

## Anti-tumor effect of antibody-drug conjugate targeting cell adhesion molecule 1 on GIST cells representing small intestinal GIST

Makoto Yoshida<sup>a</sup>, Jiayin Yuan<sup>b</sup>, Takako Kihara<sup>a</sup>, Neinei Kimura<sup>a</sup>, Takashi Yamasaki<sup>a</sup>, Mizuka Ohkouchi<sup>a</sup>, Yuka Hashikura<sup>a</sup>, Koji Isozaki<sup>a</sup>, Man Hagiya<sup>c</sup>, Akihiko Ito<sup>c,\*</sup>, Seiichi Hirota<sup>a,\*</sup>

<sup>a</sup> Department of Surgical Pathology, Hyogo Medical University School of Medicine, Nishinomiya, Hyogo, Japan

<sup>b</sup> Department of Pathology, First People's Hospital of Foshan, Foshan City, Guangdong 528000, China

<sup>c</sup> Department of Pathology, Faculty of Medicine, Kindai University, Ono-Higashi, Osaka-Sayama, Osaka, Japan

### ARTICLE INFO

Editor: Marco Giudici

#### Keywords:

Gastrointestinal stromal tumor  
Antibody-drug conjugate  
Cell adhesion molecule 1  
Anti-tumor effect  
Subcutaneous injection  
Peritoneal dissemination

### ABSTRACT

Gastrointestinal stromal tumor (GIST) is the most common mesenchymal tumor of the alimentary tract. The prognosis depends on the primary site, and small intestinal GISTs have a worse prognosis than gastric GISTs. Molecularly targeted drugs to inhibit tyrosine kinase activity of KIT were used for unresectable or recurrent GISTs. However, secondary resistance to the drugs is often acquired, and treatments based on other mechanisms are needed. Previously, we reported that cell adhesion molecule 1 (CADM1) was highly expressed in most of small intestinal GISTs but not in most of gastric GISTs. In the present study, we examined whether the antibody-drug conjugate (ADC) with anti-CADM1 antibody and monomethyl auristatin E (anti-CAD-ADC) shows anti-tumor effect on CADM1-expressing human GIST cells. The ADC adhibited in this study was previously used for CADM1-expressing human mesothelioma cells and showed anti-tumor effect for them in vitro. GIST-T1 cell line of gastric origin which scarcely expresses CADM1 and GIST-T1 cells transfected with CADM1 cDNA (GIST-T1-CAD cells) which highly expresses CADM1 and represents small intestinal GIST were used. In vitro, anti-CAD-ADC showed remarkable cytotoxic activity on GIST-T1-CAD cells, but control ADC did not. Both anti-CAD-ADC and control ADC did not show anti-tumor effect on original GIST-T1 cells. When GIST-T1-CAD cells were subcutaneously injected to the nude mice, intravenous administration of anti-CAD-ADC showed inhibitory effect for tumor enlargement. Tumor of GIST-T1 cells grew even after anti-CAD-ADC injection. When GIST-T1-CAD cells were injected into peritoneal cavity of the SCID mice, intraperitoneal administration of anti-CAD-ADC showed reduction of the peritoneal tumor. On the other hand, peritoneal tumor grew after control ADC administration. Tissue and organ damage due to administration of anti-CAD-ADC was not apparent by macroscopic and histological examinations in mice. These results indicate that anti-CAD-ADC could have apparent anti-tumor effect on CADM1-expressing human GIST cells both in in vitro and in vivo mouse models.

### 1. Introduction

Gastrointestinal stromal tumor (GIST) is the most common

mesenchymal tumor of the gastrointestinal tract (Hirota et al., 1998). We previously found that most of sporadic GISTs (80–85%) harbor gain-of-function mutations in the *KIT* gene encoding KIT protein (Hirota

**Abbreviations:** GIST, gastrointestinal stromal tumor; CADM1, cell adhesion molecule 1; ADC, antibody-drug conjugate; anti-CAD-ADC, antibody-drug conjugate with anti CADM1 antibody and monomethyl auristatin E; GIST-T1-CAD cells, GIST-T1 cells transfected with CADM1 cDNA; PDGFRA, platelet-derived growth factor receptor alpha; RTKs, receptor tyrosine kinases; SgIGSF, spermatogenic immunoglobulin superfamily; SynCAM, synaptic cell adhesion molecule; MMAE, monomethyl auristatin E; GIST-T1-CAD-Luc cells, GIST-T1-CAD cells transfected with pGL4.51[luc2/CMV/Neo] vector; DMEM, Dulbecco's minimum essential medium; FBS, fetal bovine serum; mc-vc-PAB-MMAE, chicken monoclonal anti-CADM1 ectodomain (3E1) humanized with human IgG4 (named h3E1) and conjugated with maleimidocaproyl-valine-citrulline-p-amino-benzyl linker and a MMAE payload; SCID, severe combined immunodeficiency; IVIS, in vivo imaging system; PBS, phosphate buffered saline; BW, body weight.

\* Corresponding authors.

E-mail addresses: [aito@med.kindai.ac.jp](mailto:aito@med.kindai.ac.jp) (A. Ito), [hiros@hyo-med.ac.jp](mailto:hiros@hyo-med.ac.jp) (S. Hirota).

<https://doi.org/10.1016/j.yexmp.2024.104922>

Received 27 March 2024; Received in revised form 22 July 2024; Accepted 24 July 2024

Available online 2 August 2024

0014-4800/© 2024 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

et al., 1998), and we and another group reported that 5–10% of sporadic GISTs have gain-of-function mutations in the *PDGFRA* gene encoding PDGFR $\alpha$  (Heinrich et al., 2003; Hirota et al., 2003). KIT and PDGFR $\alpha$  are the receptor tyrosine kinases (RTKs) and their mutant forms are constitutively active and can cause GIST development and proliferation (Hirota et al., 1998; Heinrich et al., 2003; Hirota et al., 2003). GISTs occur mainly in the stomach (60–70%) and small intestine (20–30%) (Miettinen and Lasota, 2001). Various different features are present between gastric GISTs and small intestinal GISTs. For example, several proteins such as connexin 43 and cell adhesion molecule 1 (CADM1) are specifically expressed in small intestinal GISTs (Yuan et al., 2021; Yuan et al., 2022) and small intestinal GISTs have a worse prognosis than gastric GISTs (Miettinen and Lasota, 2006). Molecularly targeted drugs such as imatinib, sunitinib and regorafenib to inhibit RTKs were used for unresectable or recurrent GISTs (Demetri et al., 2006; Demetri et al., 2013). However, GISTs often acquire resistance to these drugs after long-term use. Thus, treatment options based on other mechanisms are needed, and antibody-drug conjugates (ADCs) might be a candidate.

