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Prospective Feasibility Study of Amrubicin and Bevacizumab Therapy for Patients With Previously Treated Advanced NSCLC

HISAO IMAI[1], KYOICHI KAIRA[2,3*], ICHIRO NARUSE[4*], NORIMITSU KASAHARA[5], YOSUKE KAMIDE[6], AKIHIRO ONO[6], TOMOMI MASUDA[6], NORIAKI SUNAGA[6], KOICHI MINATO[1] and TAKESHI HISADA[6]

[1]*Division of Respiratory Medicine, Gunma Prefectural Cancer Center, Ota, Japan;*
[2]*Department of Innovative Immune-Oncology Therapeutics, Gunma University Graduate School of Medicine, Maebashi, Japan;*
[3]*Department of Respiratory Medicine, Comprehensive Cancer Center, International Medical Center, Saitama Medical University, Hidaka, Japan;*
[4]*Division of Respiratory Medicine, Hidaka Hospital, Takasaki, Japan;*
[5]*Innovative Medical Research Center, Gunma University Hospital, Maebashi, Japan;*
[6]*Department of Respiratory Medicine, Gunma University Graduate School of Medicine, Maebashi, Japan*

Abstract. *Background/Aim: The efficacy of the combination of amrubicin and bevacizumab against advanced non small-cell lung cancer (NSCLC), as a second or third-line treatment, was evaluated. Patients and Methods: Amrubicin was administered for 3 days to patients with previously treated advanced NSCLC, whereas bevacizumab was administered on day 1 of each cycle; this regimen was repeated every 3 weeks. Results: Among the 16 patients, an overall response rate of 12.5% (for two patients) was achieved, and the overall disease control rate was 93.7%. Progression free survival and overall survival were 8.5 and 16.6 months, respectively. Grade 3 or 4 haematological toxicities were leukopenia, neutropenia, and febrile neutropenia. Grade 3 proteinuria and infection were the non haematological adverse events. Conclusion: The combination of amrubicin and bevacizumab is a promising regimen in the second or third-line treatment for advanced non-squamous NSCLC; however, physicians must recognise the risk of proteinuria related with this regimen.*

*These Authors contributed equally to this work.

Correspondence to: Kyoichi Kaira, MD, Ph.D., Department of Innovative Immune-Oncology Therapeutics, Gunma University Graduate School of Medicine, 3-39-15, Showa-machi, Maebashi, Gunma 371-8511, Japan. Tel: +81 272208222, Fax: +81 272020212, e-mail: kkaira1970@yahoo.co.jp; Hisao Imai, MD, Ph.D., Division of Respiratory Medicine, Gunma Prefectural Cancer Center, 617-1, Takahayashinishi, Ota, Gunma 373-8550, Japan. Tel: +81 276380771, Fax: +81 276380614, e-mail: m06701014@gunma-u.ac.jp

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Lung cancer is the leading cause of cancer related mortality worldwide. It is categorised into non small-cell lung cancer (NSCLC) and small cell lung cancer (SCLC); 80% of lung cancers are of the former type (1). Patients with locoregional NSCLC are eligible for curative surgery, whereas those with advanced NSCLC are usually only eligible for chemoradiotherapy or chemotherapy.

Bevacizumab is a recombinant humanised monoclonal antibody against vascular endothelial growth factor (VEGF) (2). Several clinical trials have been performed using bevacizumab; in some of these studies, the administration of bevacizumab along together with a cytotoxic drug in the first-line chemotherapy significantly improved progression-free survival (PFS), overall survival (OS), and objective response rate (ORR) in patients with advanced non-squamous NSCLC (3-9). However, studies on the feasibility of using bevacizumab and its efficacy are rare in patients with non-squamous NSCLC who have previously been administered a platinum-combination chemotherapy.

