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Influence of using history of immune checkpoint inhibitor therapy for neutropenia caused by combination therapy of ramucirumab and docetaxel

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Recently, pretreatment with immune checkpoint inhibitors (ICIs) has been shown to enhance the therapeutic effects of the combination therapy of ramucirumab (RAM) and docetaxel (DTX); however, its influence on the drug's side effects remains unclear. This study investigated the influence of pretreatment with ICIs on the incidence of neutropenia caused by RAM + DTX therapy in patients with non-small cell lung cancer (NSCLC). Patients with NSCLC who received RAM + DTX therapy at Gifu Prefectural General Medical Center between April 2016 and December 2020 were enrolled. Retrospective data regarding age, sex, performance status and detailed treatment history, among others, at treatment initiation were collected from the patients' electronic medical records. Additionally, data on the course number of RAM + DTX therapy, supportive therapy and blood biochemical parameters, including leukocyte and neutrocyte counts, during the treatment period were collected. We identified 41 patients receiving RAM + DTX therapy. Among the more than grade 3 adverse events caused by this therapy, neutropenia was the most common (78.1 %). Despite the fact that all previous risk factors influencing this incidence rate had corresponded, the only factor influencing the incidence rate of neutropenia more than grade 3 was ICI treatment history. A difference in the incidence of neutropenia more than grade 3 in the Kaplan-Meier curve was observed between patients with and without ICI pretreatment history ($p = 0.037$). The pretreatment history of ICI therapy affects the incidence of neutropenia caused by RAM + DTX therapy in patients with NSCLC.

1. Introduction

According to the Cancer Statistics in Japan by the Foundation for Promotion of Cancer Research in Japan-2019, the prevalence of lung cancer in Japan has increased year after year. Moreover, based on the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology - Non-Small Cell Lung Cancer-Version 4. 2021, lung cancer is the leading cause of cancer-related death in the United States, and the 5-year survival rate does not exceed 19% for the combination of both non-small cell lung cancer (NSCLC) and small cell lung cancer. Additionally, a survey conducted in the United States from 2009 to 2015 has reported a 5-year relative overall survival (OS) rate of 25% for NSCLC. However, with the recent rapid advancements in cancer treatments, the survival time of patients with metastatic lung cancer, for which molecularly targeted therapy and immunotherapy are indicated, has now been prolonged compared with that in the past. Approximately 85% of patients with lung cancer in Japan have NSCLC. According to Guidelines for diagnosis and treatment of the lung cancer/malignant pleural mesothelioma/thymic tumors-2020, 6th edn., combination therapy using two platinum-based anticancer agents plus an immune checkpoint inhibitor (ICI) or ICI monotherapy is recommended as the frontline therapy for NSCLC. Moreover, for second-line therapy and further, the administration of docetaxel (DTX) ± ramucirumab (RAM) is recommended for patients with a history of treatment with ICI.

A phase III trial evaluating the additional effects of RAM combined with DTX compared with those of DTX monotherapy (REVEL study) has revealed that both the OS and progression-free survival (PFS) in the RAM + DTX group were significantly prolonged compared with those in the DTX monotherapy group (Garon et al. 2014). An exploratory analysis of REVEL study has shown that the combination of any anticancer agent in the frontline platinum-based therapy did not influence the OS and PFS of RAM + DTX therapy as the second-line therapy. Furthermore, the events of grade ≥3 hypertension, neutropenia, febrile neutropenia (FN), and leucopenia (in squamous histology only) were each reported more frequently in the RAM + DTX arm than in the placebo + DTX arm in all prior therapy subgroups (Garon et al. 2020). A phase II study involving Japanese patients also confirmed the additional effects of RAM compared with DTX alone; additionally, the incidence and severity of most adverse events were similar, but FN was more common in the RAM + DTX group (34.2%) than in the placebo + DTX group (19.8%) (Yoh et al. 2016). FN is known to be caused by a variety of factors, including chemotherapy, and NCCN Clinical Practice Guidelines in Oncology – Myeloid Growth Factors – Version 1. 2018 have reported that the elderly (particularly those aged 65 or older), previous chemotherapy or radiotherapy, a history of neutropenia or bone marrow infiltration, neutropenia, infectious disease, opening wound, recent operation career, the effectiveness of performance status, kidney failure, and liver failure (particularly bilirubin aggravation) were identified as risk factors. It has been reported that the type of cancer and disease stage, measures of pretreatment health, comorbid conditions, performance status, and age are all patient-specific risk factors for FN (Crawford et al. 2004).