CADM1 is a member of a family of intercellular adhesion molecules, also known as the spermatogenic immunoglobulin superfamily (SgIGSF) and synaptic cell adhesion molecule (SynCAM) (Biederer et al., 2002; Ito and Oonuma, 2006). Bile duct and alveolar epithelial cells (Ito et al., 2007; Ito et al., 2003), fibroblasts (Moiseeva et al., 2013), and pancreatic islet cells (Koma et al., 2008) are also known to express CADM1. While CADM1 could play as a tumor suppressor in non-small cell lung cancer, gastric cancer, breast cancer and esophageal cancer (Kuramochi et al., 2001; Honda et al., 2002; Allinen et al., 2002; Zeng et al., 2016), it might be considered to act as a tumor promoter in adult T cell leukemia/lymphoma, acute myelocytic leukemia, small cell lung cancer and small intestinal GIST probably through augmentation of tumor invasion and tumor cell adhesion to the vascular endothelium (Miettinen and Lasota, 2006; Fisser et al., 2015; Dewan et al., 2008; Funaki et al., 2021).

ADC consists of a monoclonal antibody that recognizes an antigen and a payload that is a small molecule cytotoxic agent. The antibody and payload are connected by a linker. The antibody binds to the antigen specifically expressed on the surface of the tumor cell, then the ADC is internalized into the cell. The payload is detached from the linker by lysosomal activity and exerts anti-tumor effects. In the previous study, we reported that an ADC consisting of a humanized anti-CADM1 monoclonal antibody conjugated with monomethyl auristatin E (MMAE), a tubulin polymerization inhibitor (anti-CAD-ADC), is effective for CADM1-expressing human mesothelioma cells in vitro (Hagiyama et al., 2022).

As mentioned above, CADM1 is reported to be significantly expressed in small intestinal GISTs compared to gastric GISTs (Yuan et al., 2021), suggesting that CADM1 may be a potential therapeutic target for small intestinal GISTs. Therefore, in the present study, we investigated whether the previously reported anti-CAD-ADC is effective against CADM1-expressing GIST cells. Effect of the anti-CAD-ADC was compared between GIST-T1 cells which scarcely expresses CADM1 and GIST-T1-CAD cells which expresses CADM1 (Yuan et al., 2021; Yuan et al., 2022) by in vitro and in vivo experiments.

## 2. Materials and methods

### 2.1. Cells

GIST-T1 cell line established from metastatic pleural tumor of primary gastric GIST in a Japanese woman was purchased from CosmoBio (Tokyo, Japan #GIST01C). It harbors a heterozygous *KIT* exon 11 mutation leading to an in-frame deletion of 19 amino acids from Val560 to Tyr578 in the KIT receptor. The cell line is confirmed negative for mycoplasma, HBV, HCV, HIV, HTLV, and syphilis by the manufacturer. GIST-T1-CAD cells were previously established by transfection with full length human CADM1 cDNA into GIST-T1 cells using an Amaxa Nucleofector II machine (program T-030) (Lonza, Basel, Switzerland)

(Yuan et al., 2022). pGL4.51 [luc2/CMV/Neo] vector (Promega, Madison, WI, #E1320) was transfected into GIST-T1-CAD cells to produce stably luciferase-expressing cells (GIST-T1-CAD-Luc cells). These cells were cultured in Dulbecco's minimum essential medium (DMEM, Sigma-Aldrich; Merck KGaA, St. Louis, MO, #12491015) supplemented with 10% fetal bovine serum (FBS) (Biowest, Nuaille, France, #S1400–500), 100 U/ml of penicillin G and 100  $\mu$ g/ml of streptomycin (Invitrogen; Thermo Fisher Scientific, Waltham, MA, #15140–122) at 37 °C in 5% CO<sub>2</sub>. Histopathological examination of GIST-T1, GIST-T1-CAD and GIST-T1-CAD-Luc cells in the tumors formed by subcutaneous and intraperitoneal injection without anti-CAD-ADC similarly showed atypical nuclei with multinucleated giant cells (Supplementary Fig. 1) and high proliferative indices (data not shown). All of the tumors strongly expressed KIT and DOG1, and CADM1 was expressed in the tumors of GIST-T1-CAD and GIST-T1-CAD-Luc cells but not apparently in the tumors of GIST-T1 (Supplementary Fig. 1). Imatinib sensitivity was retained in GIST-T1-CAD and GIST-T1-CAD-Luc cells like in GIST-T1 cells (data not shown). All of the experiments using recombinant DNA were approved by the committee for Recombinant DNA Experiments of Hyogo Medical University (No. 24015).

### 2.2. Antibody-drug conjugates

The preparation of anti-CAD-ADC used in cell culture experiments was described previously (Hagiyama et al., 2022). Briefly, the chicken monoclonal anti-CADM1 ectodomain (3E1) was humanized into human IgG4 (named h3E1) (Hagiyama et al., 2022), and then conjugated with maleimidocaproyl-valine-citrulline-p-amino-benzyl linker and a MMAE payload (mc-vc-PAB-MMAE) (Hagiyama et al., 2022). For the control ADC, CADM1-nonrelated human IgG4 antibody was conjugated with mc-vc PAB-MMAE (Hagiyama et al., 2022). For ADC administration to mice, 3E1 was modified into a chicken-mouse chimera antibody, in which the 3E1 original Fab portion was fused with mouse IgG1 Tc portion. The resulting chimera antibody was conjugated with mc-vc-PAB-MMAE according to the methods described previously (Hagiyama et al., 2022). As a control for this ADC, CADM1-nonrelated mouse IgG1 antibody was conjugated with mc-vc-PAB-MMAE.

### 2.3. Cell viability assay

GIST-T1 cells or GIST-T1-CAD cells ( $2.5 \times 10^3$  cells) were seeded onto 96-well plates (Corning, Corning, NY, #3595). Three days later, anti-CAD-ADC or control ADC was added to each well in the indicated concentrations. The plates were incubated at 37 °C for three days and cell viability was evaluated using cell counting kit 8 (Dojindo Laboratories, Kumamoto, Japan, #343–07623).

