Long-term survival of a feline with non-T/B large granular lymphocyte lymphoma treated with chemotherapy and activated lymphocyte therapy

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Abstract

Background: Feline large granular lymphocyte lymphoma (LGLL) is a grave prognosis. However, the effectiveness of concurrent treatment with chemotherapy and activated lymphocyte therapy for feline LGLL has not been evaluated.
Case Description: A 7-year-old, castrated male, domestic cat presented with gastrointestinal symptoms and an abdominal mass. A Tru-Cut biopsy of the mass revealed LGLL. The cat responded well to chemotherapy regimens of cyclophosphamide, vincristine, prednisolone, and L-asparaginase. Furthermore, activated lymphocyte therapy was added as an adjuvant treatment. The cat survived 982 days from the first presentation and experienced few adverse events. Necropsy was performed and immunohistochemistry revealed that the neoplastic lymphocytes were CD3$^-$/CD20$^-$ cells. The final diagnosis was non-T/B LGLL.
Conclusion: Minimal physical burden and a good initial response to chemotherapy might have contributed to long-term survival in the present case. Moreover, activated lymphocyte therapy could be performed safely and may be a feasible treatment for feline non-T/B LGLL.
Keywords: Activated lymphocyte therapy, Chemotherapy, Feline, Large granular lymphocyte, Lymphoma.

Introduction

Gastrointestinal lymphoma is a common type of cancer in cats (Sato *et al.*, 2014; Economu *et al.*, 2021). Among this type of lymphoma, the incidence of feline large granular lymphocyte lymphoma (LGLL) has been reported to be 6%–28% (Pohlman *et al.*, 2009; Moore *et al.*, 2012; Chino *et al.*, 2013; Sato *et al.*, 2014; Rau and Burgess, 2017). Feline LGLL is considered to have a more aggressive clinical course and is associated with poor prognosis [median survival time (MST) = 29–60 days] (Krick *et al.*, 2008; Sato *et al.*, 2014; Finotello *et al.*, 2018) compared with high-grade lymphomas (MST = 86 days) (Sato *et al.*, 2014). Although feline LGLL is a relatively common disease, optimal therapy remains unclear.

Activated lymphocyte therapy is an adjuvant cancer treatment that enhances antitumor immunity because of the transfer of autologous-activated cytotoxic CD8$^+$ T lymphocytes, which are stimulated *in vitro* with anti-CD3 antibodies and interleukin-2 (Itoh *et al.*, 2003; Hoshino *et al.*, 2008; Mie *et al.*, 2016; Mitani *et al.*, 2021). This therapy is generally administered in combination with chemotherapy in veterinary medicine (O'Connor and Wilson-Robles, 2014; Bujak *et al.*, 2018). In human and canine cancer patients, concurrent treatment with activated lymphocyte therapy and chemotherapy decreased recurrence risk, improved the quality of life (QOL), or prolonged survival time without serious adverse events (Takayama *et al.*, 2000; O'Connor *et al.*, 2012, 2013; López-Díaz de Cerio *et al.*, 2020; Mitani *et al.*, 2021). Furthermore, activated lymphocyte therapy was reported to be safe and feasible for postoperative QOL maintenance in older feline cancer patients (Maeta *et al.*, 2019). However, there are no reports on the administration of activated lymphocyte therapy for feline LGLL.

Herein, we report a case of a feline with LGLL that underwent concurrent treatment with chemotherapy and activated lymphocyte therapy and had a long-term favorable survival.

Case Details

A 7-year-old, castrated male, domestic cat weighing 4.2 kg was presented with a 1-week history of continuous vomiting, diarrhea, and anorexia (day 1). Clinical examination revealed fever (40.0°C) and a palpable irregular abdominal mass. Hematological (Celltac Alpha; NIHON KOHDEN, Tokyo, Japan) and serum biochemistry (VetTest Chemistry Analyzer; IDEXX, ME) measurements revealed mild leukocytosis [26,500 cells/μl; reference interval (RI) = 5500–19,500 cells/μl] and elevation of hepatic enzyme concentrations [alanine