A meta-analysis of randomized controlled trials has shown that ICI was superior to chemotherapy in treating advanced NSCLC

in all efficacy measures (i.e., OS, PFS, and objective response rate [ORR]), and the incidence rates of grade ≥3 adverse events were also lower (Khan et al. 2018). In a retrospective study about RAM + DTX therapy, the PFS in the presence of ICI pretreatment history was superior to that in the absence of ICI pretreatment history (Harada et al. 2019). Even for other cancer types, the use of nivolumab as the third- to sixth-line therapy may enhance subsequent chemosensitivity in patients with unresectable advanced or recurrent gastric cancer (Arigami et al. 2020). Thus, several recent studies have shown that salvage chemotherapy following PD-1 blockade results in high antitumor activity. Similarly, there have been a few reports on the effect of ICI pretreatment on the onset of adverse events associated with salvage chemotherapy. According to the previous report, the practical use of osimertinib in Japanese patients who received immediate prior nivolumab therapy frequently resulted in interstitial lung disease (Kotake et al. 2017). Furthermore, using osimertinib immediately after nivolumab significantly increased the frequency of grade 3 or higher hepatotoxicity in patients with advanced NSCLC harboring an EGFR mutation who developed T790M resistance was reported (Yamaguchi et al. 2020). Furthermore, the prevalence of adverse events such as fever, myalgia, arthritis, pleural effusion, and pneumonitis increased in NSCLC patients who received RAM + DTX therapy after having previously received ICI (Harada et al. 2019). However, it is unclear what effect a history of ICI pretreatment has on the onset of neutropenia caused by RAM + DTX therapy. Therefore, this study was designed to examine the influence of ICI pretreatment on the onset of grade ≥3 neutropenia caused by the combination therapy of RAM and DTX in patients with NSCLC.

2. Investigations and results

2.1. Patient characteristics

Forty-one patients were recruited as the study subjects. Patient characteristics at baseline are shown in Table 1. Among them, 78.0% were males, 61.0% had non-squamous cell carcinoma, and 70.7% had stage IV disease, all at a high percentage. Course number of RAM + DTX therapy was varied. The percentage of patients with a history of ICI use was 46.3%. The reasons for discontinuation of RAM + DTX therapy were as follows: progressive disease in 25 patients (61.0%), reduced performance status in 6 patients (14.6%), and the onset of adverse events in 10 patients (24.4%). In the 10 patients for which therapy was discontinued due to the onset of adverse events, the adverse events were as follows: feeling of fatigue in 3 patients, neutropenia in 2 patients, proteinuria in 2 patients, thrombocytopenia in 1 patient, fever in 1 patient, and pneumonia in 1 patient. Additionally, among the 10 patients, 5 patients had grade ≥3 neutropenia, and 6 patients had other grade ≥3 adverse events. Among the 27 patients in whom doses were reduced (for 37 times), reasons for dose reduction were as follows: neutropenia in 73.0%, adverse events except for neutropenia in 21.6%, and unknown in 5.4%.

Table 1: Patient characteristics at baseline

	Numerical value or number of patients (n=41)
Age (years)	69 (66-73)
Sex	
Male	32 (78.0%)
Female	9 (22.0%)
Performance status	
0-2	39 (95.1%)
3-4	2 (4.9%)
WHO classification	
Squamous cell carcinoma	14 (34.1%)
Non-squamous cell carcinoma	25 (61.0%)
Unknown	2 (4.9%)
Stage	
III	11 (26.9%)
IV	29 (70.7%)
Unknown	1 (2.4%)
Couse number of RAM + DTX therapy	
2^{nd}	10 (24.4%)
3^{rd}	14 (34.1%)
4^{th}	8 (19.5%)
$5^{th} \leq$	9 (22.0%)
Planned RDI of RAM (%)	94.5 ± 5.7
Planned RDI of DTX (%)	90.6 ± 9.0
Immunosuppressives	11 (26.8%)
With prophylactic G-CSF from the first treatment cycle	18 (43.9%)
WBC (/μL)	6100 (5400-8800)
AST (IU/L)	23 (18-27)
T-Bil (mg/dL)	0.48 (0.45-0.66)
Ccr (mL/min)	66.7 (52.2-78.3)
Radiotherapy career	22 (53.7%)
Immediately preceding chemotherapy	
Platinum	21 (51.2%)
Taxane	11 (26.8%)
Onset of grade ≥ 3 neutropenia on immediately preceding chemotherapy	15 (36.6%)
Usage history of immune checkpoint inhibitor	19 (46.3%)

RAM, ramucirumab; DTX, docetaxel, RDI, relative dose intensity; G-CSF, granulocyte colony-stimulating factor, WBC, white blood cell; AST, aspartate aminotransferase; T-Bil, total bilirubin; Ccr creatinine clearance

2.2. Adverse events

Among grade ≥3 adverse events that were confirmed in all 41 patients, neutropenia was the most common (78.1%), followed by hypertension (58.5%) and leukopenia (53.7%). Data on bleeding events were collected from the subjects' medical records, but collecting accurate information for all cases was impossible since this was a retrospective study, and for milder cases, data collection during outpatient treatment was more difficult than that during inpatient treatment. Nevertheless, adverse events have occurred in 23 cases, and all of them were grade ≤2 events. Moreover, the cycles in which grade ≥3 adverse events occurred were investigated, and the median was 1 cycle for leukopenia, neutropenia, anemia, and hypertension and 7 cycles for thrombocytopenia.

2.3. Risk factors for the onset of grade ≥3 neutropenia

The factors influencing the incidence rate and duration of grade ≥3 neutropenia were explored. There was a significant difference in the incidence rate between subjects with and without ICI treatment history (p = 0.012), however, the other reported patient-specific risk factors were unaffected (Table 2). There was a significant difference in duration between subjects with and without prophylactic granulocyte colony stimulating factor (G-CSF) administration or between subjects with and without the onset of grade ≥3 neutropenia, immediately before RAM + DTX therapy, however, the other factors containing patient-specific risk factors that had been reported were unaffected (Table 2). There were no patients with a recent surgical career.