Amrubicin hydrochloride, a completely synthetic 9-aminoanthracycline, is converted to an active metabolite, amrubicinol, upon reduction of its C-13 ketone group to a hydroxyl group by carbonyl reductase (10). Amrubicin and amrubicinol are inhibitors of DNA topoisomerase II, which exert cytotoxic effects by stabilising a topoisomerase II-mediated cleavable complex, and not by acting as DNA intercalators. Amrubicinol has been reported to be 5-100 times more active than amrubicin (11). Amrubicin has been

approved for the chemotherapy of NSCLC and SCLC in Japan. In a phase II trial, amrubicin used at 45 mg/m^2 for 3 consecutive days resulted in response rates >75% and ~20% in chemotherapy naive patients with SCLC and NSCLC, respectively (12, 13). Recent studies have focused on the chemotherapeutic efficacy of amrubicin against relapsed lung cancer. Therefore, we carried out a prospective trial to study the usefulness of concomitant administration of amrubicin and bevacizumab in patients with non-squamous NSCLC who were previously treated with a platinum-combination chemotherapy.

Patients and Methods

Patient selection. From January 2014 to March 2018, a total of 16 patients from three Japanese institutions (Gunma University Hospital, Hidaka Hospital, Gunma Prefectural Cancer Center) were enrolled in the study. The patient demographics are summarised in Table I. The eligibility criteria were as follows: 1) histologically and/or cytologically proven unresectable stage IIIB to IV or postoperative relapsed non-squamous NSCLC; 2) presence of a measurable lesion according to the Response Evaluation Criteria in Solid Tumours (RECIST), version 1.1 (14); 3) more than 4 weeks since the last administration of prior chemotherapy; 4) treatment with epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs), anaplastic lymphoma kinase inhibitors, and mesenchymal–epithelial transition factor (MET) inhibitors were not considered chemotherapy; 5) age ≥20 years; 6) an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1; 7) a life expectancy of ≥3 months; and 8) adequate bone marrow function (leukocyte count ≥3,000 mm^3, neutrophil count ≥1,500 mm^3, platelet count ≥ 100,000 mm^3, and haemoglobin ≥ 10 g/dl) and normal liver function (total serum bilirubin ≤1.5 mg/dl and aspartate transaminase and alanine transaminase <2.5× the upper limits of the normal range), regardless of the epidermal growth factor receptor (EGFR) mutation status and normal renal function (serum creatinine ≤1.2 mg/dl). The histological diagnosis and staging of NSCLC were according to the World Health Organization classification and the International Association for the Study of Lung Cancer tumour-node-metastasis staging system (15), respectively. Prior to the treatment, each patient had a physical check-up, chest X-ray, chest and abdominal computed tomography (CT), bone scintigraphy or ^{18}F-fluorodeoxyglucose positron emission tomography, and brain CT or magnetic resonance imaging to ascertain the TNM stage. Patients with symptomatic central nervous system metastases, active infections, redundant malignancies, or other serious medical problems were excluded. The study was conducted in accordance with the Declaration of Helsinki and Japanese guidelines for Good Clinical Practice. The institutional review boards of all the participating institutions approved this study, and all the patients gave informed consent for participation in the study. This study was registered at the University Hospital Medical Information Network (UMIN) (Clinical trial number: UMIN 000013350).

Treatment protocol. The present study was a prospective, multi centre, single arm study performed to investigate the efficacy of amrubicin and bevacizumab combination treatment for the chemotherapy of patients with non-squamous NSCLC. Amrubicin [35 mg/(m^2•day)] was delivered transvenously for 3 consecutive days and was followed by 3 weeks without treatment. Bevacizumab [15 mg/(m^2•day)] was delivered as an intravenous instillation on day 1 of each course. After every 3 weeks, the cycle was repeated. Although prophylactic dosing of granulocyte colony stimulating factor (G CSF) was not allowed, the dosing of G CSF was allowed in patients with grade 4 neutropenia and/or grade 3 febrile neutropenia. Later cycles of treatment were started after day 22, when the neutrocyte counts were ≥1500 m^3, and the platelet counts were ≥100,000 m^3. If the neutrocyte and platelet counts had not reverted to these levels by day 1 of the subsequent cycle of drug administration, the initiation of treatment was discontinued until complete remission. The treatment persisted until disease progression, incidence of unacceptable severe adverse events, or withdrawal agreement. When a severe adverse event was recognised, the amrubicin dose was decreased as per the protocol. In brief, if a severe adverse event was recognised, the dose was reduced to 5 mg decrements to 25 mg/day. If the protocol failed, the patients were allowed further treatment(s), as desired.

