Value of TTF-1 expression in non-squamous non-small-cell lung cancer for assessing docetaxel monotherapy after chemotherapy failure

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Abstract. Docetaxel is one of the standard second/third-line treatments for non-small-cell lung cancer (NSCLC) following a failed response to prior cytotoxic chemotherapy. The predictive biomarker for the effectiveness of docetaxel therapy remains undetermined. However, thyroid transcription factor-1 (TTF-1) is known to be a good prognostic factor for a variety of chemotherapies. To investigate the association between TTF-1 expression and docetaxel monotherapy outcome, 82 patients with non-squamous NSCLC who received second/third-line docetaxel monotherapy were retrospectively screened. All backgrounds were well-balanced whether or not tumor TTF-1 was expressed, and the present clinical outcomes were similar to those reported by previous clinical studies. A better clinical outcome was indicated in TTF-1 positive compared with TTF-1 negative patients, with disease control rates of 69% vs. 42%, respectively (P=0.03) and median overall survival of 393 days vs. 221.5 days, respectively (P<0.01). Furthermore, progression free survival tended to be longer in TTF-1 positive compared with TTF-1 negative patients (median, 100 days vs. 67 days; P=0.09). Multivariate analysis revealed that TTF-1 positivity was a unique significant predictor for assessing overall survival after docetaxel monotherapy. TTF-1 positivity may be useful for predicting survival outcome in patients who received docetaxel monotherapy after failure of prior chemotherapy.

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Introduction

Docetaxel (DTX) interferes with cell division and induces cell apoptosis via inhibition of microtubule depolymerization. Clinical trials have shown that DTX is active not only in front-line chemotherapy or chemoradiotherapy combined with platinum drugs (1-3), but also in previously treated patients (4). As a result, this has become the standard of treatment for non-small-cell lung cancer (NSCLC). Unfortunately, second-line chemotherapy is less effective compared to first-line platinum-based chemotherapy. Moreover, little is known about the relationship between the treatment outcome and tumor or the patient characteristics.

Thyroid transcription factor-1 (TTF-1) is a homeodomain transcription factor that is essential for the morphogenesis and differentiation in the thyroid, lung, and ventral forebrain. Furthermore, it has been demonstrated that TTF-1 controls the specific gene expression in the thyroid, lung, and central nervous system (5). In clinical practice, TTF-1 is commonly used to distinguish between primary lung adenocarcinoma and metastatic lung cancer. In addition, TTF-1 expression correlates with good prognostic outcomes in non-squamous (NS)-NSCLC and is considered to be a predictive marker for cytotoxic chemotherapy (6), antiangiogenic therapy (7), and kinase inhibitors (8).

The purpose of the present study was to examine whether TTF-1 expression affects the efficacy of DTX monotherapy in patients who failed to respond to prior cytotoxic chemotherapy.

Materials and methods

Participants and chemotherapy. We screened Stage IIIB or IV NS-NSCLC patients who failed to respond to platinum combination chemotherapy at the Nagoya City University Hospital between January 2010 and July 2017. Selected patients were

Table I. Patient characteristics.

Group	Overall (n=82)	TTF-1 expression		P-value
		Positive (n=58)	Negative (n=24)	
Age				0.481
Median [min-Max]	66 [38-78]	66 [38-78]	67 [52-78]	
Sex (%)				0.181
Male	59 (72)	39 (67)	20 (83)	
Female	23 (28)	19 (33)	4 (17)	
Smoke history (%)				0.278
Current or former	61 (74)	41 (71)	20 (83)	
Never	21 (26)	17 (29)	4 (17)	
Pathology (%)				0.577
Adenocarcinoma	78 (95)	56 (97)	22 (92)	
Large cell carcinoma	4 (5)	2 (3)	2 (8)	
Stage (%)				0.204
IIIB	3 (4)	1 (2)	2 (8)	
IV	79 (96)	57 (98)	22 (92)	
Driver mutation (%)				0.095
Positive	13 (16)	12 (21)	1 (4)	
Negative	69 (84)	46 (79)	23 (96)	
Treatment line (%)				0.05
Second	47 (57)	29 (50)	18 (75)	
Third	35 (43)	29 (50)	6 (25)	
Treatment cycles				0.237
Median [min-Max]	2.5 [1-28]	4 [1-28]	2 [1-12]	
TTF-1 (%)				
Positive	58 (71)			
Negative	24 (29)			