The effects of ICI treatment history on the onset of grade ≥3 neutropenia were represented as Kaplan-Meier curves (Fig. 1). Patients with ICI treatment history had a significantly lower incidence rate than those without ICI treatment history ($p = 0.037$).

2.4. Grouping based on ICI treatment history

Nineteen patients had ICI treatment history (ICI (+) group), and 22 patients did not have ICI treatment history (ICI (−) group). The specific ICI used was nivolumab in 10 patients, pembrolizumab in 6 patients, atezolizumab in 2 patients, and durvalumab in 1 patient.

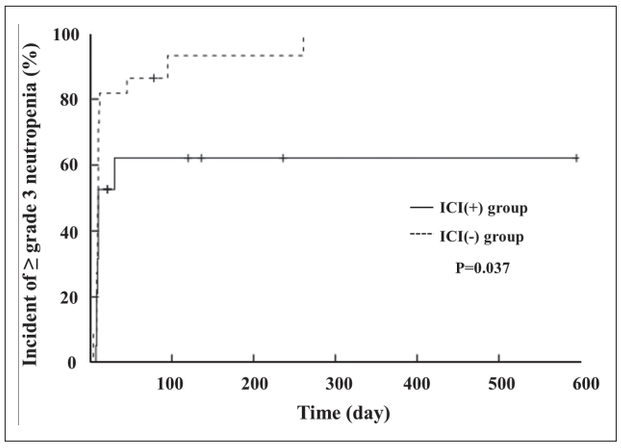

Fig. 1: Kaplan-Meier estimates of the incidence of grade ≥3 neutropenia in patients with ICI pretreatment history. ICI, immune checkpoint inhibitor; ICI (+) group, the patient group with ICI pretreatment history (n = 19); ICI (−) group, the patient group without ICI pretreatment history (n = 22).

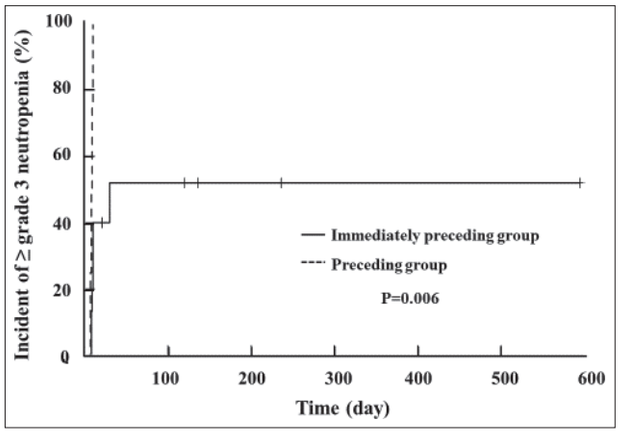

Fig. 2: Kaplan-Meier estimates between the immediately ICI treatment preceded RAM + DTX therapy group and the ICI treatment preceding ≥2 course group. Immediately preceding group, patients who received ICI treatment as the immediately preceding course of RAM + DTX therapy group (n = 15); Preceding group, patients in whom ICI treatment preceded RAM + DTX therapy by 2 or more courses group (n = 4).

No significant differences in all patient background items were observed between the ICI (+) and ICI (−) groups (Table 3).

2.5. Influence of ICI treatment history on the onset of adverse events

The incidence rates of eight grade ≥3 adverse events caused by RAM + DTX therapy were compared between the two groups (Table 4). Of these, leukopenia and hypertension, revealed high incidence rates. Furthermore, the first cycle wherein leukopenia or