Treatment assessment. The best overall response and maximum tumour shrinkage were recorded as the tumour responses. Radiographic responses were evaluated according to the RECIST, version 1.1 (14): complete response (CR), the disappearance of all target lesions; partial response (PR), a decrease in the sum of the target lesion diameter by at least 30% relative to the baseline; progressive disease (PD), an increase of at least 20% in the sum of the target lesion diameters relative to the smallest sum during the study; and stable disease (SD), insufficient shrinkage or expansion to qualify as PR or PD. The primary end point of this study was the ORR, which was the rate of patients with CR + PR, whereas the secondary end points were PFS, OS, and safety. Chest X-ray and CT were performed every 4 weeks until CR or PR was obtained. The CR and PR were re-evaluated within 4 weeks of the first assessment meeting the criteria for response. Following confirmation, images were obtained every other month until PD was recognised. PFS was calculated from the initiation of treatment until PD or death because of any reason, and OS was noted from the first day of treatment until death or was censored on the date of the last follow-up. Adverse events were assessed according to the National Cancer Institute Common Toxicity Criteria version 4.0.

Statistical analyses. The sample size was decided based on the following data. The primary end point of this research was ORR, and the secondary end points were PFS, OS, and safety. The estimated required accrual was 15 patients, supposing that an ORR of 50% in eligible patients would demonstrate potential usefulness, whereas an ORR of 20% would form the lower limit of interest, with a power of 80% and the α-level set to 5% (one sided test). The approximations were based on the 30.0%-66.7% ORR, as shown in previous studies of cytotoxic drug monotherapy regimens with bevacizumab (16-18), and 13.5%-14.4% response rates, as shown in a study of amrubicin monotherapy (19, 20). The survival curves were calculated using the Kaplan–Meier methodology. All statistical analyses were performed with JMP, version 11.0, for Windows (SAS Institute, Cary, NC, USA).

Results

Patient characteristics. The patient demographics are summarised in Table I. The median age of patients was 67 years (range=57-72 years); 11 (68.7%) patients were men

Table I. *Patient demographics*.

Characteristics	n=16
Gender (male/female)	11/5
Age (years; median (range))	67 (57-72)
ECOG PS (0/1)	0/16
Histology (adenocarcinoma/other)	15/1
Smoking history (yes/no)	11/5
Clinical stage at diagnosis (III/IV)	2/14
EGFR mutation (yes/no)	2/14
Comorbid disease (yes/no)	11/5
Treatment line	
Second-line	14
Third-line	2
Prior therapy	
Platinum combination	16
Bevacizumab combination	11
EGFR-TKI	2

PS: Performance status; EGFR: epidermal growth factor receptor; EGFR-TKI: epidermal growth factor receptor-tyrosine kinase inhibitor.

Table II. *Response rate and treatment delivery.*

	Total	95%CI
Response		
CR	0	
PR	2	
SD	13	
PD	1	
NE	0	
Response rate (%)	12.5	3.4%-36.0%
Disease control rate[a] (%)	93.7	71.6%-98.8%
No. of treatment cycles		
Median	10	
Range	2-14	
Dose reduction (yes/no)	6/10	
35→30 mg/m^2	4	
35→30→25 mg/m^2	2	

CI: Confidence interval; CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease; NE: not evaluated. [a]Calculated as the number of patients with complete response, partial response, and stable disease, divided by the whole study population.

and 5 (31.3%) were women. The histological assessment revealed that 15 (93.7%) patients had adenocarcinomas and 1 (6.3%) had another type of cancer. Two patients (12.5%) had stage IIIB and 14 (87.5%) had stage IV cancer. Other patient attributes included an ECOG PS score of 1 (100%) and presence of smoking history (68.7%). With regard to the presence of comorbid diseases, one patient had chronic obstructive pulmonary disease, eight had hypertension (medically treated), one each had diabetes mellitus and arrhythmia, and two patients had bronchial asthma (medically treated). The presence of *EGFR* mutation was investigated in all the patients; only 2 patients had mutation in this gene, whereas 14 had the wild-type *EGFR*. At the time of data cut-off for the analysis (31 August 2018), there were no patients being administered the treatment.