aminotransferase (ALT) = 370 U/l; RI = 12–130 U/l; aspartate aminotransferase (AST) = 190 U/l; RI = 0–48 U/l]. We conducted Diff-Quik-stained peripheral blood smear microscopy. We observed that segmented neutrophils were predominant in the white blood cells. Additionally, a few band neutrophils and normal morphological lymphocytes were diffused in the smear. We could not observe atypical lymphocytes, such as large or irregular shapes. Therefore, we concluded neutrophilia and no neoplastic cells infiltration in a peripheral blood smear. Feline immunodeficiency virus antibody and feline leukemia virus antigen tests yielded negative results. No abnormalities were observed in urine or fecal specimens. Thoracic radiographs were unremarkable; however, abdominal radiographs revealed an irregularly rounded, opaque, soft tissue mass (4 cm in diameter) in the mid-ventral abdomen. Abdominal ultrasonography (Apron EUB-7000HV; Hitachi Medical, Tokyo, Japan) revealed an irregularly rounded, heterogeneous, hypoechoic abdominal mass (4.25 cm in diameter) that had developed within a mesenteric lymph node. The width of the abdominal mass was measured at the widest diameter and followed up using ultrasonography throughout the treatment period (Fig. 1). A Tru-Cut biopsy was performed on the abdominal mass with the patient under sedation (day 2). The cytology was performed with Wright-Giemsa-stained smear preparations of the Tru-Cut biopsy samples at the hospital, revealing many atypical round cells with chromatin aggregation and irregular nuclear morphology. We clinically diagnosed the patient with lymphoma and began treatment before histopathological diagnosis because of the rapid deterioration of the patient's general condition, which included weight loss and anorexia.

On day 3, we began a combination chemotherapy regimen with cyclophosphamide (Endoxan; Shionogi; 50 mg/m² PO q48h), vincristine (Oncovin; Nippon Kayaku; 0.025 mg/kg IV q1w), prednisolone (Predonine; Shionogi; 2 mg/kg PO q24h) (COP-based chemotherapy), and L-asparaginase (Leunase; Kyowa Kirin; 400 U/kg SC), which is a modified version of the regimen used in a previous study (Simon *et al*., 2008). The dose of prednisolone was tapered 1 week after beginning chemotherapy.

Cytological and histopathological analyses of the Tru-Cut biopsy sample were conducted by a veterinary pathologist (J.K.C.). Cytology revealed numerous round cells with medium-sized nuclei. These cells exhibited moderate to high nucleus/cytoplasm ratios, anisocytosis, and anisokaryosis. The nuclei showed irregular, round, and fine-to-stippled chromatin. The cytoplasm often contained basophilic granules and/or vacuoles. Lymphocytes, neutrophils, and eosinophils were admixed. Histopathologically, severe infiltration by neoplastic lymphocytes with medium-sized, irregularly shaped nuclei and clear cytoplasm was observed; a small number of eosinophils were found admixed with these cells (Fig. 2). Based on these findings, the abdominal mass was diagnosed as LGLL, which had developed within the mesenteric lymph nodes.

We informed the owner that feline LGLL has a grave prognosis, even with chemotherapy. Although it had never been administered for feline LGLL, we explained to the owner that activated lymphocyte therapy is expected to have antitumor efficacy and is considered to be safe based on the reports of this treatment in other species (Takayama *et al*., 2000; O'Connor *et al*., 2012, 2013; Mie *et al*., 2016). After discussion with the owner, activated lymphocyte therapy was administered as an adjuvant therapy in combination with COP-based chemotherapy instead of intensified chemotherapy to avoid excessive patient burden.