Table 2: Onset rate and onset period of grade ≥ 3 neutropenia

	n	Onset rate (%)	p-value	Onset period (day)	p-value
Age (years)					
65>	9	88.9	0.654	9 (8-10)	0.653
65≤	32	75.0		9 (3-10)	
Sex					
Male	32	81.3	0.633	10 (8-10)	0.588
Female	9	66.7		9 (8-10)	
Performance status					
0-2	39	76.9	1.000	9 (4-10)	0.295
3-4	2	100.0		9, 30 *	
WHO classification					
Squamous cell carcinoma	14	71.4	0.831	10 (8-10)	0.880
Non-squamous cell carcinoma	25	80.0		10 (8-10)	
Stage					
III	11	63.6	0.385	10 (9-10)	0.800
IV	29	82.8		9 (8-10)	
Couse number of RAM + DTX therapy					
2nd	10	70.0	0.789	10 (6-10)	0.592
3rd ≤	31	80.6		9 (8-10)	
Planned RDI of RAM (%)					
85>	3	66.7	0.535	0, 11,261 *	0.372
85≤	38	78.9		9 (5-10)	
Planned RDI of DTX (%)					
85>	9	77.8	1.000	9 (8-11)	0.699
85≤	32	78.1		9 (4-10)	
Immunosuppressives					
Yes	11	72.7	0.680	11 (9-108)	0.249
No	30	80.0		10 (8-19)	
With prophylactic G-CSF from the first treatment cycle					
Yes	18	61.1	0.053	21 (9-70)	0.035
No	23	91.3		10 (9-10)	
WBC (/μL)					
5000>	6	83.3	1.000	8 (8-9)	0.482
5000≤	35	77.1		9 (4-10)	
AST (IU/L)					
35≥	37	75.7	0.559	9 (4-10)	0.111
35<	4	100.0		11 (10-16)	
T-Bil (mg/dL)					
1≥	40	77.5	1.000	9 (4-10)	0.780
1<	1	100.0		8 *	
Ccr (mL/min)					
60>	19	78.9	1.000	8 (4-10)	0.760
60≤	22	77.3		9 (8-10)	
Radiotherapy career					
Yes	22	77.3	1.000	10 (9-28)	0.239
No	19	78.9		10 (8-11)	
Immediately preceding chemotherapy Platinum					
Yes	21	85.7	0.277	10 (9-21)	0.579
No	20	70.0		10 (9-21)	
Taxane					
Yes	11	90.9	0.401	9 (8-10)	0.061
No	30	73.3		10 (9-41)	
Onset of grade ≥ 3 neutropenia on immediately preceding chemotherapy					
Yes	15	93.3	0.119	9 (8-10)	0.049
No	26	69.2		10 (9-41)	
Usage history of immune checkpoint inhibitor					
Yes	19	57.9	0.012	9 (8-10)	0.558
No	22	95.5		10 (8-10)	

RAM, ramucirumab; DTX, docetaxel, RDI, relative dose intensity; G-CSF, granulocyte colony-stimulating factor;
WBC, white blood cell; AST, aspartate aminotransferase; T-Bil, total bilirubin; Ccr creatinine clearance;
* measured value

Table 3: Comparison of the characteristics between the ICI (+) and ICI (–) groups

	ICI (+) group (n = 19)	ICI (–) group (n = 22)	p-value
Patient characteristic			
Age (years)	70 (65–72)	69 (67–74)	0.763
Male/female (n)	15 / 4	17 / 5	0.803
Performance status: 3 or 4 (n)	1	1	1.000
WHO classification (n)			
Squamous cell carcinoma	9	5	0.235
Non- squamous cell carcinoma	9	16	
Unknown	1	1	
Stage (n)			
III	6	5	0.418
IV	12	17	
Unknown	1	0	
Chemotherapy			
Couse number of RAM + DTX therapy	4 (2–7)	4 (4–8)	0.500
Planned RDI of RAM (%)	95.4 ± 5.6	93.7 ± 5.9	0.348
Planned RDI of DTX (%)	89.7 ± 9.2	91.3 ± 8.9	0.563
With prophylactic G-CSF			
Total course number of dosage	2 (1–5)	2 (1–5)	0.947
Usage on first treatment cycle (n)	8	10	0.829
Blood biochemical parameter at baseline			
WBC (/μL)	6800 (5350-9350)	6050 (5500-8300)	0.714
AST (IU/L)	23 (17-27)	23 (19-27)	0.937
T-Bil (mg/dL)	0.48 (0.36-0.66)	0.49 (0.46-0.64)	0.592
Ccr (mL/min)	59.7 (51.6-75.2)	71.7 (59.0-79.4)	0.296
Medication			
Immunosupressives	5	6	0.945
Medical history of immediate prior chemotherapy			
Chemotherapy			
Platinum	7	14	0.087
Taxane	5	6	0.945
Onset of grade ≥ 3 neutropenia	4	11	0.055
Medical history			
Radiotherapy	10	12	0.902

ICI (+) group, the patient group with a history of immune checkpoint inhibitor pretreatment.
ICI (-) group, the patient group without a history of immune checkpoint inhibitor pretreatment.
RAM, ramucirumab; DTX, docetaxel, G-CSF, granulocyte colony-stimulating factor; WHO, World Health Organization; WBC, white blood cell; AST, aspartate aminotransferase; T-Bil, total bilirubin; Ccr creatinine clearance

hypertension occurred was compared between ICI (+) and ICI (–) groups. For these adverse events, the median number of cycles was 1 cycle in both the ICI (+) and ICI (–) groups; thus, no significant differences in terms of these adverse events were observed between the two groups (leukopenia; p = 0.393, hypertension; 0.403).
Moreover, only in the ICI (+) group, the influence of RAM + DTX therapy on the onset of grade ≥3 neutropenia was compared

Table 4: Comparison of the ratio of grade ≥ 3 neutropenia between the ICI (+) and ICI (–) groups

Adverse event	Number of patients		p-value
	ICI (+) group (n = 19)	ICI (–) group (n = 22)	
Leukopenia	9	13	0.453
Anemia	3	1	0.321
Thrombocytopenia	1	2	0.610
AST (IU/L)	2	0	0.209
ALT (IU/L)	1	0	0.463
T-Bil (mg/dL)	0	0	1.000
Hypertension	10	14	0.476
Proteinuria	1	0	0.463

AST, aspartate aminotransferase; ALT, alanine aminotransferase; T-Bil, total bilirubin

