.009$). Patients in the favorable group on day 28 exhibited significantly longer PFS compared with patients in the unfavorable group (11.6 vs. 6.3 months; $p < 0.0001$). In univariate analysis, CTC-d0 ≥ 5 versus CTC-d0 = 0–4 was significantly associated with poor PFS and time-to-treatment failure (TTF). CTC-d28 ≥ 5 versus CTC-d28 = 0–4 was significantly associated with a poor PFS outcome. CTC-d0 and CTC-d28 remained independent poor prognostic markers in the stepwise multivariate analysis. Our study indicates that the CTC count is a prognostic factor for PFS and TTF outcomes in patients with advanced EGFR-mutant NSCLC.

Key words: EGFR-mutant non-small cell lung cancer (NSCLC); Circulating tumor cells (CTCs); Prognostic marker

INTRODUCTION

Lung cancer is the most common cause of cancer deaths among men and women worldwide[1]. Epithelial growth factor receptor (EGFR) mutations are present in 10%–26% of non-small cell lung cancer (NSCLC) tumors. Five prospective randomized clinical trials have established EGFR-targeting tyrosine kinase inhibitors (TKIs) as a first-line treatment option for EGFR-mutant NSCLC patients[2–6]. In addition to the development of novel therapies for this disease, strategies such as biomarker development are needed to predict clinical outcomes.

Circulating tumor cell (CTC) count, an easily accessed "liquid biopsy," has the potential to be a prognostic, predictive, and pharmacodynamic biomarker. Studies in breast, prostate, colorectal, and lung cancers have demonstrated the prognostic significance of CTC counts, and changes in CTC counts with standard therapy have highlighted the potential of CTC as a predictive biomarker[7–10]. However, the association between CTC count and the prognosis of EGFR-mutant NSCLC patients treated initially with EGFR TKIs remains unclear. The goal of this study was to determine the prevalence of CTCs and their relationship with progression-free survival (PFS)/time-to-treatment failure (TTF) in advanced EGFR-mutant NSCLC patients.

MATERIALS AND METHODS

Patients aged ≥18 years with radiologically confirmed stage IIIB (ineligible for sequential radiotherapy

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or concurrent chemo/radiotherapy) or stage IV disease according to tumor node metastasis (TNM) version 7, and with treatment naive, histologically, or cytologically proven NSCLC harboring common activating EGFR mutation (exon 19 deletion or Leu858Arg) were eligible for the study. Other inclusion criteria were as follows: Eastern Cooperative Oncology Group performance status 0–2; life expectancy >3 months; absence of known central nervous system involvement; no contraindications for TKI treatment; and adequate kidney and liver function. Patients with a history of prior malignancy within 5 years of study entry were excluded. The patients received erlotinib (150 mg) orally once daily or gefitinib (250 mg) orally once daily as an initial treatment.

The study was approved by the ethics committee of Luoyang Central Hospital and conducted according to the Declaration of Helsinki principles. Written informed consent was obtained from all patients prior to enrollment in the study.

Blood samples were collected in 10-ml CellSave (Veridex) preservative tubes, stored at room temperature, and processed within 96 h of collection, according to standard operating procedures and good laboratory practice. Serial blood samples (7.5 ml) were taken within 5 days of commencing treatment (defined as baseline) and on day 28 for measurement of CTC counts using the CellSearch system. The blood was processed and the CTCs were isolated and enumerated according to the manufacturer's instructions[11]. Because CTC counts of 1 have previously been reported in healthy donors or patients with nonmalignant disease[12], >1 CTC in one blood sample was considered to be positive for our study.