On day 53, we began activated lymphocyte therapy using the CD3-activated T lymphocyte culture kit
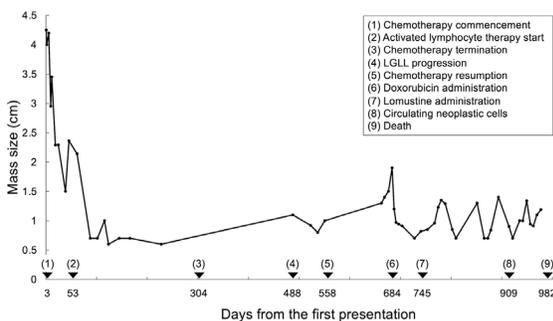
Fig. 1. The diameter of the abdominal mass over time. The widest point of the abdominal mass was measured using ultrasonography during treatment and until death. Each event is indicated by black arrows. LGLL, large granular lymphocyte lymphoma.
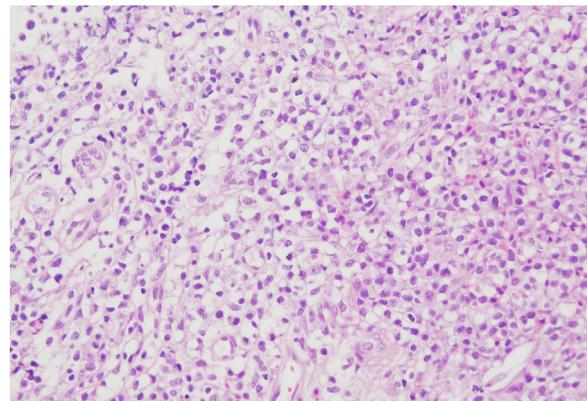
Fig. 2. Histopathology of the Tru-Cut biopsy sample from the abdominal mass. Hematoxylin and eosin staining (200× magnification). Severe infiltration by neoplastic lymphocytes with medium-sized, irregularly shaped nuclei and clear cytoplasm was observed.

(J-ARM Co., Ltd.) as per the manufacturer's protocol. Briefly, peripheral blood mononuclear cells (PBMCs) were isolated using density-gradient centrifugation of 10 ml of venous blood collected from the patient. PBMCs were cultured with a lymphocyte growth medium containing human recombinant IL-2 and 10% autologous plasma in an anti-CD3 antibody-coated flask at 37°C in 5% CO_2 for 2 weeks. The cultured cells collected using centrifugation were then suspended in 50 ml of saline and transfused into the patient intravenously over 30 minutes. Simultaneously, we assessed the number of viable cells, the presence of atypical lymphocytes, and contamination in cultured cell suspension using trypan blue staining. We conducted a total of 28 sessions of activated lymphocyte therapy with a 3–4-week interval until the first 11 sessions were completed. The mean viable cell numbers for each transfusion were 1.45×10^8 cells. There was no contamination or atypical lymphocyte proliferation in any procedures. During the treatments, the patient showed no abnormal signs. Details of the treatment regimens are presented in Table 1. From the initial chemotherapy, the patient's condition improved, and the abdominal mass was reduced to less than 10 mm at the widest diameter. On day 304, we withdrew the COP-based chemotherapy at the owner's request; however, activated lymphocyte therapy was continued with an interval of at least 2 weeks between doses with few side effects. Grade 1–2 AST level elevation and grade 1 creatinine level elevation/neutropenia, diagnosed based on the Veterinary Cooperative Oncology Group-Common Terminology Criteria for Adverse Events (LeBlanc et al., 2021), were observed temporarily during the treatments.

We observed enlargement of the abdominal mass on day 488 and concluded that there was LGLL progression. Fine-needle aspiration (FNA) of the mass was conducted on day 558, and the FNA samples were analyzed using polymerase chain reaction for antigen receptor rearrangements (PARR) (Mochizuki et al., 2011, 2012) and flow cytometry at a commercial laboratory (Canine-lab) using the antibodies listed in Table 2. The cell suspension of the culture medium and patient's blood were also analyzed with flow cytometry. PARR did not detect monoclonal amplification of T cell receptor gamma or immunoglobulin H genes in the FNA samples. Flow cytometry revealed that the aspirate largely comprised non-T/B lymphocytes (96.60%) in the FNA samples and $CD8^+$ T lymphocytes (70.38%) in the culture medium (Table 3).

After LGLL progression, we readministered COP-based chemotherapy with L-asparaginase and continued activated lymphocyte therapy; however, the size of the abdominal mass increased and the patient failed to respond to the treatment. As a rescue protocol, we administered doxorubicin (Adriacin; Kyowa Kirin; 20 mg/m² IV) and lomustine (CeeNU; Bristol-Myers Squibb; 40 mg/m² PO) with the

Table 1. The treatment time course.

