Thyroid Dysfunction Related to the Antiangiogenic VEGFR2-Binding Monoclonal Antibody Ramucirumab: A Series of 14 Cases and a Descriptive Study

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INTRODUCTION

Vascular endothelial growth factor (VEGF) has an important role in angiogenesis, one of the hallmarks of tumor development.1–3 VEGF stimulates vascular endothelial cell growth, survival, and proliferation by binding to specific VEGF receptor (VEGFR) 1-VEGFR3, especially VEGFR2 is the main signaling VEGFR in blood vascular endothelial cells, leading to subsequent signal transduction.2,3 Therefore, many anticancer drugs that target the VEGF signaling pathway have been developed. Ramucirumab, the human immunoglobulin G1 (IgG1) monoclonal antibody receptor antagonist of VEGFR2, has been developed for the treatment of solid tumors, such as gastric, colorectal, and lung cancers. Toxicities induced by anti-VEGF biologics, such as bevacizumab (anti-VEGF-A antibody), ramucirumab, and aflibercept (an Fc fusion protein consisting of the extracellular domains of VEGFR1 and VEGFR2 fused with the Fc domain of human IgG), include hypertension, proteinuria, neutropenia, hemorrhagic events, thrombotic events, and gastrointestinal perforation.4–6 In addition, blockade of the VEGF pathway may potentially cause thyroid dysfunction.7 As VEGF is crucial for vascular homeostasis and the maintenance of vascular integrity and architecture in the thyroid gland, systemic delivery of anti-VEGF drugs reduces thyroid vascular density and fenestrations, leading to hypothyroidism.7 Hypothyroidism is also a well-known toxicity of small-molecule anti-VEGF tyrosine kinase inhibitors (TKIs),8 such as sunitinib, sorafenib, pazopanib, and lenvatinib. However, it is uncertain if anti-VEGF biologics can induce hypothyroidism. CYRAMZA® package insert9 is only information available to review (case series). We also evaluated the change of thyroid-stimulating hormone (TSH) level during ramucirumab chemotherapy in 16 out of 38 patients who were regularly confirmed TSH (descriptive study). A total of 14 (36.8%) patients developed thyroid dysfunction (TSH >10 mU/L) after ramucirumab chemotherapy. Thyroid autoantibodies were detected in one of the 10 patients (10.0%) who were tested for thyroid autoantibodies. The median time to onset of thyroid dysfunction after ramucirumab initiation was 275 (range, 63–553) days. Levothyroxine replacement was needed in 10 (71.4%) patients. Sixteen patients had thyroid function regularly monitored; the mean TSH level was significantly increased after ramucirumab chemotherapy compared with that at baseline (10.7 ± 10.0 mU/L vs. 4.1 ± 2.8 mU/L; p < 0.01). Our findings indicate that ramucirumab can result in thyroid dysfunction. We propose that thyroid function testing should be performed regularly to detect hypothyroidism and guide its management in patients receiving ramucirumab chemotherapy.

Key words ramucirumab; thyroid dysfunction; vascular endothelial growth factor; anti-vascular endothelial growth factor monoclonal antibody

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reviewed the electronic medical records of Kyoto-Katsura Hospital from September 2015 to December 2018 and identified patients who were receiving ramucirumab for any reason and had thyroid function testing data available. We excluded patients who had thyroid disease at the time of ramucirumab initiation. Clinical course and features and severity of thyroid dysfunction [serum thyroid-stimulating hormone (TSH) level >10 mU/L, which generally should be treated with levothyroxine (LT4) in patients with subclinical hypothyroidism] were investigated as a case series. In addition, we performed a descriptive study of the change in TSH level over time during ramucirumab chemotherapy in patients with regularly confirmed thyroid function testing; patients’ TSH levels were followed for up to 2 months after ramucirumab discontinuation because its elimination half-life is approximately two weeks. This study was performed in accordance with the Declaration of Helsinki and its amendments and was approved by the Ethics Committee of Kyoto-Katsura Hospital (Approval number: 640). This study is a retrospective cohort study, carried out by the opt-out method of a poster presentation at our institution, and it was exempted from obtaining informed consent from each patient in consideration of the aim and methods of the study.

Descriptive statistics were used to report the results of this study (mean ± standard deviation). The paired t-test was used to compare baseline TSH level and highest TSH level measured after ramucirumab chemotherapy. Statistical analysis was performed with EZR version 1.37 (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, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics. p < 0.05 was defined as statistically significant.