TTF-1, thyroid transcription factor-1.

treated with DTX monotherapy (60 mg/m^2) every three weeks as a second- or third-line chemotherapy. Patients found to have a gene mutation and who were naïve to the corresponding kinase inhibitor were excluded from this study. DTX monotherapy was continued until the start of the progressive disease (PD) state or intolerable toxicity occurred. Dose interruption or reduction was modulated for individual patients at the physician's discretion. Our Institutional Ethics Committee approved the protocol of this study (IRB number: 1115), with all medical data anonymized.

Immunohistochemical analysis of TTF-1 expression. NS-NSCLC tissue samples were obtained at the time of diagnosis using surgeries, bronchoscopy, or computed tomography-guided biopsy. After paraffin-embedding of all of the samples, 2-4 μm thick sections were prepared. Antigen retrieval was performed by autoclaving the sections at 97˚C for 20 min in citrate buffer (pH 6.0). Sections were then incubated with mouse monoclonal anti-TTF-1 antibody clone 8G7G3/1 (Dako, Agilent) 1/100 dilution at room temperature for 2 h. Primary antibody bound to the tissue sections was detected using the EnVision FLEX kit (Dako). Immunostained sections showing nuclear staining were considered to be positive (9) and reviewed by a pathologist (YY) and a pulmonologist (AT), who were blinded to the clinical information.

Statistical analysis. Response rate (RR) was defined as the sum of the complete response (CR) and partial response (PR) rates. Disease control rate (DCR) was defined as the sum of CR, PR, and stable disease rates. RR and DCR were compared using Fisher's exact test, with $P<0.05$ considered statistically significant. Progression-free survival (PFS) was defined as the time from the first day of chemotherapy to the date of disease progression, death, or the most recent follow-up. Overall survival (OS) was defined as the time from the first day of chemotherapy to the day of death or the most recent follow-up. PFS and OS were analyzed using the Kaplan-Meier method and compared using the log-rank test, with $P<0.05$ considered statistically significant. We identified TTF-1 positivity as a significant predictor of clinical outcomes (RR, DCR, PFS, or OS), and performed multivariate analysis using the logistic regression model (RR and DCR) or the Cox

Table II. Fisher's exact test about Disease Control Rate by clinical characteristics.

Group	DCR (%)	P-value
Age		0.11
<75	57	
≥75	83	
Sex		0.21
Male	56	
Female	74	
Smoke history		0.12
Current or former	56	
Never	76	
Pathology		0.64
Adenocarcinoma	62	
Large cell carcinoma	50	
Stage		0.56
IIIB	33	
IV	62	
Driver mutation		0.56
Positive	69	
Negative	59	
Treatment line		0.26
Second	55	
Third	69	
TTF-1		0.03
Positive	69	
Negative	42	

DCR, disease control rate; TTF-1, thyroid transcription factor-1.