Regimens	Week																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Cyclophosphamide (50 mg/m², PO)	•	•	•	•	•	•	•	•	•											
Vincristine (0.025 mg/kg, IV)		•	•	•	•	•	•	•	•			•			•			•		
Prednisolone[a] (PO)		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
L-asparaginase (400 U/kg, SC)	•				•															
Activated lymphocyte therapy								•					•				•			
Lomustine (40 mg/m², PO)																				
Doxorubicin (20 mg/m², IV)										•	•	•		•	•	•		•	•	•

ᵃThe dose was 2 mg/kg for the first week of chemotherapy and then tapered off.

Table 1. The treatment time course (*cont.*).

Regimens										Week										
	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Cyclophosphamide (50 mg/m², PO)	•	•	•	•	•					•	•	•	•	•	•	•	•	•	•	•
Vincristine (0.025 mg/kg, IV)	•			•			•			•			•				•			
Prednisolone[a] (PO)	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
L-asparaginase (400 U/kg, SC)									•											
Activated lymphocyte therapy		•			•			•												
Lomustine (40 mg/m², PO)											•		•					•		•
Doxorubicin (20 mg/m², IV)																				

[a]The dose was 2 mg/kg for the first week of chemotherapy and then tapered off.

Table 1. The treatment time course (*cont.*).

Regimens										Week										
	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Cyclophosphamide (50 mg/m², PO)	•	•	•																	
Vincristine (0.025 mg/kg, IV)	•																			
Prednisolone[a] (PO)	•	•	•																	
L-asparaginase (400 U/kg, SC)																				
Activated lymphocyte therapy		•			•									•						
Lomustine (40 mg/m², PO)																		•		
Doxorubicin (20 mg/m², IV)																				

[a]The dose was 2 mg/kg for the first week of chemotherapy and then tapered off.

Table 1. The treatment time course (*cont.*).

Regimens																				
Week	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
Cyclophosphamide (50 mg/m², PO)																				
Vincristine (0.025 mg/kg, IV)																				
Prednisolonea (PO)																				
L-asparaginase (400 U/kg, SC)																				
Activated lymphocyte therapy		•											•						•	
Lomustine (40 mg/m², PO)																				
Doxorubicin (20 mg/m², IV)																				

[a] The dose was 2 mg/kg for the first week of chemotherapy and then tapered off.

Table 1. The treatment time course (*cont.*).

Regimens																				
Week	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
Cyclophosphamide (50 mg/m², PO)			•	•	•	•	•	•	•	•	•	•	•	•	•	•				
Vincristine (0.025 mg/kg, IV)			•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•		•
Prednisolonea (PO)			•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
L-asparaginase (400 U/kg, SC)		•																•		
Activated lymphocyte therapy		•		•			•			•			•			•			•	
Lomustine (40 mg/m², PO)																				
Doxorubicin (20 mg/m², IV)																	•			

[a] The dose was 2 mg/kg for the first week of chemotherapy and then tapered off.

Table 1. The treatment time course (*cont.*).

Regimens	Week																			
	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120
Cyclophosphamide (50 mg/m², PO)																				
Vincristine (0.025 mg/kg, IV)	•																			
Prednisolonea (PO)		•	•	•	•	•	•	•		•	•	•	•	•	•	•	•	•	•	•
L-asparaginase (400 U/kg, SC)	•													•						
Activated lymphocyte therapy						•			•											
Lomustine (40 mg/m², PO)						•									•				•	
Doxorubicin (20 mg/m², IV)																				

[a]The dose was 2 mg/kg for the first week of chemotherapy and then tapered off.

Table 1. The treatment time course (*cont.*).

Regimens	Week																		
	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139
Cyclophosphamide (50 mg/m², PO)																			
Vincristine (0.025 mg/kg, IV)		•																	
Prednisolonea (PO)	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
L-asparaginase (400 U/kg, SC)		•								•				•		•		•	
Activated lymphocyte therapy															•				
Lomustine (40 mg/m², PO)			•				•				•				•				•
Doxorubicin (20 mg/m², IV)																			

[a]The dose was 2 mg/kg for the first week of chemotherapy and then tapered off.

Table 2. Antibodies used in flow cytometry.