RESULTS

Case Series We identified 38 eligible patients who were receiving ramucirumab for any reason and had thyroid function testing data available for review during the study period. The incidence of thyroid dysfunction was 36.8% (14/38 patients). Details of these 14 patients are shown in Table 1. Three (21.4%) patients had a history of receiving an immune checkpoint inhibitor. In three patients with lung cancer with prior immunotherapy (two patients, second line nivolumab; one patient, third line nivolumab), the times to onset of TSH >10 mU/L after final immunotherapy were 126, 364, and 465 d, respectively. Ten patients were tested for the thyroid autoantibodies thyroid peroxidase antibody (TPO-Ab) or thyroglobulin antibody (Tg-Ab); one (10.0%) of these 10 patients was positive. Ten of fourteen patients (71.4%) were administered LT4, of which 10 patients received continued LT4 administration during ramucirumab chemotheraphy. The clinical manifestations associated with thyroid dysfunction during ramucirumab chemotheraphy were as follows: fatigue (78.6%, 11/14) and edema (35.7%, 5/14). Therapy timelines for patients in relation to the onset of TSH >10 mU/L are presented in Fig. 3. Descriptive Study In 16 patients, thyroid function testing was regularly monitored over a median follow-up period of 154 (range, 54–577) days. The median TSH level gradually increased throughout the period of ramucirumab chemotherapy and was over the upper normal limit of TSH at the time of 3 months after ramucirumab initiation (Fig. 2). LT4 supplementation was required in 5 (31.3%) of the 16 patients. Also, of the 12 patients with TSH level elevated above the normal upper reference range (5 mU/L), TSH levels returned to normal reference range within 2 months after final ramucirumab administration in 3 (Fig. 3). The mean TSH level significantly increased after ramucirumab chemotherapy compared with that at baseline (10.7 ± 10.0 mU/L vs. 4.1 ± 2.8 mU/L; p < 0.01) (Fig. S2). Thus, we then conducted post-hoc analysis to evaluate the change of free thyroxine (FT4) level between baseline and lowest level during ramucirumab chemotherapy over a median follow-up period of 140 (range, 54–577) days. The mean FT4 level significantly decreased after ramucirumab chemotherapy compared with that at baseline (1.0 ± 0.1 ng/dL vs. 1.2 ± 0.2 ng/dL; p < 0.001).

DISCUSSION

The results of this study provided the detailed information on the effect of anti-VEGFR2 monoclonal antibody ramucirumab chemotherapy on thyroid function. TSH level significantly increased gradually over time during ramucirumab chemotherapy. Possible mechanisms of cancer treatment-related hypothyroidism may be divided into two categories: 1) autoantibody-associated thyroiditis induced by immune checkpoint inhibitors and interferon-alfa and 2) blockade of the VEGF signaling pathway. Systemic delivery of anti-VEGF drugs has the potential to adversely affect the vasculature in healthy tissues and organs, including the density and structure of capillary networks in endocrine organs. In the thyroid gland, systemic delivery of anti-VEGF drugs reduces thyroid vascular density and fenestrations, leading to reduced glandular uptake of iodine and production of thyroid hormone. In the present study, we did not detect thyroid autoantibodies in most patients with thyroid dysfunction, which is in line with previous studies regarding small-molecule anti-VEGF TKI-induced hypothyroidism.

In the modern immunotherapy era, healthcare professionals should be able to address hypothyroidism in patients with cancer because hypothyroidism is the most frequent immune-
Table 1. Clinical Details of 14 Patients Who Developed Thyroid Dysfunction during Ramucirumab Chemotherapy