Statistical Analysis

Patients were divided into the favorable group (CTC < 5/7.5 ml) and the unfavorable group (CTC ≥ 5/7.5 ml) according to a previous report of the prognostic significance of CTCs in NSCLC[10]. Baseline (CTC-d0), day 28 (CTC-d28), and standard clinical factors, including performance status (PS), age, stage, gender, EGFR mutation type, smoking status, treatment received, and sites of metastasis, were subjected to univariate Cox proportional hazards regression analysis for both PFS and TTF. Univariately significant parameters were then included in a multivariate Cox proportional hazards regression analysis. PFS was measured from the date of baseline blood sample to the date of radiological progression according to Revised Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 or was censored at the last follow-up. TTF was measured from the date of treatment initiated to the date of treatment discontinuation for any reason such as disease progression, treatment toxicity, or death. Statistical analyses were performed using the SPSS version 18.0 software (SPSS, Inc., Chicago, IL, USA) and GraphPad Prism (GraphPad Software, San Diego, CA, USA), where a value of $p < 0.05$ was considered to be statistically significant.

RESULTS

Prevalence of CTC

From March 2014 to April 2015, 107 consecutive patients signed informed consent and were enrolled in the trial. Patient characteristics are shown in Table 1. At the time of analysis, 62/107 (60%) of the patients had experienced disease progression, and 23/107 (20%) of patients continued treatment beyond radiological progression.

The prevalence of CTCs before TKI therapy is shown in Table 2: 47/107 (44%) had CTC-d0 of ≥2 (2 to 80) (CTC-d0 2–4 and CTC-d0 ≥ 5 were 29% and 15%, respectively).

Prognostic Value of the CTC-d0

Patients were divided into favorable (CTC-d0 of 0–4, $n = 90$) and unfavorable (CTC-d0 of ≥5, $n = 17$) prognostic groups. The median PFS time in the favorable versus unfavorable groups (11.1 vs. 6.8 months) was significantly longer [hazard ratio (HR): 4.763, 95% CI: 2.615–8.716, $p = 0.009$] (Fig. 1A). Overall, 31 (35%) of the 90 patients in the favorable group and 7 (41%) of the 17 patients in the unfavorable group continued treatment beyond radiological progression. The median TTF was also significantly longer for patients with CTC-d0 of 0–4 versus CTC-d0 of ≥5 (12.4 vs. 8.0 months, HR: 6.981, 95% CI: 3.197–12.703, $p = 0.0107$) (Fig. 1B).

Table 1. Patient Characteristics

Characteristics	Patients [*n* (%)]
Total evaluable patients	107
Age (years)	
Median	65
Range	51–72
Gender	
Female	48 (45%)
Male	59 (55%)
Tumor stage	
IIIB	42 (39%)
IV	65 (61%)
Performance status	
0–1	77 (74%)
2	30 (26%)
No. of affected organs	
0–1	63 (59%)
>1	44 (41%)
EGFR status	
Exon 19 deletion	44 (41%)
Leu858Arg	63 (59%)
Smoking status	
Ever smoker	44 (40%)
Never smoker	63 (60%)

Prognostic Value of the CTC-d28

A CTC-d28 analysis was not available in 17 patients; 9 had drug-related adverse effects resulting in the treatment being paused, and 8 had sample processing errors. The changes in numbers of CTCs between day 0 and day 28 are summarized in Figure 2. Of the 30 patients with a CTC-d0 of 2–4, 28 had a CTC-d28 of 0–4 on day 28, whereas 2 had a CTC-d28 of ≥5. The two patients who demonstrated an increase in CTC number had a median PFS (6.3 months) compared with the favorable group (11 months). Of 17 patients with a CTC-d0 of ≥5, 10 had a CTC-d28 of ≥5 and 7 had a CTC-d28 of 0–4. In exploratory analyses, a change in CTC number was highly predictive for PFS (11.3 vs. 5.7 months; $p<0.001$) in favor of a reduction in CTC number compared with the unfavorable group.

Overall, the 12 patients with a CTC-d28 of ≥5 had significantly shorter PFS compared with the 78 patients with a CTC-d28 of 0–4 (6.3 vs. 11.6 months; $p<0.0001$) (Fig. 3). TTF was not significantly different between the two groups (7.1 vs. 10.6 months; $p=0.061$).

