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ORIGINAL ARTICLE

Circulating tumor cells predict prognosis following second-line AZD 9291 treatment in EGFR-T790M mutant non-small cell lung cancer patients

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Summary

Purpose: *AZD9291 has been developed as third-generation epithermal growth factor receptor (EGFR)- tyrosine kinase inhibitor (TKI) with activities against T790M mutation. This study aimed to isolate and quantify the circulating tumor cells (CTCs) in non-small cell lung cancer (NSCLC) patients after first-line EGFR TKIs and investigate their role in providing prognostic information.*

Methods: *Enrolled patients confirmed with EGFR T790M mutation received AZD9291 80 mg orally once daily as second-line treatment. Serial blood samples were taken at baseline (CTC-d0) and on day 28 (CTC-d28) following the initiation of AZD9291 for detection of CTCs using the CellSearch system.*

Results: *The CTC measurements were dichotomized as favorable (<5 CTCs) and unfavorable (≥5 CTCs) groups. Patients in the favorable group at baseline exhibited significantly longer median progression-free survival (PFS) compared with patients in the unfavorable group (9.3 vs. 6.5 months; p=0.0002). The PFS interval for patients in the favorable group on day 28 was 9.7 months, significantly longer than the median PFS time of 6.2 months achieved by patients in the unfavorable group (p=0.011). In univariate and multivariate analysis, CTC-d0 ≥5 vs CTC-d0=0-4 was significantly associated with poor PFS.*

Conclusions: *This is the first report over the presence of CTCs and their prognostic role in patients with EGFR T790M-positive NSCLC following disease progression on an EGFR TKI. The use of serial CTC evaluation as a surrogate biomarker needs further validation in larger samples of patients.*

Key words: *AZD 9291, circulating tumor cell, lung cancer*

Introduction

Lung cancer is the most common cause of cancer related deaths worldwide [1]. Epithelial growth factor receptor (EGFR) mutations are present in approximately 10% of White non-small-cell lung cancers (NSCLC) patients and in 30% of Asian NSCLC patients [2,3]. Previous clinical trials have established EGFR-targeting tyrosine kinase inhibitors (TKIs) as a first-line treatment option for positive EGFR-mutant NSCLC patients [4-7]. Despite an initial favorable response, the vast majority of patients will have disease progression and acquire resistance to these EGFR-TKIs [8]. T790M as the secondary mutation in EGFR is the most common mechanism of acquired resistance [9]. In addition to the development of new-generation targeted therapies for this disease, biomarker researches are needed to provide prognostic information.

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CTCs, as an easily non-invasive accessed "liquid biopsy," can be identified in peripheral blood from patients with serious cancers, such as prostate, colorectal, breast, and lung cancer [10-13]. More and more research focuses not only on improving analyses of these CTCs but also on identifying therapeutic and prognostic applications during the treatment of a specific cancer. In NSCLC, CTCs have been detected in blood at different time points throughout disease treatment and changes in CTC numbers have demonstrated their prognostic significance [14].

Recently, one study found that the CTC measurement could predict the efficacy and prognosis of EGFR-TKIs in NSCLC patients [15]. However, the association between CTC count and prognosis of NSCLC patients harboring T790M with second-line AZD 9291 treatment is unclear.

Methods

Inclusion criteria

Eligible patients aged 18 years and beyond with radiologically confirmed stage IIIB (ineligible for sequential radiotherapy or concurrent chemo/radiotherapy) or stage IV disease according to TNM system, version 7, and with first-line TKIs treatment failure, histologically or cytologically proven EGFR-T790M positive were enrolled in this study. The patients received AZD9291, a third-generation EGFR-TKI used to treat NSCLC with T-790M mutation in the gene coding for EGFR. The drug was administered orally at a dose of 80mg once daily as second-line treatment until objective disease progression. AZD 9291 was discontinued if patients had grade 4 non-disease related adverse events or unacceptable toxicity. Other inclusion criteria were as follows: Eastern Cooperative Oncology Group performance status (ECOG PS) 0-2; life expectancy >3 months; absence of known central nervous system involvement; no contraindications for AZD 9291 treatment; and adequate kidney and liver function. Patients with a history of prior malignancy within 5 years of study entry were excluded. The study was approved by the ethics committee of Luoyang Central Hospital and conducted according to the Declaration of Helsinki principles.