Treatment delivery, efficacy, and survival. All the 16 patients were administered a total of 131 cycles; the median number of cycles was 10 (range=2-14 cycles). Following disease progression, 11 patients received subsequent line chemotherapy. All the 16 patients completed >2 cycles of chemotherapy. CR was not achieved in any of the patients, whereas PR was achievable in two patients, with an ORR of 12.5% [95% confidence interval (CI)=3.4%-36.0%]. The disease control rate (DCR) (CR + PR + SD) was 93.7% (95%CI=71.6%-98.8%; Table II). At the time of data cut-off for analysis, the median follow-up time was 15.5 months, the median PFS was 8.5 months (95%CI=3.2-11.1), and the PFS rates at 3 and 6 months were 81.2% and 68.7%, respectively (Figure 1A). Thirteen patients (81.2%) died. The median OS was 16.6 months (95%CI=6.9-24.7), and the OS rates at 6 and 12 months were 73.6% and 60.2%, respectively (Figure 1B). The duration of amrubicin plus bevacizumab treatment and post-progression survival period for the selected patients is shown in Figure 2.

Safety and toxicity. Adverse events were evaluated for all the 16 patients. Haematological and non haematological adverse events are summarised in Table III. Grade 3 or 4 haematological toxicities were neutropenia (68.7%), leukopenia (50%), anaemia (6.2%), and thrombocytopenia (6.2%). Febrile neutropenia was recognised in 4/16 (25%) of the treated patients. The non haematological adverse events observed were grade 3 proteinuria (31.2%) and infection (6.2%). No pulmonary adverse events, including interstitial lung disease, or treatment related deaths were observed.

Discussion

We report the results of the first study performed to assess the effects of a combination chemotherapeutic regimen of amrubicin and bevacizumab for advanced NSCLC. The safety and effectiveness of bevacizumab administered together with amrubicin were assessed in patients receiving second- or third-line treatment for recurrent NSCLC. We observed an ORR of 12.5% and a DCR of 93.7%. The median PFS was 8.5 months, and the median OS was 16.6 months.

There have been some studies on therapies with cytotoxic drugs, including bevacizumab, as pre-treatment chemotherapies for non-squamous NSCLC (16-18, 21). A randomised phase II trial for pre-treated NSCLC that assessed the effectiveness of