### 2.4. Animals, experimental group design, and ethics committee approval

A total of 30 female BALB/c nude mice (4-week-old female) were purchased from Japan SLC (Hamamatsu, Japan) and a total of 20 female severe combined immunodeficiency (SCID) mice (4-week-old female) were purchased from CLEA Japan (Tokyo, Japan). Mice were kept in cages under specific pathogen free conditions (temperature; 23 °C, humidity; 50%) and exposed to a 12-h light/dark cycle. Sterilized water and standard food were offered ad libitum. In whole-body IVIS (in vivo imaging system), mice were fluorescently imaged under 1–2% isoflurane inhalation. Humane endpoints were defined as tumor volume >2000 mm<sup>3</sup> or apparent physical activity deterioration. Mice were euthanized by cervical dislocation.

To create a mouse subcutaneous injection model,  $1 \times 10^7$  GIST-T1 cells or GIST-T1-CAD cells in 200  $\mu$ l of phosphate buffered saline (PBS) were inoculated subcutaneously into nude mice. The volume of tumor formed subcutaneously was measured twice a week using a caliper before treatment with anti-CAD-ADC or PBS. The following formula was adopted: tumor volume = length  $\times$  (width)<sup>2</sup>  $\times$  1/2, where

length is the longitudinal axis direction and width is the short axis direction. When the tumor volume exceeded 100 mm<sup>3</sup>, anti-CAD-ADC of 5 mg/kg body weight (BW) ( $n = 5$ ), that of 10 mg/kg BW ( $n = 5$ ), or PBS ( $n = 5$ ) was intravenously injected once from tail vein. Tumor volume was measured twice a week after the injection.

To create a peritoneal seeding mouse model,  $2 \times 10^7$  GIST-T1-CAD-Luc cells in 200  $\mu$ l of PBS were seeded into the peritoneal cavity of SCID mice. The bioluminescence signal (photons/s) of abdominal area of mice after intraperitoneal injection of luciferin (150 mg/kg BW) was measured in 15 min using IVIS lumina II (Caliper Life Sciences, Hopkinton, MA). When the signal of mice became  $>1 \times 10^5$  photons/s (range:  $1.0\text{--}1.5 \times 10^5$  photons/s), anti-CAD-ADC (5 mg/kg BW,  $n = 5$ ), anti-CAD-ADC (10 mg/kg BW,  $n = 5$ ), control ADC (5 mg/kg BW,  $n = 5$ ), or control ADC (10 mg/kg BW,  $n = 5$ ) was intraperitoneally administered once. Measurement of bioluminescence signal was performed once a week before anti-CAD ADC or PBS treatment and once every week after the treatment.

Residual subcutaneous and intraperitoneal tumors after single administration of anti-CAD-ADC were resected and examined histopathologically and immunohistochemically.

Animal experiment protocols were approved by the Hyogo Medical University Animal Experiment Committee (No. 20-063), and all animal experiments were conducted according to the institutional ethical guidelines in Hyogo Medical University.

## 2.5. Histopathological evaluation of influence of anti-CAD-ADC to murine organs and tissues other than tumors

The organ damage of anti-CAD-ADC was examined in a mouse subcutaneous injection model (5 mg/kg BW and 10 mg/kg BW). From each mouse, the cerebrum, cerebellum, lung, heart, liver, adrenal gland, kidney, spine, and spinal cord were removed. Tissues were fixed with 10% neutral buffered formalin, embedded in paraffin, and cut 3  $\mu$ m thick. Hematoxylin and eosin specimens were prepared and examined under a light microscope.

## 2.6. Statistical analysis

Cell viability assay was repeated at least three times. GraphPad Prism 9.4.1 software (GraphPad Software, Inc., Boston, MA) was used for all experimental data, and the results were presented as the mean  $\pm$  SD. Statistical analysis was performed using two tailed  $t$ -test with Welch's  $t$ -test.  $p < 0.05$  was considered to indicate a statistically significant

difference.

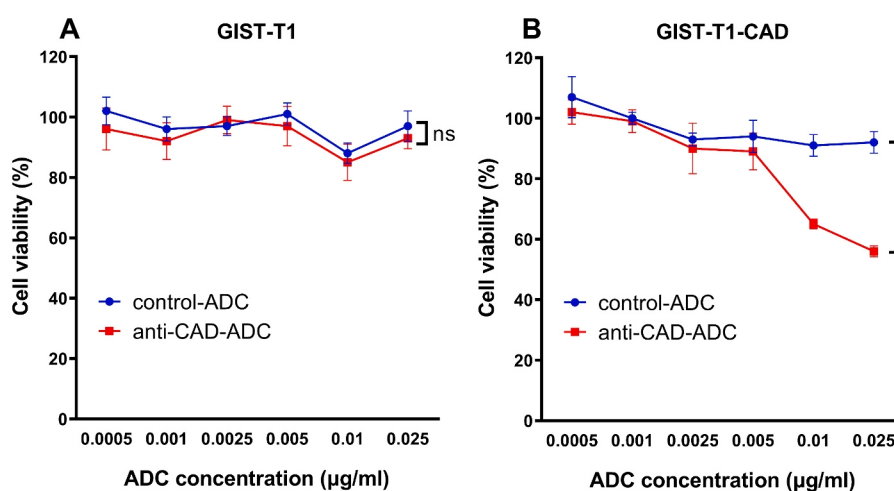
## 3. Results

### 3.1. Anti-CAD-ADC shows cytotoxic activity on GIST-T1-CAD cells in vitro

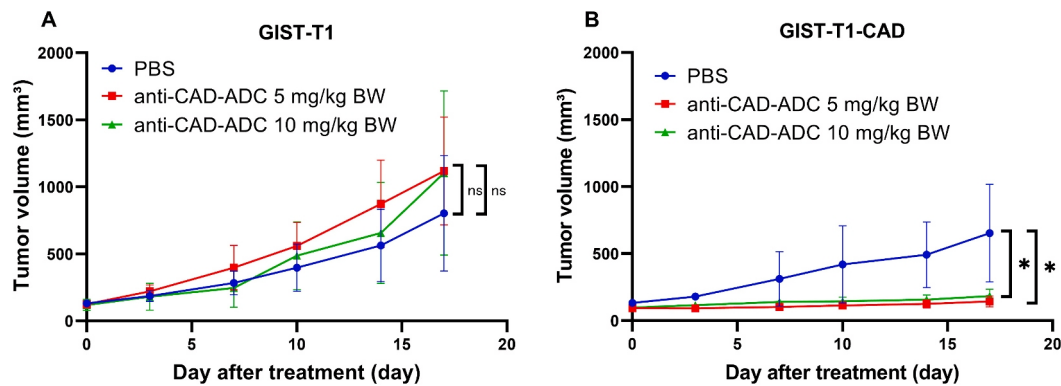
Effect of anti-CAD-ADC and control ADC on original GIST-T1 and GIST-T1-CAD cells was evaluated at 3 days after the ADC addition in vitro. Both anti-CAD-ADC and control ADC showed no significant cytotoxic activity at any concentration examined on GIST-T1 cells (Fig. 1A). On the other hand, anti-CAD-ADC inhibited growth of GIST-T1-CAD cells at concentrations of 0.025 and 0.01  $\mu$ g/ml dose-dependently, whereas control ADC did not inhibit growth of them at any concentration examined (Fig. 1B).