Univariate and Multivariate Analyses

In the univariate analysis, CTC-d0, stage, PS, and smoking status were significantly associated with both PFS and TTF outcomes (Table 3). For the PFS outcome, a CTC-d28 of ≥5 was the most significant prognostic

Table 2. Prevalence of Circulating Tumor Cells (CTCs) at Baseline

	Patients With CTCs at Baseline [*n* (%)]	
Characteristics	2–4	≥5
Gender		
Female	13 (12%)	7 (7%)
Male	17 (16%)	10 (9%)
Age		
<60	11 (10%)	6 (6%)
≥60	19 (18%)	11 (10%)
Tumor stage		
IIIB	12 (11%)	7 (7%)
IV	18 (17%)	10 (9%)
EGFR status		
Leu858Arg	13 (12%)	10 (9%)
Exon 19 deletion	17 (16%)	7 (7%)
Baseline performance status		
0–1	11 (10%)	5 (5%)
PS = 2	19 (18%)	12 (11%)
Smoking status		
Never smoker	12 (11%)	6 (6%)
Ever smoker	18 (17%)	11 (10%)
No. of affected organs		
0–1	13 (12%)	5 (5%)
>1	17 (16%)	12 (11%)

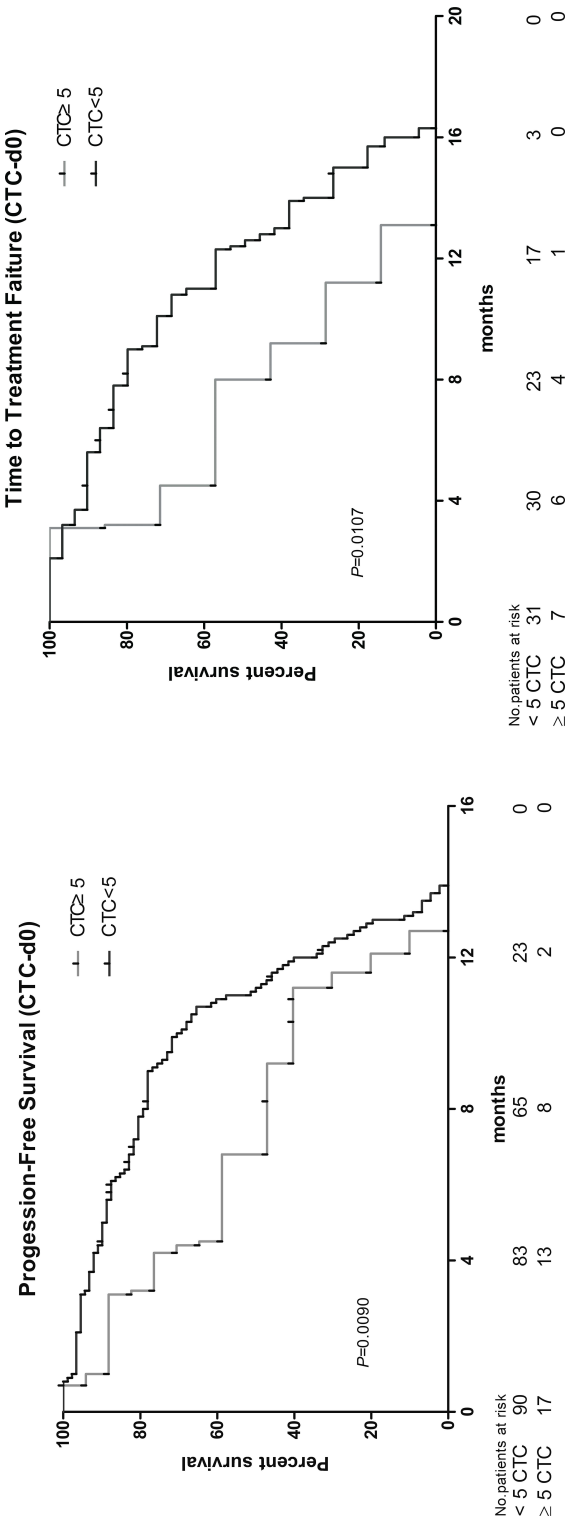

Figure 1. (A) Progression-free survival outcome according to circulating tumor cell (CTC) count at baseline. (B) Time-to-treatment failure outcome according to CTC count at baseline.