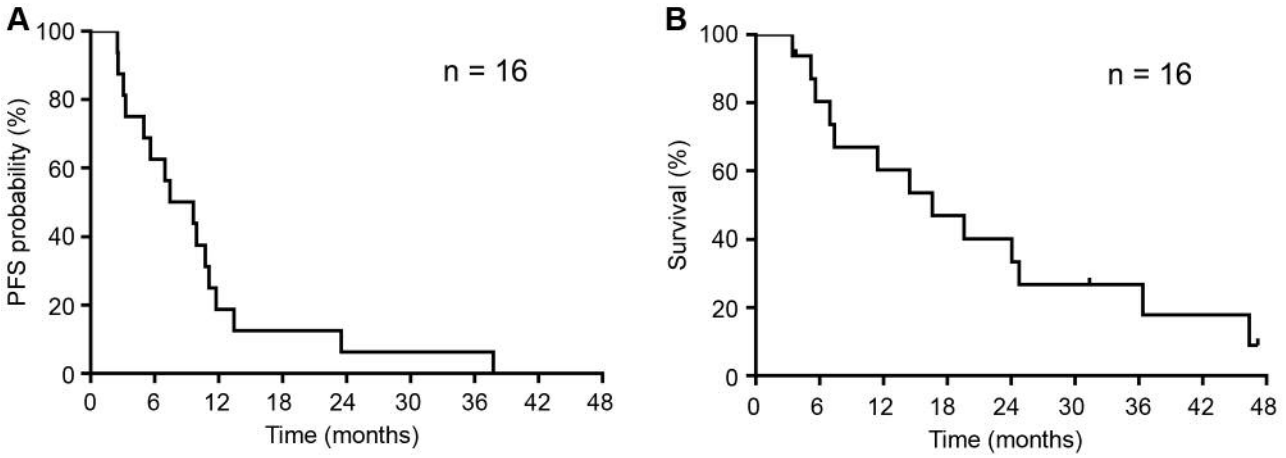

Figure 1. *Kaplan–Meier analysis of progression-free survival and overall survival. A. Kaplan–Meier analysis of the progression-free survival of 16 patients. Median progression-free survival was 8.5 months. B. Kaplan–Meier analysis of the overall survival of 16 patients. Median overall survival was 16.6 months.*

bevacizumab in combination with standard second-line cytotoxic drugs, including docetaxel or pemetrexed, revealed an ORR of 12.5%, a DCR of 52.5%, a median PFS of 4.8 months, and an OS of 12.6 months (21). Furthermore, a Japanese single-arm phase II trial for pre-treated NSCLC that examined the efficacy of bevacizumab plus pemetrexed or docetaxel revealed an ORR of 30.0%, a DCR of 66.7%, a median PFS of 5.0 months, and an OS of 15.8 months (17). The values for ORR (12.5%), DCR (93.7%), median PFS (8.5 months), and median OS (16.6 months) obtained in this study are similar to or better than those in the abovementioned studies for subsequent-line regimen.

The adverse events observed in this study were comparable to those in previous studies. In a previous study, bevacizumab was shown to have high toxicity potential and also enhanced the efficacy of another cytotoxic drug when administered concomitantly (18). Bevacizumab targets VEGF and causes transient normalisation of the tumour vessel environment, thereby enhancing the intratumoural absorption of drugs (22, 23). In a Japanese phase III study, the addition of bevacizumab enhanced the toxicity (hypertension and proteinuria) to some degree (24). The combination therapy used in this study increased the typical adverse events related to cytotoxic drugs, although it did not particularly affect those related to bevacizumab, such as proteinuria. These results are comparable to the drug toxicity observed for bevacizumab in combination with other drugs (3-9, 16-18, 24). The bevacizumab and amrubicin combination chemotherapy was regarded as a high risk therapy from the perspective of the occurrence of proteinuria or febrile neutropenia. Furthermore, neutropenia was high (incidence of grade 3 or higher), affecting 68.7% of the patients, and 25.0% of the patients developed febrile neutropenia. Although G-CSF was not prophylactically administered in this trial, its therapeutic use was required. Severe neutropenia has been demonstrated in clinical studies when bevacizumab was used in combination with other cytotoxic drugs (16-18). These results suggest that prophylactic administration of G-CSF might be necessary in this regimen.

Our study demonstrates that the combination of amrubicin and bevacizumab as a VEGF inhibitor might improve the DCR. VEGF immunoreactivity is related to topoisomerase II expression and is prognostically relevant in ovarian serous adenocarcinomas (25). Although not for NSCLC, VEGF and topoisomerase II have been shown to serve as markers of aggressive tumour behaviour (25). In light of these observations, the relationship between VEGF and topoisomerase II might be an interesting topic for further studies to investigate the clinical and therapeutic relevance of these factors in combination therapies for cancer. Bevacizumab is a recombinant humanised monoclonal antibody targeting VEGF. The open-label, randomised phase IIIb Avastin in All Lines Lung (AvaALL) trial evaluated the effectiveness and toxicity of bevacizumab beyond the first progression in advanced NSCLC, following bevacizumab maintenance therapy (26). A considerable advantage of bevacizumab treatment beyond the aggravation in patients with NSCLC was not observed, but some benefits regarding the effectiveness were noticed. The median PFS from randomisation at the first progression to the second progression (PFS2) was numerically longer for bevacizumab plus standard chemotherapy than for standard chemotherapy alone. Moreover, the median PFS from randomisation at the first progression to the third progression (PFS3) was significantly longer for bevacizumab plus SOC than for SOC