| Case no. | Age  | Sex   | Tumor type | No. of prior therapy | Combined regimen | ICPi prior to Ramab | How TFT confirmed | Thyroid antibodies positivity (Tg-Ab/TPO-Ab) | Time to onset of thyroid dysfunction (TSH >10) after initial Ramab administration (days) | Overt hypothyroidism (required LT4 supplementation) | Starting/maintenance\(a\) dose of LT4 (mg/d) | Toxicity grades of hypothyroidism (CTCAE version 4.0\(b\)) | Clinical manifestations associated with hypothyroidism |
|----------|------|-------|------------|----------------------|-------------------|---------------------|-------------------|-----------------------------------------------|------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|---------------------------------------------------------|-----------------------------------------------------------------------|
| 1        | 67   | Female | Lung       | 3                    | DTX               | Yes                 | Routine monitoring post ICPi therapy | Unconfirmed       | 395                                                                 | No                                                                 | —                                                                       | 25/75                                                          | 1 Fatigue                                                                 | Appetite loss  
Atrial fibrillation |
| 2        | 79   | Male   | Lung       | 2                    | DTX               | Yes                 | Routine monitoring post ICPi therapy | Not detected      | 109                                                                 | Yes                                                                  | 25/75                                                      | 2 Fatigue                                                                 | Cold intolerance  
Supraventricular tachycardia |
| 3        | 62   | Male   | Lung       | 2                    | DTX               | Yes                 | Signs or symptoms suspicious for hypothyroidism | Unconfirmed       | 341                                                                 | No                                                                  | —                                                                       | 2 Fatigue                                                                 | Edema  
Sonnolence |
| 4        | 66   | Male   | Colorectal | 1                    | FOLFIRI           | No                  | Signs or symptoms suspicious for hypothyroidism | Positive: Tg-Ab | 553                                                                 | Yes                                                                  | 25/25                                                      | 2 Fatigue                                                                 | Voice disturbance  
Edema  
Hypotension (127 mEq/L)  
Forgetfulness |
| 5        | 78   | Female | Gastric    | 2                    | nab-PTX           | No                  | Routine monitoring          | Not detected      | 118                                                                 | Yes                                                                  | 25/50                                                      | 2 Fatigue                                                                 | |
| 6        | 74   | Male   | Colorectal | 3                    | FOLFIRI           | No                  | Signs or symptoms suspicious for hypothyroidism | Not detected      | 126                                                                 | Yes                                                                  | 25/25                                                      | 2 Fatigue                                                                 | |
| 7        | 65   | Male   | Gastric    | 1                    | nab-PTX           | No                  | Routine monitoring          | Unconfirmed       | 98                                                                  | No                                                                  | —                                                                       | 2 Fatigue                                                                 | |
| 8        | 69   | Female | Colorectal | 1                    | FOLFIRI           | No                  | Signs or symptoms suspicious for hypothyroidism | Not detected      | 63                                                                  | Yes                                                                  | 25/25                                                      | 2 Fatigue                                                                 | |
| 9        | 82   | Female | Colorectal | 1                    | FOLFIRI           | No                  | Signs or symptoms suspicious for hypothyroidism | Unconfirmed       | 345                                                                 | No                                                                  | —                                                                       | 2 Fatigue                                                                 | |
| 10       | 56   | Male   | Gastric    | 1                    | Ramab monotherapy | No                  | Signs or symptoms suspicious for hypothyroidism | Not detected      | 397                                                                 | Yes                                                                  | 50/75                                                      | 2 Edema                                                                 | Dry mouth  
Voice disturbance |
| 11       | 63   | Female | Gastric    | 1                    | PTX               | No                  | Baseline assessment before starting ICPi therapy | Not detected      | 245                                                                 | Yes                                                                  | 25/25                                                      | 2 Edema                                                                 | |
| 12       | 65   | Female | Colorectal | 1                    | FOLFIRI           | No                  | Routine monitoring          | Not detected      | 0                                                                   | Yes                                                                  | 25/50                                                      | 2 Edema                                                                 | |
| 13       | 82   | Male   | Gastric    | 1                    | nab-PTX           | No                  | Routine monitoring          | Not detected      | 0                                                                   | Yes                                                                  | 12.5/25                                                   | 2 Fatigue                                                                 | |
| 14       | 70   | Female | Gastric    | 1                    | PTX               | No                  | Baseline assessment before starting ICPi therapy | Not detected      | 304                                                                 | Yes                                                                  | 25/75                                                      | 2 Fatigue                                                                 | |