Antibody to	Host	Catalogue number	Source	Specificity
CD3ε	Mouse	ABS-070002	Ab Science	T lymphocytes
CD4	Mouse	8130-01	SouthernBiotech	Helper T lymphocytes
CD8	Mouse	9535-01	SouthernBiotech	Cytotoxic T lymphocytes
CD21	Mouse	MCA1781R	Bio-Rad Laboratories	B lymphocytes
sIgG	Goat	AAI26	Bio-Rad Laboratories	B lymphocytes

(CD3ε): CD3 epsilon; (sIgG): surface immunoglobulin G.

Table 3. Percentage of lymphocyte phenotypes.

Phenotype	Phenotype of lymphocytes (%)		
	Peripheral blood	Cultured medium	Abdominal mass
CD3+	19.19	91.01	2.90
CD4+/CD8−	7.67	4.66	0.69
CD4−/CD8+	7.37	70.38	1.16
CD21+/sIgG+	60.40	1.37	0.33
Others	16.69	8.24	96.60

(sIgG): Surface immunoglobulin G; (CD3+): T lymphocytes; (CD4+/CD8−): CD4+ helper T lymphocytes; (CD4−/CD8+): CD8+ cytotoxic T lymphocytes; (CD21+/sIgG+): B lymphocytes; (others): Non-T/B lymphocytes.

owner's consent on days 684 and 745, respectively. Then, lomustine administration was continued with a 1-month interval between treatments. We discontinued cyclophosphamide treatment after day 684. Temporal grade 1–3 AST level elevation, grade 1 creatinine level elevation, and grade 1 alopecia were observed after the rescue protocol. The patient's condition was stable and the abdominal mass rapidly decreased in size after administration of doxorubicin. However, the abdominal mass size increased subsequently, and we found circulating neoplastic cells in the patient's peripheral blood on day 909. The cat presented with diarrhea, rapidly decreasing appetite, and anorexia beginning on day 972 and died at home on day 982.

A postmortem examination was performed. Cytology was performed by staining with Wright-Giemsa. The visceral organs were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 4-μm thickness, and stained with hematoxylin and eosin for histopathology. Immunohistochemistry was performed using primary antibodies as previously described (Hirabayashi *et al*., 2019). Stamp cytology of the liver, spleen (Fig. 3), axillary lymph nodes, and bone marrow revealed large numbers of round cells with large cytoplasmic granules. Histopathology revealed infiltration of neoplastic lymphocytes in the liver, spleen, heart, ileum (Fig. 4a), and pancreaticoduodenal and axillary lymph nodes. Neoplastic lymphocytes were medium-sized with moderate anisocytosis and anisokaryosis. The nuclear membranes were often irregular, and the nuclei had a distinct nucleolus and fine-to-stippled chromatin. Mitotic count (MC) was 11 per 2.37 mm² [equal to 10 high power fields (HPF) at 400× magnification] in the tumor regions of the ileum

based on a previously established guideline (Meuten *et al*., 2021). Immunohistochemistry revealed abundant granzyme B-positive, cytoplasmic granules in the neoplastic lymphocytes, which were also negative for CD3 and CD20 (Fig. 4b–d).

Ethical approval
All procedures were performed for an owned cat. Informed consent was obtained from the owner and they approved the management of the case and procedures. Therefore, additional ethical approval was not required.