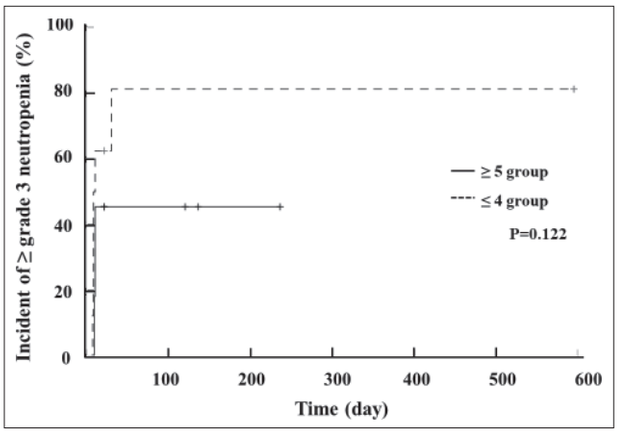

Fig. 3: Kaplan-Meier estimates between the previously received ≤4 cycles group and ≥5 cycles group.
≤4 cycles group, patients who previously received up to 4 cycles (n = 8); ≥5 cycles group, patients who received at least 5 cycles (n = 11).

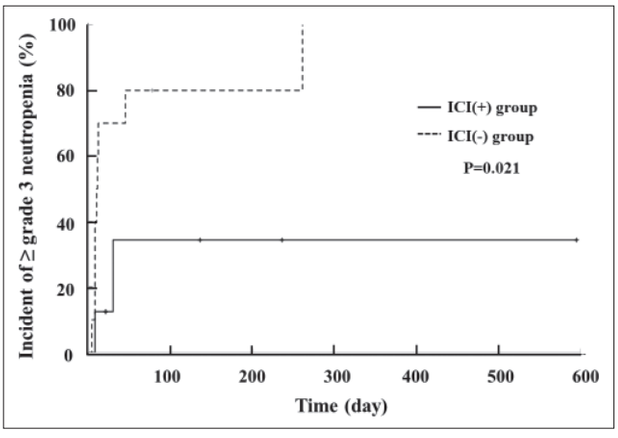

Fig. 4: Kaplan-Meier estimates between ICI pretreatment history group and non-pretreatment group on patients with prophylactic G-CSF from the first course. ICI, immune checkpoint inhibitor; ICI (+) group, the patient group with ICI pretreatment history (n = 8); ICI (–) group, the patient group without ICI pretreatment history (n = 10).

between patients who received ICI treatment as the immediately preceding course of chemotherapy (immediately preceding group) and patients in whom ICI treatment preceded RAM + DTX therapy by 2 or more courses (preceding group) using the Kaplan–Meier method (Fig. 2). The immediately preceding group had 15 patients,

whereas the preceding group had 4 patients. The incidence rate of neutropenia in the first course in the immediately preceding group was lower than that in the preceding group ($p = 0.006$). A similar analysis was performed between patients who previously

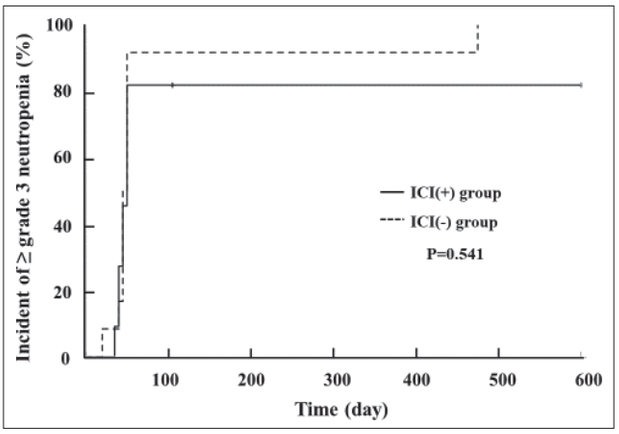

Fig. 5: Kaplan-Meier estimates between ICI pretreatment history group and non-pretreatment group on patients without prophylactic G-CSF from the first course. ICI, immune checkpoint inhibitor; ICI (+) group, the patient group with ICI pretreatment history (n = 11); ICI (−) group, the patient group without ICI pretreatment history (n = 12).

Abbreviations

DCR	disease control rate
DTX	docetaxel
FN	febrile neutropenia
G-CSF	granulocyte colony stimulating factor
ICI	immune checkpoint inhibitor
NSCLC	non-small cell lung cancer
ORR	objective response rate
OS	overall survival
PD-1	programmed death-1
PD-L1	programmed death-ligand 1
PFS	progression-free survival
RAM	ramucirumab
RDI	relative dose intensity
ULN	upper limit of normal
VEGF	vascular endothelial growth factor

received up to 4 cycles (≤4 group) and those who received at least 5 cycles (≥5 group) of ICI administration (Fig. 3). The ≤4 group had 8 patients, whereas the ≥5 group had 11 patients. No significant differences were observed between the 2 groups ($p = 0.122$).