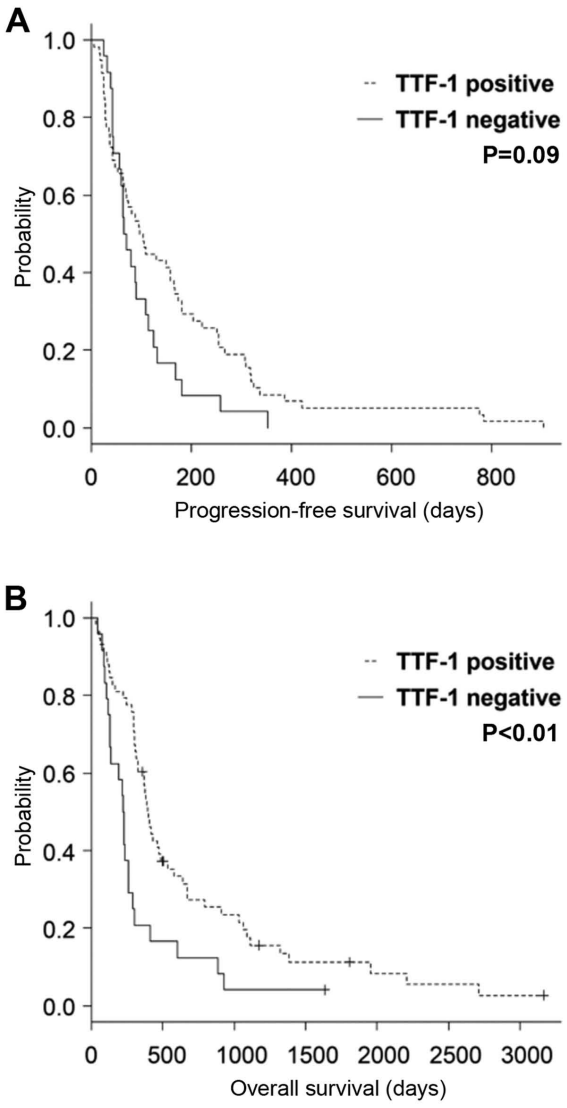

Figure 1. Kaplan-Meier curves showing (A) progression-free survival and (B) overall survival of patients who received DTX monotherapy. Results are shown for TTF-1-positive and TTF-1-negative patient groups, which were compared using the log-rank test. DTX, docetaxel; TTF-1, thyroid transcription factor-1.

proportional hazards model (PFS or OS) to identify the association between clinical outcomes and clinical characteristics. These analyses used a probability of P=0.10 as a threshold in the Fisher's exact test or the log-rank test for the addition or removal of a covariant from the model, with P<0.05 considered statistically significant. All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, the currently used program was a modified version of R commander that was designed to incorporate statistical functions that are frequently used in biostatistics (10).

Results

This study evaluated a total of 82 patients with NS-NSCLC. Table I summarizes the clinical backgrounds. DTX was administered as an earlier treatment in 58 patients with TTF-1-positive tumors. Clinical outcomes of the present study were similar to those reported previously (4) [RR, 13%; DCR, 61%; median PFS, 88 days (95% confidence interval (CI), 62-113 days); and median OS, 322.5 days (95% CI 285-403 days)].

We classified patients according to TTF-1 positivity and investigated the relationship between the TTF-1 positivity and the DTX outcome. There were no associations between the TTF-1 expression and grade of pathological differentiation of the tumor in this study. We also found that there was no significant difference in the RR between TTF-1-positive and TTF-1-negative patients (14% vs. 13%, respectively; P>0.99). Conversely, DCR was significantly higher in the TTF-1-positive vs. the TTF-1-negative patients (69% vs. 42%, respectively; P=0.03). Other clinical characteristics did not affect DCR (Table II). Subsequently, we analyzed the relationship between PFS/OS and TTF-1 positivity. The PFS in TTF-1-positive patients appeared to be longer than that observed in the TTF-1-negative patients (median PFS, 100 days [95% CI 64-165 days] vs. 67 days [95% CI 44-108 days], respectively; P=0.09) (Fig. 1A). The OS in TTF-1-positive patients was significantly longer than that observed in TTF-1-negative patients (median OS, 393 days [95% CI 322-483 days] vs. 221.5 days

Table III. Univariate analysis about overall survival by log-rank test.