\(a\) Levothyroxine maintenance dosage was investigated from levothyroxine initiation up to 2 months after final ramucirumab administration. \(b\) Grade 1: asymptomatic; clinical or diagnostic observations only; intervention not indicated. Grade 2: symptomatic; thyroid replacement indicated; limiting instrumental ADL. Grade 3: severe symptoms; limiting self-care ADL.; hospitalization indicated. Grade 4: life-threatening consequences; urgent intervention indicated. Grade 5: death. Abbreviations: Ramab, ramucirumab; ICPi, immune checkpoint inhibitor; TFT, thyroid function test; Tg-Ab, thyroglobulin antibody; TPO-Ab, thyroid peroxidase antibody; TSH, thyroid stimulating hormone; LT4, levothyroxine; VEGF, vascular endothelial growth factor; DTX, docetaxel; FOLFIRI, leucovorin plus fluorouracil plus irinotecan; nab-PTX, nanoparticle albumin-bound paclitaxel; PTX, paclitaxel.
related adverse event in the endocrine system and is observed in up to 10% of patients treated with programmed death-1/programmed death-ligand 1 (PD-1/PD-L1) blockade. In the IMpower150 study, the addition of atezolizumab, anti-PD-L1 antibody, to bevacizumab plus chemotherapy significantly improved progression-free survival and overall survival among patients with metastatic lung cancer.20 Interestingly, the prevalence of all grades of hypothyroidism was 3.8% in the control group patients treated with bevacizumab plus carboplatin plus paclitaxel (BCP).20 The experimental group (receiving atezolizumab in addition to BCP) had a 12.7% prevalence of all grades of hypothyroidism. This reported prevalence of hypothyroidism in the experimental group was considerably higher than the 3.9% prevalence previously reported in a meta-analysis of patients treated with anti-PD-L1 antibody.21 Therefore, two important points are raised here. First, hypothyroidism may occur during anti-VEGF monoclonal antibody bevacizumab therapy. Second, immunotherapy-related hypothyroidism incidence may be increased in patients with the addition of bevacizumab. The microvascular structures in endocrine organs are hypersensitive to VEGF owing to expression of high levels of VEGFR2 in the fenestrated endothelium.7

Fig. 2. The Change in TSH Level from Baseline during 6 Months of Ramucirumab Therapy in 16 Patients Treated with Ramucirumab

Five patients treated with levothyroxine were included in the descriptive analysis. The closed circle represents the median TSH level at each month. The gray area represents the interquartile range of TSH level. The dotted line represents the upper normal limit of TSH level. The dashed line represents the lower normal limit of TSH level.

Fig. 3. The Change in Individual TSH Levels Over Time in 16 Patients Treated with Ramucirumab

Each line represents the TSH levels of each individual patient. The closed circles represent the measured TSH levels during ramucirumab therapy. The open circles represent the measured TSH levels when off ramucirumab. The dotted lines represent the periods during levothyroxine supplementation. The gray area represents the normal reference range of TSH level (0.5–5 mU/L). About 3 patients with TSH level elevated above the normal upper reference range, TSH levels returned to normal reference range within 2 months after final ramucirumab administration (arrow).
Although, ramucirumab is more likely to cause hypothyroidism than bevacizumab as it directly blocks the functions of ligands that bind to VEGFR2, further studies are warranted to clarify the change of thyroid function during anti-VEGF biologic therapy.

There are some limitations to this study. We were unable to investigate the association between ramucirumab-associated thyroid dysfunction and prior chemotherapy, cytotoxics combined with ramucirumab, and other ramucirumab-induced toxicities because of small study sample size. In addition, we were unable to precisely investigate the rate of thyroid autoantibody positivity, details of thyroid ultrasound imaging studies, whether LT4 improved clinical symptoms, and whether hypothyroidism during ramucirumab chemotherapy resulted in treatment interruption or discontinuation due to the retrospective nature of the study. To overcome these limitations, a large prospective observational study among the patients treated with ramucirumab compared to those without should be conducted.

In conclusion, our findings suggest that anti-VEGFR2 monoclonal antibody ramucirumab, as well as small-molecule anti-VEGF TKIs results in thyroid dysfunction. We propose that thyroid function testing should be performed during ramucirumab chemotherapy to detect hypothyroidism early as follows: 1) check baseline TSH level, 2) monitor TSH level once every three months because of low healthcare cost, and 3) re-check TSH level in patients with symptoms of fatigue or edema. Future research that expands on the findings of this study may further elucidate the prevalence and clinical manifestations of anti-VEGF biologics-induced hypothyroidism.

**Conflict of Interest** The authors declare no conflict of interest.

**Supplementary Materials** The online version of this article contains supplementary materials.

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