All patients provided written informed consent and underwent a baseline blood draw for assessment of CTCs using the CellSearch system. Samples were collected in 10-ml CellSave (Veridex, Raritan, NJ, USA) preservative tubes, stored at room temperature, and processed within 96 hrs of collection, according to standard operating procedures and good laboratory practice. Blood draws for CTC analysis were re-evaluated at the completion of first 28-day treatment cycle. The blood was processed and the CTCs were isolated and enumerated according to the manufacturer's instructions [16]. Considering CTC counts of 1 have previously been reported in healthy donors or patients with nonmalignant diseases [17], >1 CTCs in one blood sample was considered to be positive for this study.

Statistics

Patients were divided into the favorable group (CTC <5 per sample) and the unfavorable group (CTC ≥5 per sample) according to one previous report of the prognostic significance in NSCLC [13]. Baseline (CTC-d0), day 28 (CTC-d28), and standard clinical factors, including performance status (PS), age, stage, gender, smoking status, previous treatment, and sites of metastasis, were subjected to univariate Cox proportional hazards regression analysis for progression-free survival (PFS), while no overall survival (OS) data were evaluated because there were not mature. 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. 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 CTCs at baseline

From October 2015 to December 2016, 68 consecutive patients were enrolled in this trial. Patient characteristics are shown in Table 1. At the time of analysis, all of the patients had experienced disease progression, and 7 of 68 (11%) patients died. Sixty-two patients had CTC-d0 of ≥2 at baseline (range, 2-80); among the patients that were positive for ≥2 CTCs per sample, 51 had CTC- d0 <5 and 17 patients had CTC-d0 ≥5, respectively. We observed no significant correlation between the different baseline CTC count and patient clinical characteristics. The prevalence of CTCs and patient characteristics before treatment are shown in Table 1.

Prognostic value of CTCs

Patients were divided into favorable (CTC-d0 of 0-4, n=51) and unfavorable (CTC-d0 of ≥5, n=17) prognostic groups. The median PFS in the favorable group was 9.3 months, significantly longer than the unfavorable group of 6.5 months, [hazard ratio (HR): 5.712, 95% CI: 3.781-9.577, p=0.0002; Figure 2). A CTC-d28 analysis was not available in 13 patients; 5 had drug-related adverse effects resulting in treatment termination, and 8 had blood sample processing errors. The changes in the number of CTCs between day 0 and day 28 are summarized in Figure 1. Of the 51 patients with a CTC-d0 of 0-4, 38 had a CTC-d28 of 0-4, whereas 5 had a CTC-d28 of ≥5. The 5 patients who demonstrated an increase in the CTC number had a median PFS of 5.7 months

compared with the favorable group (9.3 months, p<0.001). Of 17 patients with a CTC-d0 of ≥5, 10 had a CTC-d28 of ≥5 and 2 had a CTC-d28 of 0-4. In total, 40 patients with a CTC-d28 of 0-4, and 15 patients with a CTC-d28 of ≥5, had in exploratory analyses a change in the CTC number which was highly predictive for PFS (9.7 vs. 6.2 months, p=0.011) in favor of a reduction in CTC number compared with the unfavorable group (Figure 3).

Univariate and multivariate analyses

In the univariate analysis, CTC-d0, PS, tumor stage, affected organ numbers, and CTC-d28 were significantly associated with PFS (Table 2). In the stepwise multivariate analyses, a CTC-d0 of ≥5 was the most significant prognostic factor among all the poor prognostic markers, demonstrating a lower risk of disease progression in the favorable group (HR: 6.835, 95% CI: 2.347-11.707, p <0.001).