Discussion
We report a case of a feline with LGLL treated with combination treatment with chemotherapy and activated lymphocyte therapy that survived for 982 days after initial presentation.
Flow cytometry, PARR, and immunohistochemistry revealed that the abdominal mass in the present case was LGLL of non-T/B lymphocyte origin, which type of lineage existence is consistent with the findings of previous studies (Roccabianca *et al*., 2006; Pohlman *et al*., 2009). In a previous study, the mean survival time of patients with non-T/B LGLL was 17.5 days (Roccabianca *et al*., 2006). However, several feline LGLL patients survived for over 180 days, suggesting the existence of an LGLL subtype with a good prognosis (Krick *et al*., 2008; Sapierzyński *et al*., 2015; Rau and Burgess, 2017; Finotello *et al*., 2018).
The possible reasons for the long-term favorable survival in the present case are presented below. first, there was minimal impact on organ systems. Retrospective studies reported that poor prognostic factors for feline LGLL were bicavitary involvement and hypoalbuminemia or the presence of symptoms and increased serum
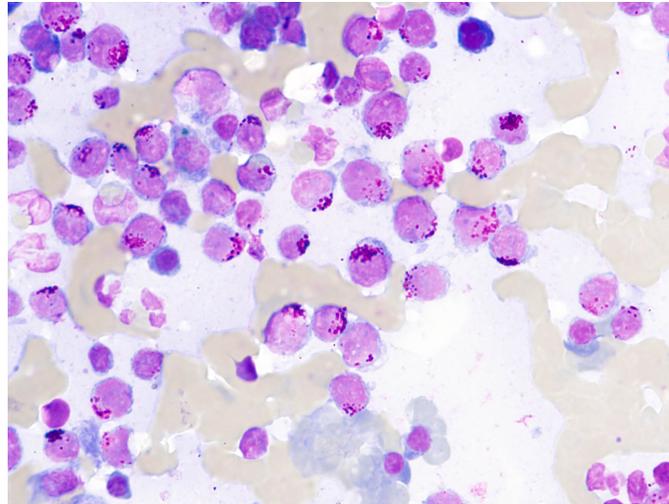
Fig. 3. Infiltration of neoplastic lymphocytes in the spleen. Cytology staining with Wright-Giemsa (1000× magnification). Numerous neoplastic round cells with medium-sized nuclei in the spleen. The cytoplasm contained numerous amphophilic granules (1–2 μm in diameter).
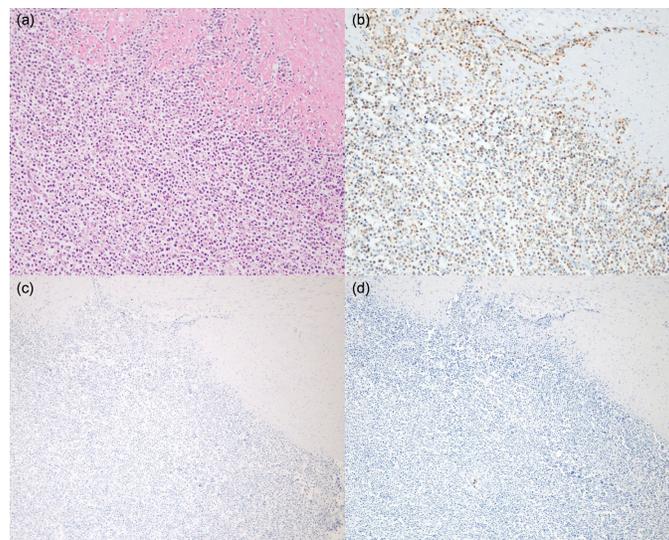
Fig. 4. Infiltration of neoplastic lymphocytes in the ileum. (a) Hematoxylin and eosin staining (200× magnification). (b) Granzyme B-positive cytoplasmic granules in the neoplastic lymphocytes. Immunohistochemistry (IHC) for granzyme B (200× magnification). Neoplastic lymphocytes were negative for (c) CD3 and (d) CD20. IHC for CD3 and CD20 (× 40 magnification).

levels of lactate dehydrogenase (Krick *et al*., 2008; Finotello *et al*., 2018). During most of the treatment period, the cat in the present case was asymptomatic and had temporal low-grade adverse events without bicavitary involvement, hypoalbuminemia, or lactate dehydrogenase level elevation; the low physical burden may have contributed to the long-term survival. Second, there was a good initial response to chemotherapy. A retrospective study demonstrated

that 7.3% of feline LGLL patients survived over 180 days; 75.0% of these patients were treated with cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP)-based chemotherapy; and a lack of response to chemotherapy was a poor prognostic factor (Finotello *et al*., 2018). These results suggested the existence of a feline LGLL subtype that could respond well to chemotherapy and result in long-term survival. Moreover, in feline lymphoma, a good initial