Group	Number	Median OS	95% CI	P-value
Age				0.981
<75	70	318	284-408	
≥75	12	346	219-603	
Sex				0.0105
Male	59	299	255-377	
Female	23	458	224-1,110	
Smoke history				0.0133
Current or former	61	298	246-368	
Never	21	579	369-1,110	
Pathology				0.0902
Adenocarcinoma	78	345.5	285-415	
Large cell carcinoma	4	244	104-NA	
Stage				0.633
IIIB	3	230	91-NA	
IV	79	323	291-403	
Driver mutation				0.0583
Positive	13	639	384-1,958	
Negative	69	299	246-377	
Treatment line				0.224
Second	47	291	219-323	
Third	35	403	322-603	
TTF-1				0.00248
Positive	58	393	322-483	
Negative	24	221.5	126-255	

OS, overall survival; CI, confidence interval; TTF-1, thyroid transcription factor-1.

Table IV. Multivariate analysis about overall survival by Cox-proportional hazard model.

Factor	Group	Hazard ratio	95% CI	P-value
Sex	Male	1.15	(0.535-2.474)	0.7198
Smoke history	Current or former	1.569	(0.7842-3.137)	0.2031
Pathology	Adenocarcinoma	0.5097	(0.1801-1.443)	0.2043
Driver mutation	Positive	0.7084	(0.342-1.467)	0.3534
TTF-1	Positive	0.5823	(0.3404-0.9962)	0.0484

CI, confidence interval; TTF-1, thyroid transcription factor-1.

[95% CI 126-255 days], respectively; P<0.01) (Fig. 1B). The univariate analysis demonstrated that sex, smoking history, pathology, driver mutations, and TTF-1 positivity were significant predictors of OS (Table III). Multivariate analysis showed that TTF-1 was an isolated significant prognostic predictor of survival (Table IV).

Discussion

This retrospective study showed that the DCR and OS of DTX-treated TTF-1-positive patients with NS-NSCLC had a better prognosis as compared to DTX-treated TTF-1-negative patients with NS-NSCLC. Multivariate analysis particularly demonstrated that TTF-1 positivity was the only significant prognostic predictor of OS. Therefore, our findings suggest that TTF-1 expression might be a potential predictor of sensitivity to second-line DTX treatment.

During monotherapy (11) or combination therapy (12-14), immune checkpoint inhibitors (ICIs) have been reported to play more significant roles in patients with NSCLC, particularly those having high (>50%) tumor PD-L1 expression. Since the efficacy of ICIs after primary ICI strategy failure is unclear,

cytotoxic chemotherapy plays an important role in these types of cases. DTX is one of the standard anticancer drugs used in NS-NSCLC treatment (4), and is frequently utilized in patients with a failed response to prior chemotherapy. In order to improve the therapeutic efficacy of DTX, it is necessary to further identify predictive biomarkers. A previous Phase III study showed there was a significantly longer survival among female, stage IIIB patients who had good performance statuses and responses to prior chemotherapy (15). In addition, it has also been reported that there is a correlation between high class III β-tubulin expression and taxane resistance (16,17). Polymorphisms in Cytochrome P450 1B1 (18), STMN1 (19), and multidrug resistance proteins (20), as well as plasma levels of CEA and CYFRA 21-1 (21) have been shown to be associated with DTX outcomes. However, the mechanism via which these factors affect clinical outcomes remains unknown, and thus, the definitive predictive biomarker associated with the benefit of DTX remains unidentified.