### 3.2. Anti-CAD-ADC inhibits tumor growth of GIST-T1-CAD cells in the mouse subcutaneous injection model

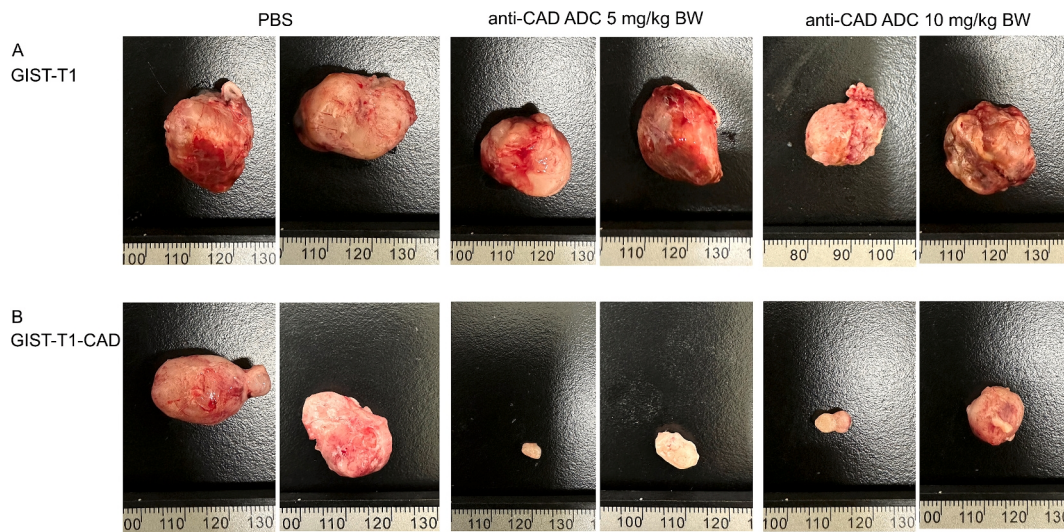
To evaluate the anti-tumor effect of anti-CAD-ADC in vivo, GIST-T1 and GIST-T1 CAD cells were injected subcutaneously in nude mice. After forming tumor over 100 mm<sup>3</sup> volume, anti-CAD-ADC (5 mg/kg BW or 10 mg/kg BW) or PBS was administered. Tumors of GIST-T1 cells grew constantly even after the ADC and PBS treatments (Fig. 2A), and the tumor volume of GIST-T1 cells was not significantly different among anti-CAD-ADC (5 mg/kg BW), anti-CAD-ADC (10 mg/kg BW) and PBS treatment groups (Fig. 2A). Tumors of GIST-T1-CAD cells grew after PBS treatment. In contrast, tumor of GIST-T1-CAD cells did not grow after anti-CAD-ADC (5 mg/kg BW) and anti-CAD-ADC (10 mg/kg BW) treatments. Tumor volume of GIST-T1-CAD cells administered anti-CAD-ADC (5 mg/kg BW or 10 mg/kg BW) was significantly smaller than that administered PBS (Fig. 2B). Five mg/kg BW administration and 10 mg/kg BW administration of anti-CAD-ADC showed similar results on tumor growth inhibition of GIST-T1-CAD cells (Fig. 2B). Macroscopic images of the representative tumors removed of GIST-T1 cells and GIST-T1-CAD cells at 17 days after PBS and anti-CAD-ADC (5 mg/kg BW and 10 mg/kg BW) administration were shown (Fig. 3A and B). Tumors of GIST-T1 cells at 17 days after PBS and anti-CAD-ADC (5 mg/kg BW and 10 mg/kg BW) administration were similar in size (Fig. 3A). Tumor size of GIST-T1-CAD cells was apparently smaller in anti-CAD-ADC (5 mg/kg BW and 10 mg/kg BW) groups than in PBS group (Fig. 3B).



**Fig. 1.** Humanized anti-CAD-ADC exhibits cytotoxic activity on GIST-T1-CAD cells in vitro. (A) Both anti-CAD-ADC and control ADC did not show anti-tumor effect to GIST-T1 cells. (B) Control ADC did not show cytotoxic activity to GIST-T1-CAD cells but anti-CAD-ADC did.  $P$ -values were calculated using GraphPad Prism. The two-tailed Welch's  $t$ -test was used to determine statistical difference between groups. The data are expressed as the mean  $\pm$  SD ( $n = 5$ /each group). \* $p < 0.05$ ; ns, not significant.



**Fig. 2.** Anti-CAD-ADC inhibits tumor growth of GIST-T1-CAD cells in subcutaneously injected nude mouse model. (A) Tumor growth of GIST-T1 cells did not inhibit after anti-CAD-ADC (5 mg/kg BW or 10 mg/kg BW) or PBS treatment, suggesting that anti-CAD-ADC did not show anti-tumor effect to GIST-T1 cells. (B) Tumor growth of GIST-T1-CAD cells did not inhibit after PBS treatment, but anti-CAD-ADC (5 mg/kg BW and 10 mg/kg BW) did, suggesting that anti-CAD-ADC showed anti-tumor effect to GIST-T1-CAD cells. P-values were calculated using GraphPad Prism. The two-tailed Welch's t-test was used to determine statistical difference between groups. The data are expressed as the mean  $\pm$  SD ( $n = 5$ /each group). \* $p < 0.05$ ; ns, not significant.



**Fig. 3.** Macroscopic views of subcutaneous tumors of GIST-T1-CAD cells and GIST-T1 cells after anti-CAD-ADC treatment are shown. (A) Macroscopic subcutaneous tumors of GIST-T1 cells after PBS treatment or anti-CAD-ADC (5 mg/kg BW or 10 mg/kg BW) treatment were similarly large in size. (B) Subcutaneous tumors of GIST-T1-CAD cells after PBS treatment were large, but those after anti-CAD-ADC (5 mg/kg BW or 10 mg/kg BW) treatment were apparently small. Representative specimens resected 17 days after PBS or anti-CAD-ADC treatment were shown in each group.