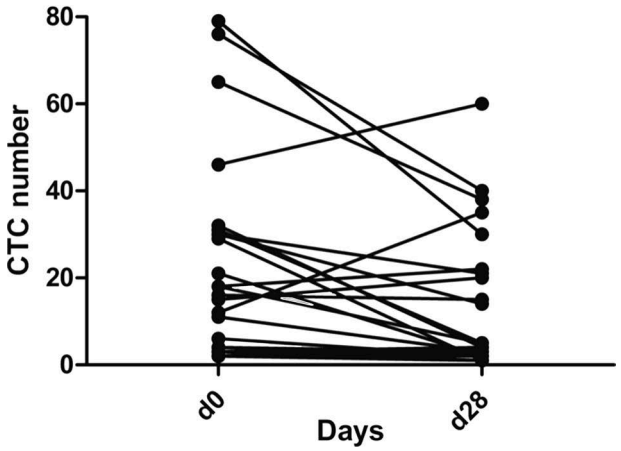

Figure 2. Of the 30 patients with CTC-d0 of 2–4, 28 had CTC-d28 of 0–4 on day 28, whereas 2 had CTC-d28 of ≥5. Of 17 patients with CTC-d0 of ≥5, 10 had CTC-d28 of ≥5 and 7 had CTC-d28 of 0–4.

factor among all the poor prognostic markers in the stepwise multivariate analysis (HR: 8.017, 95% CI: 4.421–13.733, $p<0.001$). For TTF, a CTC-d0 of ≥5, PS, ever smoker, and >1 affected organs were independent poor prognostic factors.

DISCUSSION

Previous studies have reported that 30% of patients with NSCLC had CTC counts >1 in 7.5 ml of blood, as measured by CellSearch, and that 15% had five or more CTCs[13]. A recent study by Punnoose et al. showed that one or more CTCs were detected at baseline in 76% of relapsed NSCLC patients[14]. Similarly, in our study 44% of patients demonstrated at least two CTCs per sample at baseline. These two findings indicate that advanced-stage NSCLC patients with mutations may have a trend toward higher baseline CTC counts than those who do not have mutations.

Several studies in recent years have shown that the presence of five or more CTCs at baseline is indicative of a poor prognosis in patients receiving standard chemotherapy regimens. In lung cancer, a meta-analysis performed by Wang et al. showed that the presence of CTCs was associated with a poorer outcome than a lack of CTCs, and CTCs were strongly associated with reduced survival[15]. In addition, Zhang et al. reported baseline CTC counts as an independent negative prognostic factor for NSCLC patients[16]. Targeted therapies have become a mainstay option for NSCLC patients with mutations; however, data on the relationship between CTCs and prognostic significance in patients receiving targeted therapy are limited. Recently, Punnoose et al. reported that decreases in CTC count with targeted therapy combinations were associated with a longer PFS in patients with relapsed NSCLC[14]. Our study demonstrated that CTC ≥5 at baseline was a strong negative predictor of PFS and TTF. We also found that five or more CTCs on day 28 were strongly associated with a poor PFS outcome for the patients.

However, there are several limitations in our study. It should be noted that the number of patients with samples at both time points was small, and the small sample size may have introduced bias in the results. Regarding the CellSearch technology, because it uses epithelial cell adhesion molecule (EpCAM) expression to detect CTCs,