TTF-1 is considered a good predictive factor for NSCLC. To the best of our knowledge, this is the first report to demonstrate the association between TTF-1 expression and the benefit of DTX in patients who have relapsed after prior chemotherapy. TTF-1 is mainly expressed in the alveolar type II cells and Clara cells in the epithelium at the terminal respiratory unit of the normal lung (5). TTF-1 is a potential lineage-survival oncogene in a subset of lung adenocarcinoma (22). Although the mechanism behind the relationship between the TTF-1 positivity and the better outcome remains unclear, it is thought that tumor pathogenesis might be correlated with clinical outcomes. Although most adenocarcinomas express TTF-1, we found that the expression frequency differed from its known historical pathology. In particular, low TTF-1 expression has been observed in invasive mucinous adenocarcinoma (23) that originates from the non-terminal respiratory unit (non-TRU) (24) of the lung. In another study, TRU and non-TRU adenocarcinoma exhibited differences in the gene expression and clinical features (25). When taken together, this suggests that TTF-1 expression might be a surrogate biomarker of TRU adenocarcinoma, with the differences in the molecular pathogenesis resulting in different DTX sensitivity. Moreover, most NSCLCs with epidermal growth factor receptor mutations have been shown to originate from the TRU (26). In a previous study, it was reported that ICIs might be less effective in driver mutation-positive patients (27). Although the mechanism remains unclear, DTX might be of benefit in these patients after targeted molecular therapy failure.

There were several limitations for our current study. First, our results may have been affected by selection bias, as this was a single-institute, retrospective study. However, our clinical outcomes did not significantly differ from previous reports (28,29), and the clinical benefit of TTF-1 positivity was assessed by multivariate analysis. In order to validate our current findings, future prospective studies will need to investigate the relationship between the DTX efficacy and TTF-1 positivity. Furthermore, our current study did not assess the change of the TTF-1 expression before and after treatment, which is an important limitation. However, it would be impractical to rebiopsy all of the patients who were failures to prior therapy. Additionally, except for patients who had an EGFR sensitive mutation and were treated with 1st or 2nd generation EGFR-TKIs, rebiopsies would have had little effect on the treatment strategy. Thus, we believe that the important point is that the pretreatment analysis of TTF-1 expression may predict the treatment outcome of DTX in treatment failure situations, without additional intervention. We demonstrated a clinical benefit for TTF-1 positivity in the DCR but not in the RR. Patients who relapsed after prior chemotherapy exhibited more variability with regard to their backgrounds, performance status, and shorter prognosis as compared to the treatment-naïve patients. The effectiveness of chemotherapy in patients with recurrent NSCLC is lower, with the response duration generally shorter than that observed for the prior chemotherapy. In second-line chemotherapy, it is important to not only decrease the tumor volume but also to prevent disease progression or metastasis. In cases of prior chemotherapy failure, ramucirumab can improve clinical outcomes in patients with NSCLC in addition to DTX (30). We previously reported on the additional benefits when using bevacizumab combined with cytotoxic chemotherapy in TTF-1-positive patients (7). Moreover, TTF-1 positivity might predict the efficacy of DTX and ramucirumab combination therapy. To validate the clinical relevance of our hypothesis, future prospective randomized studies will need to be undertaken.

In conclusion, TTF-1 positivity was significantly associated with better clinical outcomes in patients with recurrent NS-NSCLC treated with second/third-line DTX monotherapy. Since immunohistochemical analysis of TTF-1 expression in tumor tissue is commonly used to diagnose lung adenocarcinoma, assessing TTF-1 expression can also be used as a conventional, cost-free method for predicting the benefit of DTX treatment.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

AT and TO designed the study and wrote the initial draft of the manuscript. AT, TO, KS, SF, OT, TU and KM contributed to the analysis and interpretation of data and assisted in the preparation of the manuscript. AT and YY performed the histological diagnoses. TO, SF, KM, YIn, SY, HN, KF, YK, TT, HO, MT, YIt and AN contributed to data collection and interpretation. All authors have contributed to data collection and interpretation, and critically reviewed the manuscript. All authors approved the definitive version of the manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity

of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

The protocol of this study was approved by Institutional Ethics Committee of Nagoya City University Hospital (IRB no. 1115). We disclosed the study design and announced the opportunity to opt out in web page.

Patient consent for publication

Not applicable.

Competing interests

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