### 3.3. Anti-CAD-ADC reduces tumor growth of GIST-T1-CAD-Luc cells in the mouse model of peritoneal dissemination

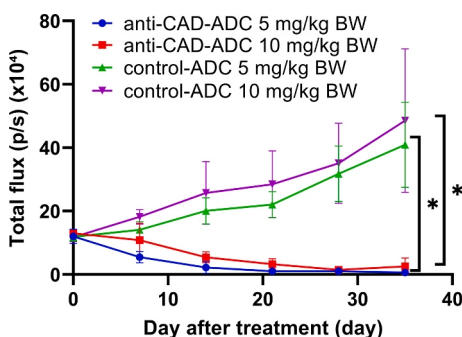
The anti-tumor effect of anti-CAD-ADC and control ADC was examined in a peritoneal seeding model of GIST-T1-CAD-Luc cells. After GIST-T1-CAD-Luc cells were injected to peritoneal cavity of SCID mice and the bioluminescence intensity reached over  $1 \times 10^5$  photons/s, administration of control ADC (5 mg/kg BW or 10 mg/kg BW) or anti-CAD-ADC (5 mg/kg BW or 10 mg/kg BW) was done. Thereafter, bioluminescence intensity of the abdominal area of the mice was measured once a week. SCID mice treated with control ADC (5 mg/kg BW or 10 mg/kg BW) showed gradual enhancement of bioluminescence intensity (Fig. 4). On the other hand, bioluminescence intensity reduced in 1 week after administration of the anti-CAD-ADC (5 mg/kg BW or 10 mg/kg BW), and it was barely detectable after 5 weeks (Fig. 4). The bioluminescence images of each mouse are shown (Fig. 5).

### 3.4. Histopathological examination of residual GIST-T1-CAD and GIST-T1-CAD-Luc cells after administration of anti-CAD-ADC shows retention of CADM1 expression

Tumors formed by subcutaneous injection of GIST-T1-CAD cells and by peritoneal seeding of GIST-T1-CAD-Luc cells were histopathologically examined after single administration of anti-CAD-ADC. Both of the residual tumors contained viable tumor cells without apparent scar tissue, and the cells showed atypical nuclei with multinucleated giant cells like the tumors without anti-CAD-ADC administration (Fig. 6). Expression of KIT, DOG1 and CADM1 was retained in the residual tumors of GIST-T1-CAD and GIST-T1-CAD-Luc cells even after single administration of anti-CAD-ADC (Fig. 6).

### 3.5. Anti-CAD-ADC shows no influence on mouse organs and tissues

Each organ (lung, heart, liver, pancreas, spleen, kidney, adrenal gland, cerebellum, bone marrow) was removed and macroscopically and histologically examined. There was no apparent macroscopic abnormality detected. Histological examination of the resected



**Fig. 4.** Anti-CAD-ADC reduces tumor growth of GIST-T1-CAD cells in intra-peritoneally injected SCID mouse model. Bioluminescence activity constantly increased in both control ADC (5 mg/kg BW) and control ADC (10 mg/kg BW) groups, suggesting that control ADC did not show anti-tumor effect to GIST-T1-CAD cells. On the other hand, bioluminescence activity decreased similarly in both anti-CAD-ADC (5 mg/kg BW) and anti-CAD-ADC (10 mg/kg BW) groups, suggesting that anti-CAD-ADC showed similar anti-tumor effect to GIST-T1-CAD cells. P-values were calculated using GraphPad Prism. The two-tailed Welch's t-test was used to determine statistical difference between groups. The data are expressed as the mean  $\pm$  SD ( $n = 5$ /each group). \* $p < 0.05$ .

organs showed no obvious abnormal change (Fig. 7).

#### 4. Discussion

For the treatment of RTK-resistant GISTs, therapeutic ways based on mechanisms other than RTK inhibition are considered to be promising. Since most of small intestinal GISTs show high level of CADM1 expression (Yuan et al., 2021), we supposed that CADM1 may be a good target for small intestinal GISTs when the ADC system is used. We investigated the efficacy of anti-CAD-ADC, which have a specific binding to CADM1 and was previously reported to be effective for CADM1-expressing mesothelioma cells (Hagiya et al., 2022), against CADM1-expressing human GIST cells. Since there is no available cell line for small intestinal GISTs which has been proved to express abundant CADM1, we used GIST-T1-CAD cells, which is transfected with full length of human CADM1 cDNA into GIST-T1 cell line which were derived from the stomach and scarcely express CADM1 (Yuan et al., 2021). We consider

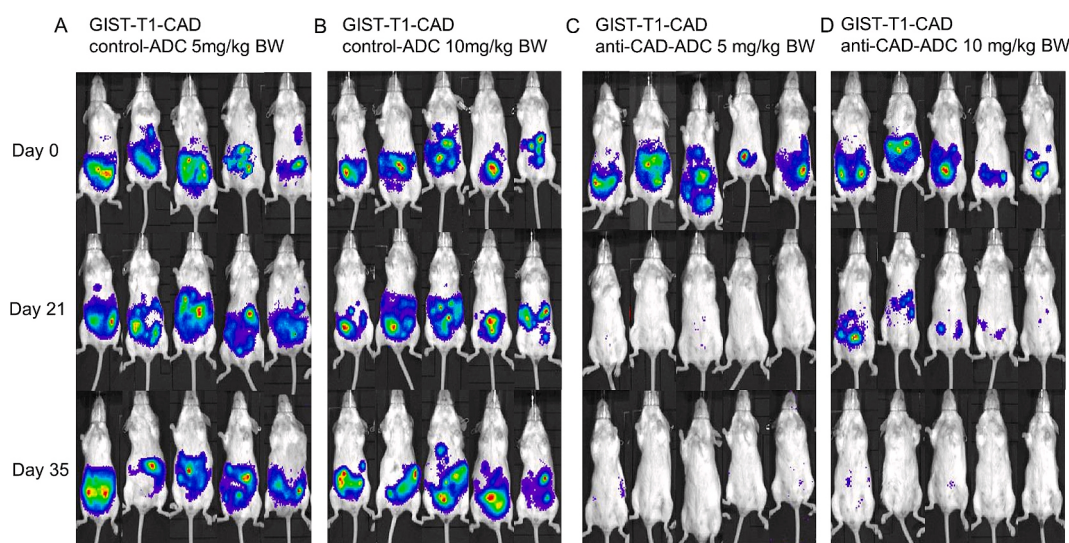
that GIST-T1-CAD cells represents CADM1-expressing small intestinal GIST. Although the anti-CAD-ADC did not show anti-tumor effect to original GIST-T1 cells and control ADC did not show anti-tumor effect to GIST-T1-CAD cells, the anti-CAD-ADC specifically showed prominent anti-tumor effect to highly CADM1-expressing GIST-T1-CAD cells in vitro and in vivo.