2.6. Influence of ICI treatment history on neutropenia in patients with prophylactic G-CSF from the first course

Among the 18 patients with prophylactic G-CSF, the influence of ICI treatment history on grade ≥3 neutropenia was compared between the ICI (+) and ICI (−) groups. The ICI (+) group had 8 patients, whereas the ICI (−) group had 10 patients. The Kaplan-Meier analysis showed that the incidence rate in the first cycle in the ICI (+) group was lower than that in the ICI (−) group, and the difference was observed in the Kaplan-Meier curves ($p = 0.021$) (Fig. 4). No differences in age, the ratios of sexes/cancer types/stages, the number of RAM + DTX therapy courses, the ratio of the actual dose to the planned dose of RAM, and the ratio of actual dose/planned dose of DTX were observed between the two groups (data not shown).

2.7. Influence of ICI treatment history on neutropenia in patients without prophylactic G-CSF from the first course

Among the 23 patients without prophylactic G-CSF, the influence of ICI treatment history on grade ≥3 neutropenia was compared between the ICI (+) and ICI (−) groups. The ICI (+) group had 11 patients, whereas the ICI (−) group had 12 patients. The Kaplan-Meier analysis revealed no differences between the ICI (+) and ICI (−) groups ($p = 0.541$) (Fig. 5). No differences in age, the ratios of sexes/cancer types/stages, the number of RAM + DTX therapy courses, the ratio of the actual dose to the planned dose of RAM, and the ratio of the actual dose to the planned dose of DTX were observed between the two groups (data not shown).

3. Discussion

The results of this retrospective study demonstrated that pretreatment with ICI decreased the incidence rate of grade ≥3 neutropenia after RAM + DTX therapy in patients with NSCLC. Furthermore, prophylactic administration of G-CSF as part of and ICI treatment prior to the course of RAM + DTX therapy revealed remarkable neutropenia-preventing effects.

The REVEL study, which proved the additional effects of RAM + DTX therapy on DTX monotherapy, has shown that grade ≥3 neutropenia occurred in 49% of the subjects and was the most common grade ≥3 adverse event (Garon et al. 2014). A phase II study involving Japanese patients with NSCLC has shown that FN was more common with RAM + DTX (34.2%) than with placebo + DTX (19.8%) (Yoh et al. 2016). In this study, the incidence rate of grade ≥3 neutropenia associated with RAM + DTX therapy was high (89.5%). Because of such a high incidence rate of neutropenia in Japanese patients who received this therapy, prophylactic G-CSF administration is common nowadays (Hata et al. 2018a; Mouri et al. 2019; Kasahara et al. 2020; Hata et al. 2018b). The incidence rate of grade ≥3 neutropenia in Japanese patients with gastric cancer who received the combination therapy of RAM and paclitaxel was 46.2% (Kadokawa et al. 2018). It has been reported that the incidence rates of grade ≥3 neutropenia and FN in Japanese patients with NSCLC receiving paclitaxel plus carboplatin therapy was significantly higher than that in patients with NSCLC in the United States receiving the same therapy (Gandara et al. 2009). These data suggest that caution is particularly necessary to efficiently perform RAM + DTX therapy in Japanese patients for the onset of neutropenia and FN. The results of this study also showed that neutropenia was the most common grade ≥3 adverse event caused by RAM + DTX therapy and was the most common reason for treatment discontinuation and dose reduction. It has been reported that the onset of high-grade neutropenia was most common in the first cycle, whereas some patients had high-grade neutropenia in the second and third cycles (Kadokawa et al. 2018). In this study, neutropenia also frequently occurred in the first cycle, and this is consistent with the results of previous studies. In many cases, the standard dose was used in the first cycle, and then, the dose was reduced in subsequent cycles. Thus, we must be attentive for the onset of neutropenia and FN in the first cycle when RAM + DTX therapy is administered.

This study was showed that a history of ICI pretreatment was associated with a reduced incidence rate of grade ≥3 neutropenia. Additionally, the effect was remarkable from the first cycle. A study investigating the incidence rate of grade ≥3 neutropenia in Japanese patients with gastric cancer undergoing the combination therapy of RAM and paclitaxel has reported that pretreatment regimens had no effect (Kadokawa et al. 2018). However, the pretreatment regimens under study did not include ICI, and the effects of ICI pretreatment was not considered. Meanwhile, 19 of the 41 patients in this study received ICI treatment before RAM + DTX therapy because the Japanese guidelines recommend ICI monotherapy or the combination therapy of an ICI and other anticancer agents as the frontline therapy for NSCLC. There have been some reports about the impact of ICI pretreatment on the onset of adverse events in other places. According to the previous report, immediate prior nivolumab therapy frequently resulted in interstitial lung disease in patients receiving osimertinib (Kotake et al. 2017). It has been reported that using osimertinib immediately after nivolumab significantly increased the frequency of grade 3 or higher hepatotoxicity in patients with advanced NSCLC harboring an EGFR mutation who developed T790M resistance (Yamaguchi

et al. 2020). These studies suggest that ICI pretreatment may promote some adverse events.