Table 1. Prevalence of CTCs before AZD 9291 treatment

Characteristics	*Total*	*CTCs at baseline*		*p value*
	(n=68) n (%)	0-4 (n=51) n (%)	≥5 (n=17) n (%)	
Gender				0.579
Male	30 (45)	23 (77)	7 (23)	
Female	38 (55)	28 (74)	10 (26)	
Age, years				0.691
≥60	40 (59)	30 (72)	12 (28)	
<60	28 (41)	23 (82)	5 (18)	
Tumor stage				0.673
IIIB	27 (40)	19 (70)	8 (30)	
IV	41 (60)	32 (79)	9 (21)	
Tumor grade				0.491
High/moderate differentiated	33 (49)	23 (70)	10 (30)	
Low-differentiated	35 (51)	28 (80)	7 (20)	
Baseline ECOG PS				0.551
0-1	45 (66)	34 (76)	11 (24)	
2	23 (34)	17 (74)	6 (26)	
Treatment received				0.712
Erlotinib	31 (46)	24 (77)	7 (23)	
Gefitinib	37 (54)	27 (73)	10 (27)	
Smoking status				0.933
Ever smoker	29 (43)	23 (79)	6 (21)	
Never-smoker	39 (57)	28 (72)	11(28)	
No. of affected organs				0.892
0-1	30 (45)	22 (73)	8 (27)	
>1	38 (55)	29 (76)	9 (24)	

CTC: circulating tumor cells

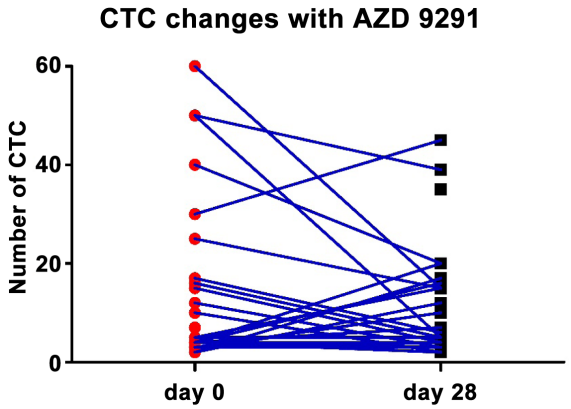

Figure 1. Circulating tumor cells (CTC) changes with AZD 9291 treatment for 28 days.

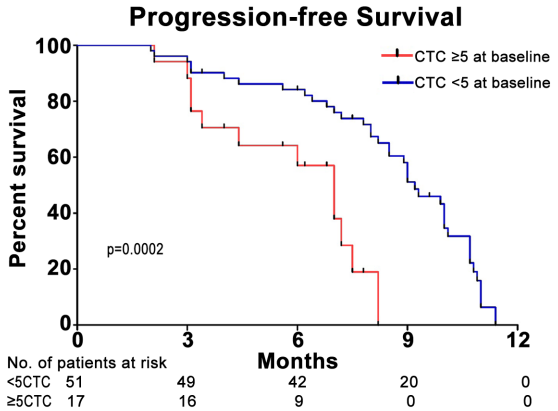

Figure 2. Progression-free survival outcome according to circulating tumor cell (CTC) count at baseline.