In the current study, anti-tumor effect of anti-CAD-ADC was not significantly different between 5 mg/kg BW administration and 10 mg/kg BW administration in the subcutaneously implanted and peritoneally seeded mouse models. The result might indicate that 5 mg/kg BW administration is enough to be administrated in mice. Anti-CAD-ADC did not show any macroscopic and microscopic abnormal findings in model mice. Since humans and mice have different biological dynamics, it has to be clarified whether the anti-CAD-ADC is less toxic and how much dose is effective enough to control proliferation of CADM1-expressing GISTs in human.

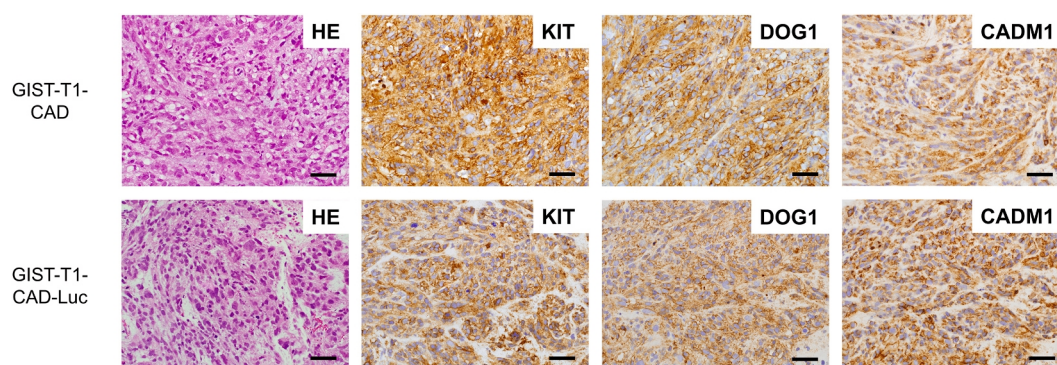
We used two injection mouse models, i.e. subcutaneous inoculation model and peritoneal seeding model using GIST-T1 cells, GIST-T1-CAD cells and GIST-T1-CAD-Luc cells. In clinical situation, GISTs often show liver metastasis and peritoneal dissemination, and metastasis to the skin is very rare. Peritoneal seeding model resembles peritoneal dissemination in GIST patients, and it may be more realistic than the subcutaneous inoculation model.

The anti-CAD-ADC used in the present study was administered only once in vivo studies, but anti-tumor effect continued for a considerable period of time. Skin tumors which was formed by subcutaneous injection of GIST-T1-CAD cells did not grow for at least 2 weeks, and peritoneal tumors which was formed by peritoneal injection of GIST-T1-CAD cells diminished and did not regrow for at least 5 weeks. We have to examine how long the anti-tumor effect of the single administration of anti-CAD-ADC continues. Residual tumor cells were viable with high mitotic figures and high Ki-67 proliferative indices. The tumor may be just in the process of regrowth after initial control by the single ADC administration. Since CADM1 expression was retained after the single ADC administration, we expected that the second administration of ADC may inhibit proliferation of residual tumors again. We have to clarify whether the second administration of the ADC can control tumor growth again if the tumors enlarge later.

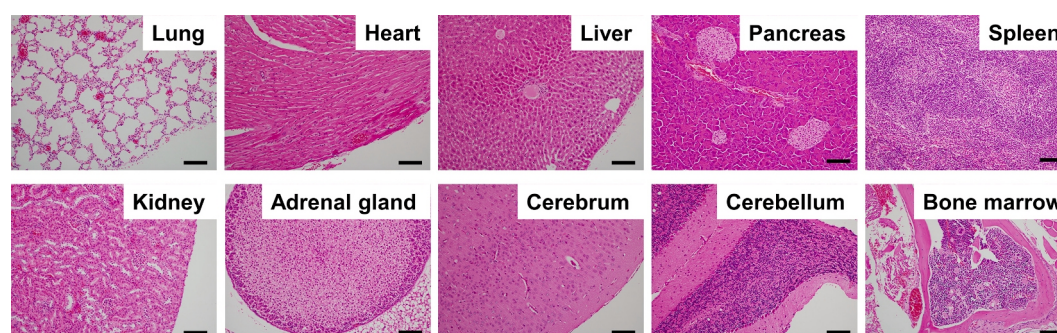
In the present study, we examined the anti-tumor effect of the anti-CAD-ADC for GIST-T1-CAD cells which are CADM1-expressing GIST



**Fig. 5.** Bioluminescence images of peritoneal tumors of GIST-T1-CAD cells and those of GIST-T1 cells are shown before and after anti-CAD-ADC treatment. (A) Bioluminescence intensity was not reduced in control ADC (5 mg/kg BW) treatment. (B) It was not also reduced in control ADC (10 mg/kg BW) treatment. (C) Bioluminescence intensity decreased and was not apparent 21 and 35 days after anti-CAD-ADC (5 mg/kg BW) treatment. (D) It decreased, and weak signals were seen 21 days after anti-CAD-ADC (10 mg/kg BW) treatment. However, it was not apparent 35 days after anti-CAD-ADC (10 mg/kg BW) treatment. Data at 0, 21 and 35 days of all mice are shown.



**Fig. 6.** Histopathological examination of residual GIST-T1-CAD and GIST-T1-CAD-Luc cells after administration of anti-CAD-ADC shows retention of KIT, DOG1 and CADM1 expression. Tumors formed by subcutaneous injection of GIST-T1-CAD cells and by peritoneal seeding of GIST-T1-CAD-Luc cells after the single administration of anti-CAD-ADC contained viable tumor cells without apparent scar tissue, and the cells showed atypical nuclei with multinucleated giant cells. The tumors of both GIST-T1-CAD and GIST-T1-CAD-Luc cells retained expression of KIT, DOG1 and CADM1. Original magnification,  $\times 400$ . Scale bar = 50  $\mu\text{m}$ .