In this study, however, we led to results that differed from these reports. A phase III trial involving patients with hepatocellular carcinoma (IMbrave150) has demonstrated that the combination therapy of atezolizumab, classified as an ICI, and bevacizumab, classified as a vascular endothelial growth factor (VEGF) inhibitor, improved both the PFS and OS (Kudo et al. 2020). It has been reported that the combination of antiangiogenic therapy and immunotherapy may enhance the efficacy of immunotherapy and reduce the risk of immune-related adverse reactions (Fukumura et al. 2018). Based on these reports, the possibility that ICI pretreatment somehow influenced the effects of the VEGF inhibitors subsequently used, as shown in the present study, and should not be ruled out. In addition, this consideration may be supported because serious neutropenia by RAM + DTX therapy was reduced when ICI is used immediately before this therapy. It has been reported the ORR, DCR, and median PFS of patients who received DTX-based chemotherapy or S-1 after nivolumab, especially immediately after nivolumab, tended to be better than those of patients with NSCLC who received DTX or S-1 without ICIs (Tamura et al. 2019). Moreover, this study showed that the incidence rate of neutropenia in patients who received ICI treatment in the immediately preceding course was lower than that in patients whose ICI treatment preceded by 2 or more courses. This result suggests that the use of ICI in the immediately preceding course has a stronger impact on the onset of neutropenia; however, more detailed analyses could not be performed unfortunately because the number of cases was insufficient. It has been hypothesized that the early dynamics of the blood neutrophil-to-lymphocyte ratio and absolute neutrophil count during nivolumab are associated with the late efficacy of subsequent salvage chemotherapy (Soda et al. 2019). It has been reported that ICIs induce durable tumor regression by restoring the normal ability of the immune system to suppress tumor cells, achieving good results in treating NSCLC with few adverse events (Qu et al. 2020). In these reports, ICI pretreatment increased the antitumor potency and improved the therapeutic efficacy of the RAM + DTX combination; however, there were concerns about the onset of adverse events. A study that investigated RAM + DTX therapy has shown the occurrence of adverse events, such as fever, myalgia, arthritis, pleural effusion, and pneumonitis, tended to increase in patients with ICI pretreatment history (Harada et al. 2019). However, neutropenia and hypertension were higher in patients without ICI pretreatment history. Thus, despite increased toxicity concerns, RAM + DTX therapy in patients with ICI pretreatment history showed a trend for tumor regression and statistically significant prolongation of PFS. The REVEL study has showed higher incidence rates of FN caused by RAM + DTX therapy than those caused by DTX monotherapy (Garon et al. 2014). Therefore, the availability of a method for reducing the onset of serious neutropenia and FN in patients receiving RAM + DTX therapy is ideal. Note that our findings suggest prior treatment with ICI as such a method. However, further studies are required to determine the mechanisms underlying the reducing effect of ICI treatment history, which was newly discovered in this study, on the incidence rate of grade ≥3 neutropenia.

According to the Cancer Statistics in Japan by the Foundation for Promotion of Cancer Research in Japan-2019, the median age of the subjects in this study was 69 years, and in terms of sex, the subjects were male-dominated; thus, the subjects nearly replicate the age and sex distributions in the Japanese population. Meanwhile, this study had several limitations as it was a retrospective study. Because data were collected from the subjects' electronic medical records, there were missing data about some variables, such as smoking history, bleeding events, and body temperature, and thus, their effects could not be discussed. Only ICI treatment history influenced the incidence rate of grade ≥3 neutropenia. The factors influencing the onset period of grade ≥3 neutropenia, on the other hand, were prophylactic G-CSF administration and the history of onset of grade ≥3 neutropenia on immediate prior chemotherapy. There was a difference in the onset of grade ≥3 neutropenia on immediate prior chemotherapy between the ICI (+) and ICI (−) groups. This could explain why 12 patients were given ICI monotherapy for immediate prior chemotherapy in the ICI (+) group and grade ≥3 neutropenia did not develop in all of them. However, this study is required to increase the number of cases and consider them thoroughly because we may not be able to remove the influence of patient-specific risk factors, which has been reported extensively. Normally, we should use multivariate analysis to investigate risk factors because the effects of confounding factors are taken into account. However, because this was a single-center study, the number of cases collected was insufficient for detailed analysis, although the findings were based on therapeutic results at a 600-bed hospital. As a result, the number of cases in this study is insufficient to conduct such an analysis. We may need to consider using a multicenter study design in the future.

It has been reported that PD-L1 expression in tumors is not associated with the efficacy of RAM + DTX therapy in patients with NSCLC (Yoshimura et al. 2019). Thus, the use of RAM + DTX therapy after ICI may be useful, regardless of PD-L1 expression. Potentially, it can be applied to other cancer types for which ICI administration and antiangiogenic therapy are indicated. Our findings add to the knowledge of the effectiveness of RAM + DTX therapy after ICI in reducing the incidence of serious adverse events and can support the results of previous studies on treatment patterns to be recommended for patients with NSCLC.

4. Experimental

4.1. Subjects

The study subjects included patients who received a combination therapy of RAM and DTX as cancer chemotherapy for NSCLC at Gifu Prefectural General Medical Center between April 2016 and December 2020. All patients who did not agree with the main purpose of the study were excluded.

To administer the combination therapy of RAM and DTX, 50-mg diphenhydramine and 6.6-mg dexamethasone were pre-administered, orally and intravenously, respectively, and 10-mg/kg RAM (1 h intravenous infusion) and 60 mg/m^2 DTX (1 h intravenous infusion) were successively administered. The interval between two cycles of RAM and DTX therapies was 3 weeks. All of the study subjects for this study were recruited. The following cases, however, were excluded from the analysis: any period when RAM, DTX, or RAM + DTX therapy was temporarily or permanently stopped.