Table 2. Univariate and multivariate analysis for progression-free survival outcomes

Covariates	*Univariate analysis*			*Multivariate analysis*		
	HR	*95% CI*	*p value*	*HR*	*95% CI*	*p value*
CTC-d0 (≥5 vs. <5)	4.713	2.613-8.769	<0.001	6.835	2.347-11.707	<0.001
CTC-d28 (≥5 vs. <5)	3.937	2.131-7.629	<0.001	6.037	2.103-10.711	<0.001
Tumor stage (IV vs. IIIB)	2.136	1.011-6.371	0.015	3.051	1.173-7.201	0.023
ECOG PS (2 vs. 0-1)	4.107	2.917-7.041	0.007	5.131	2.707-8.795	0.033
Affected organs (≥2 vs.1)	3.071	1.715-7.207	0.021	3.930	1.153-5.703	0.401

CI: confidence interval, HR: hazard ratio, CTC: circulating tumor cell

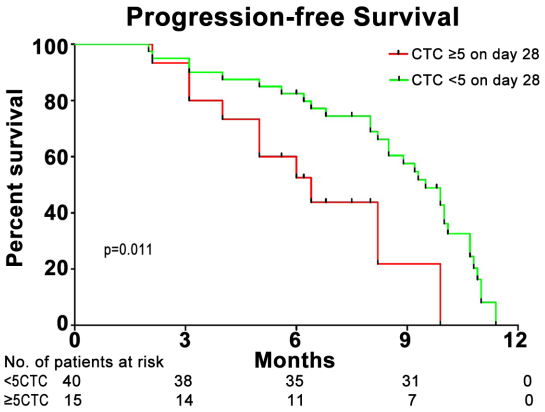

Figure 3. Progression-free survival outcome according to circulating tumor cells (CTC) count on day 28.

Discussion

In this study, we found that late-stage NSCLC patients demonstrated at least two CTCs at baseline. With a threshold of 5 CTCs per sample, there were more patients with CTC-d0 <5 than patients with CTC-d0 ≥5. In 2012, Krebs et al. used the cell search technology to emanate CTCs from 101 patients with stage III-IV NSCLC and reported increased numbers of CTCs in stage IV than in stage III patients. A cut-off value of >5 correlated with shorter PFS and OS, and in addition, decrease of CTC numbers with one cycle of standard chemotherapy corresponded to improved PFS and OS [13]. Another study conducted by Tanaka et al. found that the numbers of CTCs were higher in metastatic NSCLC patients compared to non-metastatic patients. These two findings suggest that the numbers of CTCs may be related to the stage of cancer in NSCLC patients [18].

Several studies have demonstrated the prognostic utility of CTCs in lung cancer. Zhang et al. reported baseline CTC counts as an independent negative prognostic factor for NSCLC patients [19]. Additionally, 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 [20]. Targeted therapies have become a main-stay option for NSCLC patients with mutations. Recently, He et al. reported the CTC measurement could be used to predict the efficacy of first-line EGFR-TKI treatment and prognosis of advanced NSCLC [15]. However, data on the relationship between CTCs and second-line treatment with AZD9291 after first-line TKI in NSCLC patients was lacking. We first demonstrated that patients treated with AZD9291 who had ≥5 CTCs at baseline was a strong negative predictor of PFS. In addition, 5 or more CTCs on day 28 were strongly associated with a poor PFS outcome for the patients.

It should be noted that the number of patients with samples at both time points was relatively small, and the small sample size may have introduced bias in the results. Regarding the CellSearch system, since it uses epithelial cell adhesion molecule (EpCAM) expression to detect CTCs, those that have low or absent expression of CellSearch capture antigen may be potentially missed [21]. Besides, during the epithelial-mesenchymal transition (EMT) process, cells that lose epithelial markers will not be detected by CellSearch [22]. Undoubtedly the technology for the measurement of CTCs will continue to develop, and there has been a recent move from CTC counts to molecular and genetic analysis of CTCs and the use of CTCs as potential real-time liquid biopsies to facilitate personalized medicine. Additionally, sequencing-based evaluation of CTCs or cfDNA analysis may ultimately become a clinically more meaningful tool than merely isolation of CTCs.

In summary, this is the first report over the presence of CTCs and their prognostic role in NSCLC patients harboring EGFR-T790M mutation. The use of serial CTC evaluation as a surrogate biomarker needs further validation in larger samples of patients.

Conflict of interests

The authors declare no conflict of interests.

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