**Fig. 7.** Representative microscopic images of various organs which were resected from subcutaneously injected mice with anti-CAD-ADC (5 or 10 mg/kg BW) treatment are shown. Lung, heart, liver, spleen, kidney, adrenal gland, cerebrum, cerebellum and bone marrow did not show any apparent abnormal changes, suggesting that anti-CAD-ADC did not influence mouse organs and tissues. Original magnification,  $\times 200$ . Scale bar = 100  $\mu\text{m}$ .

cells with imatinib-sensitive *KIT* mutation and showed significant anti-tumor effect to them. Since imatinib is established to be the first line drug for unresectable and recurrent GISTs, we need new drugs for imatinib-resistant GISTs. We have to clarify whether the anti-CAD-ADC has similar anti-tumor effect for imatinib-resistant GIST cells such as GISTs with the imatinib-resistant second *KIT* mutation.

## 5. Conclusion

The RTK inhibitors such as imatinib, sunitinib and regorafenib target the mutant *KIT* and *PDGFR* which are directly involved in GIST development and growth. ADC targeting *KIT* has been previously reported (Kim et al., 2022). Although *CADM1* is not a molecule that might be directly involved in the major pathways of GIST development and growth, anti-CAD-ADC showed significant effect in *CADM1*-expressing GIST cells. The results show that antigens specifically expressed on GIST cells could have the potential to be targeted. The theory might broaden the therapeutic spectrum in GISTs. In summary, we consider that the anti-CAD-ADC used in the present study may be a promising agent for *CADM1*-expressing GISTs such as small intestinal ones.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.yexmp.2024.104922>.

## Funding

This work was supported by Pharma Foods International Co., Ltd. (Kyoto, Japan), and JSPS KAKENHI Grant Numbers JP20K07434 and JP21K06978.

## CRediT authorship contribution statement

**Makoto Yoshida:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jiayin Yuan:** Investigation, Data curation. **Takako Kihara:** Writing – review & editing, Validation, Formal analysis. **Neinei Kimura:** Investigation. **Takashi Yamasaki:** Investigation. **Mizuka Ohkouchi:** Investigation. **Yuka Hashikura:** Investigation. **Koji Isozaki:** Methodology, Conceptualization. **Man Hagiya:** Resources, Investigation. **Akihiko Ito:** Validation, Resources, Funding acquisition. **Seiichi Hirota:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare no competing interests.

## Data availability

Data will be made available on request.

## Acknowledgements

Not applicable.

## References

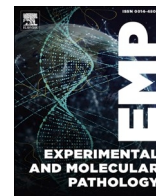
- Allinen, M., Peri, L., Kujala, S., Domenici, J.L., Outila, K., Karppinen, S.M., et al., 2002. Analysis of 11q21-24 loss of heterozygosity candidate target genes in breast cancer.