response to COP-based chemotherapy is correlated with prolonged overall survival time (Waite *et al.*, 2013). In our case, tumor regression and improvement of clinical signs were observed soon after beginning COP-based chemotherapy; therefore, a good initial response might have been associated with this patient's long-term survival. Third, there was relatively low mitotic activity [MC = 11 per 2.37 mm^2 (10 HPF)] in the ileum at postmortem examination. This MC can be converted to 1.1 per 1 HPF. Furthermore, the present MC could be categorized as low grade (0–5 per 1 HPF) according to the World Health Organization system of classification of canine lymphomas (Valli *et al.*, 2011). A retrospective study revealed that higher MC (> 20 per 1 HPF) indicated poor survival probability in canine lymphoma (Valli *et al.*, 2013). Furthermore, a case report of a feline with T LGLL surviving for 1 month from the onset had higher MC (4–5 per 1 HPF) (Tsuboi *et al.*, 2010) than the present case. Therefore, relatively low mitotic activity might have resulted in a good prognosis in this case. However, we should note the several limitations below. First, the statistical association between MC and prognosis in feline LGLL remains unclear in veterinary medicine. Second, autopsy specimens (posttreatment) were used in this MC evaluation, hence, the MC of pretreatment conditions remains unknown. Third, MC evaluation methods often differed in magnifications and number of fields among studies, leading to a lack of comparability. Activated lymphocyte therapy was administered multiple times in combination with chemotherapy without serious adverse events over a long period in the present case. Based on flow cytometry results that CD8$^+$ T lymphocytes were mainly composed in the culture medium, we assumed an enhancement effect of antitumor immunity, referred to the findings of previous studies (Itoh *et al.*, 2003; Hoshino *et al.*, 2008; Mie *et al.*, 2016; Mitani *et al.*, 2021). Several studies indicated that chemotherapy could synergistically enhance the effect of the activated lymphocyte for the following reasons. First, the chance of tumor-antigen sensitization to immune cells increases by chemotherapy cytotoxicity (Mitchell *et al.*, 2012). Second, a low-dose metronomic cyclophosphamide protocol induces selective reduction of circulating regulatory T lymphocyte levels, which contributes to immune tolerance (Ghiringhelli *et al.*, 2007; Burton *et al.*, 2011). Based on the findings of these studies, concurrent treatment with chemotherapy and activated lymphocyte therapy could be preferred to activated lymphocyte therapy alone; however further controlled studies are required to clarify the efficacy of activated lymphocyte therapy for feline LGLL. Moreover, the potential toxicity and safety issues of the activated lymphocyte therapy make the use of this therapy controversial, although some reports have described that this therapy is safe in cats and other species (Takayama *et al.*, 2000; Hoshino *et al.*, 2008; O'Connor *et al.*, 2012; Maeta *et al.*, 2019; López-Díaz

de Cerio *et al.*, 2020; Gareau *et al.*, 2021; Mitani *et al.*, 2021). In a human randomized trial, compared with surgical resection alone, activated lymphocyte therapy after surgical resection of hepatocellular carcinoma decreased the frequency of recurrence, prolonged the time to first recurrence, improved the recurrence-free survival, and improved the disease-specific survival (Takayama *et al.*, 2000). Furthermore, in a controlled study of canines, compared with chemotherapy alone, activated lymphocyte therapy after chemotherapy for lymphoma prolonged tumor-free and overall survival time (O'Connor *et al.*, 2012). In these studies, the patients had few adverse side effects. Additionally, the safety of activated lymphocyte therapy was described in some veterinary articles: a retrospective study described that no adverse events were observed in 10 dogs with lymphoma that received multiple doses of activated lymphocyte therapy after chemotherapy (Gareau *et al.*, 2021). Furthermore, two cats treated with activated lymphocyte therapy after surgical resection of carcinomas over several months maintained their QOL and showed no adverse events (Maeta *et al.*, 2019).

Long-term survival was observed in a feline patient with non-T/B LGLL treated concurrently with chemotherapy and activated lymphocyte therapy. Using a treatment regimen for feline non-T/B LGLL with a minimal physical burden and having a good initial response to treatment may prolong survival. Activated lymphocyte therapy may be a feasible adjuvant treatment for feline non-T/B LGLL.

Conflict of interest
The authors declare that there is no conflict of interest.
Authors' contributions
Shimon Furusato and Yu Tamura contributed to the analysis and interpretation of the data and writing of the first draft. James K. Chambers performed the pathological diagnosis and wrote the section regarding the pathological analysis in the manuscript. Takahiro Ushigusa and Yu Tsuyama contributed to the clinical case resolution, data collection, and critical revision of the manuscript. All of them reviewed and revised the final draft.

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