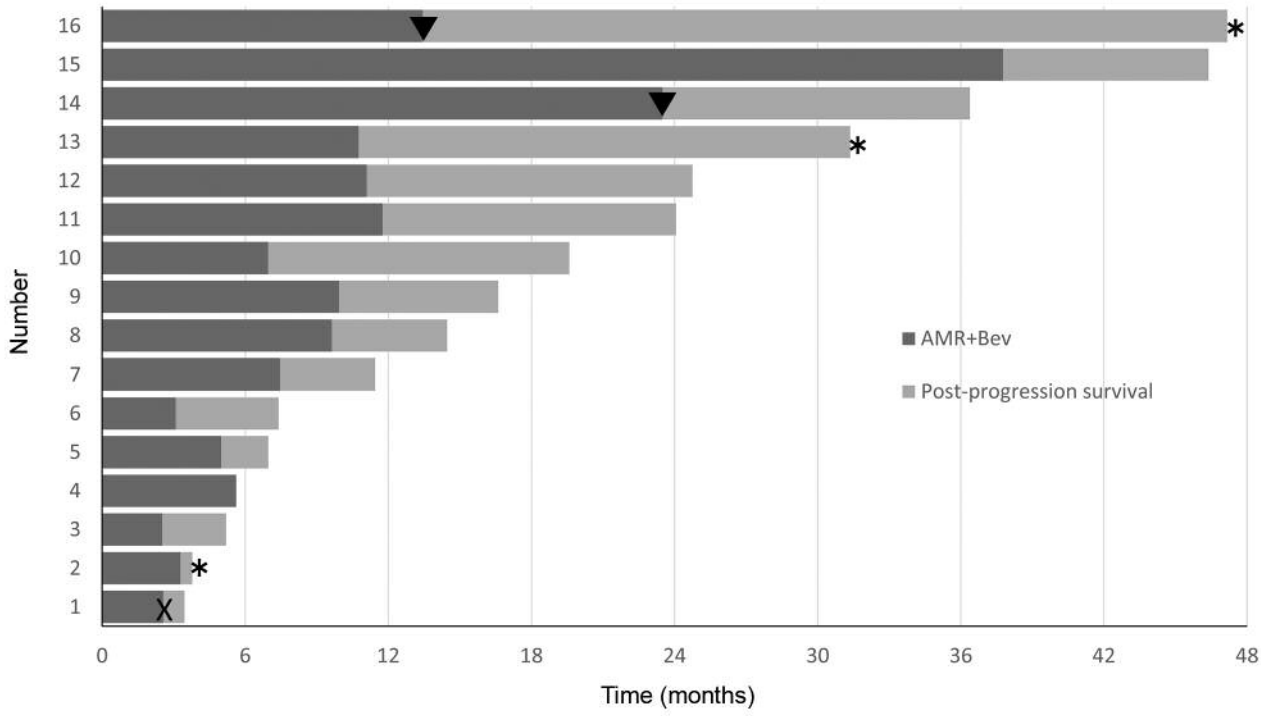

Figure 2. *Treatment duration of amrubicin plus bevacizumab and post-progression survival period for the individuals. ▼: case of PR; X: case of PD; *: censor or alive at data cut-off.*