- Indications of TSLC1 promoter hypermethylation. *Genes Chromosom. Cancer* 34, 384–389. <https://doi.org/10.1002/gcc.10079>.
- Biederer, T., Sara, Y., Mozhayeva, M., Atasoy, D., Liu, X., Kavalai, E.T., et al., 2002. SynCAM, a synaptic adhesion molecule that drives synapse assembly. *Science* 297, 1525–1531. <https://doi.org/10.1126/science.1072356>.
- Demetri, G.D., Van-Oosterom, A.T., Garrett, C.R., Blackstein, M.E., Shah, M.H., Verweij, J., et al., 2006. Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: A randomised controlled trial. *Lancet* 368, 1329–1338. [https://doi.org/10.1016/S0140-6736\(06\)69446-4](https://doi.org/10.1016/S0140-6736(06)69446-4).
- Demetri, G.D., Reichardt, P., Kang, Y.K., Blay, J.Y., Rutkowski, P., Gelderblom, H., et al., 2013. Efficacy and safety of regorafenib for advanced gastrointestinal stromal tumours after failure of imatinib and sunitinib (GRID): An international, multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet* 381, 295–302. [https://doi.org/10.1016/S0140-6736\(12\)61857-1](https://doi.org/10.1016/S0140-6736(12)61857-1).
- Dewan, M.Z., Takamatsu, N., Hidaka, T., Hatakeyama, K., Nakahata, S., Fujisawa, J.I., et al., 2008. Critical role for TSLC1 expression in the growth and organ infiltration of adult T-cell leukemia cells in vivo. *J. Virol.* 82, 11958–11963. <https://doi.org/10.1128/JVI.01149-08>.
- Fisser, M.C., Rommer, A., Steinleitner, K., Heller, G., Herbst, F., Wiese, M., et al., 2015. Induction of the proapoptotic tumor suppressor gene cell adhesion molecule 1 by chemotherapeutic agents is repressed in therapy resistant acute myeloid leukemia. *Mol. Carcinog.* 54, 1815–1819. <https://doi.org/10.1002/mc.22252>.
- Funaki, T., Ito, T., Tanei, Z.I., Goto, A., Niki, T., Matsubara, D., et al., 2021. CADM1 promotes malignant features of small-cell lung cancer by recruiting 4.1R to the plasma membrane. *Biochem. Biophys. Res. Commun.* 534, 172–178. <https://doi.org/10.1016/j.bbrc.2020.11.121>.
- Hagiyama, M., Mimae, T., Wada, A., Takeuchi, F., Yoneshige, A., Inoue, T., et al., 2022. Possible therapeutic utility of anti-cell adhesion molecule 1 antibodies for malignant pleural mesothelioma. *Front. Cell Dev. Biol.* 10, 945007. <https://doi.org/10.3389/fcell.2022.945007>.
- Heinrich, M.C., Corless, C.L., Duensing, A., McGreevey, L., Chen, C.J., Joseph, N., et al., 2003. PDGFRA activating mutations in gastrointestinal stromal tumors. *Science* 299, 708–710. <https://doi.org/10.1126/science.1079666>.
- Hirota, S., Isozaki, K., Moriyama, Y., Hashimoto, K., Nishida, T., Ishiguro, S., et al., 1998. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science* 279, 577–580. <https://doi.org/10.1126/science.279.5350.577>.
- Hirota, S., Ohashi, A., Nishida, T., Isozaki, K., Kinoshita, K., Shinomura, Y., et al., 2003. Gain-of-function mutations of platelet-derived growth factor receptor alpha gene in gastrointestinal stromal tumors. *Gastroenterology* 125, 660–667. [https://doi.org/10.1016/S0016-5085\(03\)01046-1](https://doi.org/10.1016/S0016-5085(03)01046-1).
- Honda, T., Tamura, G., Waki, T., Jin, Z., Sato, K., Motoyama, T., et al., 2002. Hypermethylation of the TSLC1 gene promoter in primary gastric cancers and gastric cancer cell line. *Jpn. J. Cancer Res.* 93, 857–860. <https://doi.org/10.1111/j.1349-7006.2002.tb01329.x>.
- Ito, A., Oonuma, J., 2006. Direct interaction between nerves and mast cells mediated by the SgIGSF/SynCAM adhesion molecule. *J. Pharmacol. Sci.* 102, 1–5. <https://doi.org/10.1254/jphs.cpj06014x>.
- Ito, A., Okada, M., Uchino, K., Wakayama, T., Koma, Y., Iseki, S., et al., 2003. Expression of the TSLC1 adhesion molecule in pulmonary epithelium and its down regulation in pulmonary adenocarcinoma other than bronchioloalveolar carcinoma. *Lab. Invest.* 83, 1175–1183. <https://doi.org/10.1097/01.lab.0000081391.28136.80>.
- Ito, A., Nishikawa, Y., Ohnuma, K., Ohnuma, I., Koma, Y., Sato, A., et al., 2007. SgIGSF is a novel biliary-epithelial cell adhesion molecule mediating duct/ductule development. *Hepatology* 45, 684–694. <https://doi.org/10.1002/hep.21501>.
- Kim, J.O., Kim, K.H., Baek, E.J., Park, B., So, M.K., Ko, B.J., et al., 2022. A novel anti-c-kit antibody-drug conjugate to treat wild type and activating-mutant c-kit positive tumors. *Mol. Oncol.* 16, 1290–1308. <https://doi.org/10.1002/1878-0261.13084>.
- Koma, Y., Furuno, T., Hagiyama, M., Hamaguchi, K., Nakanishi, M., Masuda, M., et al., 2008. Cell adhesion molecule 1 is a novel pancreatic-islet cell adhesion molecule that mediates nerve-islet cell interactions. *Gastroenterology* 134, 1544–1554. <https://doi.org/10.1053/j.gastro.2008.01.081>.
- Kuramochi, M., Fukuhara, H., Nobukuni, T., Kanbe, T., Maruyama, T., Ghosh, H.P., et al., 2001. TSLC1 is a tumor-suppressor gene in human non-small-cell lung cancer. *Nat. Genet.* 27, 427–430. <https://doi.org/10.1038/86934>.
- Miettinen, M., Lasota, J., 2001. Gastrointestinal stromal tumors-definition, clinical, histological, immunohistochemical, and molecular genetic features and differential diagnosis. *Virchows Arch.* 438, 1–12. <https://doi.org/10.1007/s004280000338>.
- Miettinen, M., Lasota, J., 2006. Gastrointestinal stromal tumors: Pathology and prognosis at different sites. *Semin. Diagn. Pathol.* 23, 70–83. <https://doi.org/10.1053/j.semdp.2006.09.001>.
- Moiseeva, E.P., Roach, K.M., Leyland, M.L., Bradding, P., 2013. CADM1 is a key receptor mediating human mast cell adhesion to human lung fibroblasts and airway smooth muscle cells. *PLoS One* 8, e61579. <https://doi.org/10.1371/journal.pone.0061579>.
- Yuan, J., Kihara, T., Kimura, N., Hashikura, Y., Ohkouchi, M., Isozaki, K., et al., 2021. Differential expression of CADM1 in gastrointestinal stromal tumors of different sites and with different gene abnormalities. *Pathol. Oncol. Res.* 27, 602008. <https://doi.org/10.3389/pore.2021.602008>.
- Yuan, J., Kihara, T., Kimura, N., Yamasaki, T., Yoshida, M., Isozaki, K., et al., 2022. CADM1 promotes adhesion to vascular endothelial cells and transendothelial migration in cultured GIST cells. *Oncol. Lett.* 23, 86. <https://doi.org/10.3892/ol.2022.13206>.
- Zeng, D., Wu, X., Zheng, J., Zhuang, Y., Chen, J., Hong, C., et al., 2016. Loss of CADM1/TSLC1 expression is associated with poor clinical outcome in patients with esophageal squamous cell carcinoma. *Gastroenterol. Res. Pract.* 2016, 6947623. <https://doi.org/10.1155/2016/6947623>.



Contents lists available at ScienceDirect

## Experimental and Molecular Pathology

journal homepage: [www.elsevier.com/locate/yexmp](http://www.elsevier.com/locate/yexmp)

## Corrigendum to “Anti-tumor effect of antibody-drug conjugate targeting cell adhesion molecule 1 on GIST cells representing small intestinal GIST” [Experimental and Molecular Pathology 139 (2024) 104922]

Makoto Yoshida<sup>a</sup>, Jiayin Yuan<sup>b</sup>, Takako Kihara<sup>a</sup>, Neinei Kimura<sup>a</sup>, Takashi Yamasaki<sup>a</sup>, Mizuka Ohkouchi<sup>a</sup>, Yuka Hashikura<sup>a</sup>, Koji Isozaki<sup>a</sup>, Man Hagiya<sup>c</sup>, Akihiko Ito<sup>c,\*</sup>, Seiichi Hirota<sup>a,\*</sup>

<sup>a</sup> Department of Surgical Pathology, Hyogo Medical University School of Medicine, Nishinomiya, Hyogo, Japan

<sup>b</sup> Department of Pathology, First People's Hospital of Foshan, Foshan City, Guangdong 528000, China

<sup>c</sup> Department of Pathology, Faculty of Medicine, Kindai University, Ono-Higashi, Osaka-Sayama, Osaka, Japan

In the original article by Makoto Yoshida, et al., entitled “Anti-tumor effect of antibody-drug conjugate targeting cell adhesion molecule 1 on GIST cells representing small intestinal GIST”, the authors regret that the protocol number approved by the Hyogo Medical University Animal Experiment Committee was incorrect. It was described as “No. 20–063”,

but the correct approval number is “No. 22–022AG”.

The authors confirm that all the results and conclusions of the article remain unchanged. The authors would like to apologize for any inconvenience caused.

DOI of original article: <https://doi.org/10.1016/j.yexmp.2024.104922>.

\* Corresponding authors.

E-mail address: [hiros@hyo-med.ac.jp](mailto:hiros@hyo-med.ac.jp) (S. Hirota).

<https://doi.org/10.1016/j.yexmp.2024.104934>

0014-4800/© 2024 The Author(s). Published by Elsevier Inc. All rights are reserved, including those for text and data mining, AI training, and similar technologies.