4.2. Data collection

This was a retrospective study based on patient data collected from electronic patient files available in the databases of Gifu Prefectural General Medical Center.

At the start of treatment, the following variables were collected: age, sex, performance status, World Health Organization classification of NSCLC, cancer stage, planned relative dose intensity (RDI) (%) of RAM and DTX, detailed treatment history (past chemotherapy, radiotherapy career, recent operation career, and history of ICI therapy), and the onset of grade ≥3 neutropenia on immediate prior chemotherapy. The RDI (%) for RAM and DTX was calculated using the following formula: actual dose /planned dose, i.e., 3.3 mg/kg/week and 20 mg/m^2/week respectively, multiplied by 100. The administration period for the planned dose was set to 3 weeks there. Data on the following variables were extracted during the treatment period: body height; body weight; body surface area; course number of the combination therapy of RAM and DTX; dose interval of RAM and DTX therapies; RAM dosage; DTX dosage; usage of immunosuppressive agent; history of prophylactic G-CSF; blood biochemical parameters including leukocyte count (×10^4/μL), neutrocyte count ratio (%), hemoglobin level (g/dL), platelet count (×10^4/μL), serum aspartate aminotransferase level (IU/L), serum alanine aminotransferase level (IU/L), serum total bilirubin level (mg/dL) and serum creatinine (mg/dL) ; systolic blood pressure (mmHg); diastolic blood pressure (mmHg); and bleeding. Creatinine clearance (Ccr) was calculated using Cockcroft–Gault formula : Ccr (mL/min): {140 − age (years)} × weight (kg) × 0.85 (in females) / (serum Cr × 72) (Cockcroft et al. 1976).

4.3. Assessment

Adverse events were determined according to the Common Terminology Criteria for Adverse Events, version 5.0 (CTCAE Ver.5). Data on the adverse events with the highest grade were extracted during the treatment period. The 2016 data were assessed again by CTCAE Ver.5. The assessment using CTCAE Ver.5 is as follows: grade 3 of leukopenia is determined as the criteria where white blood cell count is <2000/μL, grade 3 of neutropenia is determined as the criteria where neutrophil count is <1000/μL, grade 3 of anemia is determined as the criteria where hemoglobin level is <8.0 g/dL, grade 3 of thrombocytopenia is determined as the criteria where platelet count is <50000/μL, grade 3 of serum aspartate aminotransferase (AST) level is determined as the criteria where AST level is upper limit of normal (ULN) × 5–20 if baseline is normal and baseline × 5–20 if baseline is abnormal, serum alanine aminotransferase (ALT) level is determined as the criteria where ALT level is ULN × 5–20 if baseline is normal and baseline × 5–20 if baseline is abnormal, serum total bilirubin (T-Bil) level is determined as the criteria where T-Bil level is ULN × 3–10 if baseline is

normal and baseline × 3–10 if baseline is abnormal, hypertension is determined as the criteria where systolic blood pressure (BP) is ≥160 mmHg or diastolic BP is ≥100 mmHg or is the need for two or more medications or stronger treatment than before, and proteinuria is determined as the criteria where urinary protein is ≥3.5 g/24 h or 4 + proteinuria. The predicting risk factor for the onset grade ≥3 neutropenia referred to the past report, i.e., ≤65 years old, defective performance status, planned RDI ≥ 85, and primary G-CSF prophylaxis (Lyman et al. 2011; Kukec et al. 2015).

4.4. Statistical analysis

Normally distributed variables were expressed as mean±standard deviation; non-normally distributed variables were expressed as median (interquartile range). Parametric and nonparametric pairwise comparisons were performed using the t-test and Mann–Whitney U-test, respectively. Ratios were compared using the chi-square test and Fisher's exact test. The onset period of adverse events was estimated using the Kaplan–Meier method, and the log-rank test was used for comparisons between 2 groups. All statistical analyses were performed using Statistical Package for the Social Sciences (version 22.0; IBM Corporation, Armonk, NY, USA). Differences and associations with p-values of less than 0.05 were considered statistically significant.

4.5. Ethics

All procedures performed in this study involving human participants were performed according to the ethical standards of the institutional and/or national research committee and to the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.
The study protocol was approved by the Epidemiology and Clinical Research Ethics Review Board of Gifu Prefectural General Medical Center.

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Authors' contributions: Takahiro Hayashi supervised the study. Hiroyuki Ohno, Tomoyuki Hirashita, and Takahiro Hayashi planned and designed the study. Hiroyuki Ohno and Takahiro Hayashi collected and analyzed the data. Hiroyuki Ohno, Tomoyuki Hirashita, and Takahiro Hayashi wrote the original draft.
Nanae Katagiri, Hiroyuki Ohno, and Shota Mano collected data. Nanae Katagiri, Atsunori Masuda, Akifumi Tsuzuku, Fumihiro Asano, and Kotoe Inoue helped to conduct the literature review. Ryosuke Oguri, Kosuke Miyazaki, Kenji Ito, and Yasuaki Sekiya helped to analyze the data. All authors read and approved the final version.

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