Table III. *Non-haematological and haematological adverse events.*

	NCI-CTCAE Grade (Ver 4.0)					
	1	2	3	4	5	≥3 (%)
Non-haematologic adverse events						
Malaise	5	2	-	-	-	0 (0)
Skin hyperpigmentation	2	1	-	-	-	0 (0)
Alopecia	2	0	-	-	-	0 (0)
Anorexia	5	1	0	0	0	0 (0)
Constipation	5	2	0	0	0	0 (0)
Mucositis	4	1	0	0	0	0 (0)
Nausea	1	1	0	-	-	0 (0)
Vomiting	2	0	0	0	0	0 (0)
Proteinuria	2	4	5	-	-	5 (31.2)
Infection	-	1	1	0	0	1 (6.2)
Pneumonitis	0	0	0	0	0	0 (0)
Thromboembolic event	0	1	0	0	0	0 (0)
Haematologic or laboratory adverse events						
Anaemia	5	5	1	0	0	1 (6.2)
Leukopenia	3	2	4	4	-	8 (50)
Neutropenia	0	3	3	8	-	11 (68.7)
Platelet count decreased	4	3	0	1	0	1 (6.2)
Febrile neutropenia	-	-	4	0	0	4 (25)
ALT increased	5	0	0	0	-	0 (0)
AST increased	8	0	0	0	-	0 (0)
Creatinine increased	2	0	0	0	-	0 (0)

AST: Aspartate aminotransferase; ALT: alanine aminotransferase.

alone. Ramucirumab is a human immunoglobulin G1 monoclonal antibody against the VEGF receptor 2 (VEGFR2). Docetaxel and ramucirumab combination chemotherapy has been shown to be more effective than the therapy with docetaxel alone in a phase III study (REVEL study) (27). Ramucirumab was also veritably active, with an ORR of 28.9%, when combined with docetaxel in a Japanese phase II study (JVCG study) (28). As amrubicin can be expected to be as effective as docetaxel in second-line treatment (20), amrubicin and bevacizumab combination therapy might be promising in the future.

In relation to the immune checkpoint inhibition therapy, the combination chemotherapy used in this study may be important with respect to the blockade of VEGF-mediated immunosuppression with bevacizumab. The results of a phase III randomised trial have shown that the addition of atezolizumab to bevacizumab plus chemotherapy (carboplatin plus paclitaxel) as a first-line chemotherapy resulted in significant improvements in the PFS and OS in patients with advanced non-squamous NSCLC (29). Furthermore, docetaxel plus ramucirumab had a higher ORR when administered immediately after the failure of nivolumab than that in regimens without prior nivolumab treatment (30). These results suggest a promising combination of immunotherapy and angiogenesis inhibitors and studies have previously been conducted on this premise. In a previous study, it was shown that the anti-PD-L1 therapy may increase the sensitivity of the tumour to antiangiogenic therapy and prolong its effectiveness, and antiangiogenic therapy improved the efficacy of anti-PD-L1 antibodies in pre-clinical models (31). The chemo-sensitising effects induced by immunotherapy may be better with the combination of a single agent and an anti-VEGFR2 antibody than with the single agent alone. The synergistic effect of anti-angiogenesis and immunotherapy has previously been discussed at the pre-clinical level (32). Angiogenic agents boost the immune system. Conversely, immunotherapy has been suggested to be antiangiogenic and VEGF plays an important role in mediating the immunosuppressive microenvironment. Given these findings, it is not surprising that chemotherapy has a synergistic effect on immunotherapy, and anti-angiogenics enhance the immune response.

There are several limitations in this study. First, the enrolment of eligible patients for this study took a long time to complete (from 2014 to 2018 for only 16 patients). Meanwhile, several new strategies have become available for treating patients with advanced non-squamous NSCLC that may have biased the survival in current studies. Second, the relationship between the effectiveness of amrubicin plus bevacizumab and any of the biomarkers, including topo II or VEGF, was not evaluated. Finding predictive biomarkers improves post-treatment outcomes. It is important to determine whether the efficacy of the regimen used in this study improves with the expression of any predictive marker. Finally, the sample size in current study was small, which could have resulted in bias in the results.

Conclusion

The combination chemotherapy with amrubicin and bevacizumab might prove to be effective and feasible for patients with previously treated non-squamous NSCLC.

Conflicts of Interest

All Authors declare no conflicts of interest regarding this study.

Authors' Contributions

HI and KK designed the study. IN, NK, YK, AO, TM and NS collected the materials and data. HI and KK performed the statistical analysis. KM and TH reviewed the results, and HI and KK interpreted the data and wrote the manuscript. All Authors read and approved the final version of the paper.

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