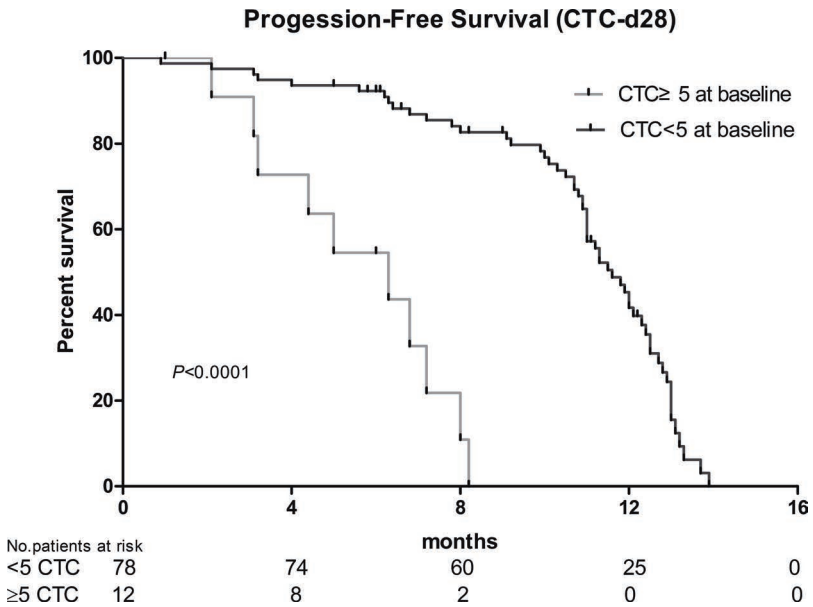

Figure 3. Progression-free survival outcome according to CTC count on day 28.

Table 3. Univariate and Multivariate Analysis for Progression-Free Survival and Time-to-Treatment Failure Outcomes

	Univariate Analysis			Multivariate Analysis		
	HR	95% CI	*p* Value	HR	95% CI	*p* Value
Progression-free survival						
Performance status (2 vs. 0–1)	5.037	2.341–8.573	0.013	5.215	1.317–8.973	0.021
Affected organs (≥2 vs.1)	3.756	1.174–6.312	0.023	1.197	0.712–6.557	0.713
CTC-d0 (≥5 vs. <5)	4.763	2.615–8.716	0.0090	6.833	3.947–13.572	<0.001
CTC-d28 (≥ 5 vs. <5)	5.785	3.171–11.801	<0.0001	8.017*	4.421–13.733	<0.001
Tumor stage (IV vs. IIIB)	4.179	2.701–9.137	0.014	6.011	3.716–9.727	0.001
Smoking (ever vs. never)	5.172	2.347–10.156	<0.001	6.033	3.911–10.537	<0.001
Time-to-treatment failure						
Performance status (2 vs. 0–1)	5.113	2.313–9.535	0.015	7.637	4.356–11.532	0.001
Affected organs (≥2 vs. 1)	2.007	0.946–7.177	0.023	3.357	1.945–7.063	0.010
CTC-d0 (≥5 vs. <5)	6.981	3.197–12.703	0.0107	8.635*	2.341–15.613	<0.001
Tumor stage (IV vs. IIIB)	5.137	2.947–9.903	<0.001	6.391	3.052–11.727	<0.001
Smoking (ever vs. never)	5.903	1.925–11.733	<0.001	6.873	2.907–10.772	<0.001

HR, hazard ratio; CI, confidence interval; CTC, circulating tumor cell.
*Most significant prognostic factor in stepwise multivariate analysis.

it may potentially miss those CTCs that have low or absent expression of CellSearch capture antigen[17]. In addition, tumor cells that lose epithelial markers during the epithelial–mesenchymal transition (EMT) process will not be detected by CellSearch. Undoubtedly the technology for the evaluation of CTCs will continue to develop, and there has been a recent move from CTC counts to molecular and functional characterization of CTCs and the use of CTCs as potential real-time liquid biopsies to facilitate personalized medicine. Also, sequencing-based evaluation of CTCs or cfDNA analysis may ultimately become a more clinically meaningful tool than enumeration of CTCs.

In summary, this is the first report over the presence of CTCs and its prognostic role in EGFR-mutant NSCLC patients. The use of serial CTC evaluation as a surrogate biomarker needs further validation in larger samples of patients.

ACKNOWLEDGMENT: John E. Anderson, M.D., from Johns Hopkins University provided sincere help in the revision of the manuscript. The authors declare no conflicts of interest.

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