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Adjuvant Immunotherapy of Feline Fibrosarcoma with Recombinant Feline Interferon-ω

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Background: Recombinant feline interferon-ω (rFeIFN-ω) was tested as a treatment option for cats with fibrosarcoma to assess safety and feasibility.

Hypothesis: Treatment with rFeIFN-ω in cats with fibrosarcoma is safe and feasible.

Animals: Twenty domestic cats.

Methods: In an open-labeled uncontrolled clinical trial 12 injections of 1×10^6 U/kg rFeIFN-ω were administered over a 5-week period: the 1st through 4th injections were given intratumorally, and the 5th through 12th injections were administered subcutaneously at the tumor excision site. Wide surgical excision of the tumors was carried out after the 4th injection and before the 5th injection of rFeIFN-ω. A Common Terminology Criteria for Adverse Events (CTCAE) analysis was conducted. Flow cytometry of fibrosarcoma cells after incubation with rFeIFN-ω and recombinant feline interferon-γ was performed to assess the biological effect of rFeIFN-ω.

Results: Changes in blood cell count, increases in serum aspartate-amino-transferase activity, serum bilirubin concentration, serum creatinine and serum electrolyte concentrations, weight loss, anorexia, increased body temperature, and reduced general condition were observed but were mostly minor (grade 1 and 2) and self limiting. Eosinophilia ($P = .025$), neutropenia ($P = .021$), and weight loss ($P < .001$) were statistically correlated with rFeIFN-ω-treatment (analysis of parameters before treatment and after 3 injections of rFeIFN-ω). Flow cytometry of 5 unrelated feline fibrosarcoma cell lines showed increased expression of major histocompatibility complex (MHC) class I molecules ($P = .026$) in response to in vitro incubation with rFeIFN-ω, whereas expression of MHC class II molecules was not affected significantly.

Conclusions and Clinical Importance: RFeIFN-ω for the treatment of feline fibrosarcoma is safe, well tolerated, and can be easily performed in practice. To assess the efficacy of the treatment, it should be tested in a placebo-controlled trial.

Key words: Antitumor immunity; Cat; Cytokines; Major histocompatibility complex; Soft-tissue sarcoma.

Fibrosarcomas are common in the cat and comprise over 40% of all skin tumors in this species.[1] Because of the invasive nature of fibrosarcomas, the recurrence rate ranges from 30 to 70%,[2,3] and metastasis occurs in approximately 10 to 20% of affected cats.[4–7] Currently, there is no successful treatment for fibrosarcoma in the cat.[7] Although surgery is the treatment of choice, complete excision often is difficult. Wide surgical excision extends the tumor-free interval and increases survival time.[8,9]

Pre- or postoperative radiation therapy extends the median tumor-free time up to 422 days[10] and may reduce the recurrence rate to 42% in cats with clean surgical margins.[11] In the study by Cohen et al,[4] a recurrence rate of 41% after tumor excision, electron-beam radiation, and, in some cases, chemotherapy was documented. Chemotherapy regimens that have been investigated include doxorubicin[12,13] and carboplatin.[11] Martano et al[14] showed that the combination of surgery and doxorubicin does not increase the disease-free interval or reduce tumor recurrence or the rate of metastasis.

Cancer immunotherapy refers to using the power and the specificity of the immune system for the treatment of a malignancy. This therapy is based on the use of biologically active proteins with the aim of altering the specific and nonspecific immune responses of the patient. The immune system is capable, under certain circumstances, of recognizing and eliminating tumor cells.[15]

Interferons (IFN) are cytokines that have antiviral, antiproliferative, and immunomodulatory effects. The family of IFNs consists of 2 major classes, type I and type II. Type I IFNs include IFN-α, β, δ, τ, and ω, whereas IFN-γ is the only type II IFN. The biological activities of type I IFNs include growth inhibition of tumor cells,[16,17] induction of apoptosis,[17,18] natural killer cell activation,[19] and an increase in the expression of major histocompatibility complex (MHC) class I molecules.[20,21] The amino acid sequence of recombinant feline IFN-ω has approximately 60% homology to that of human IFN-ω.[22] The antitumor efficacy of human IFN-ω has been described in vitro[23,24] and in vivo.[25] Recombinant feline IFN-ω has approximately 60% homology to human IFN-α.[22,26] Human IFN-α has been used in Germany since 1999 for the adjuvant therapy of malignant melanoma in humans. Because of the close relationship of IFN-ω and IFN-α, adjuvant immunotherapy of feline fibrosarcoma by using rFeIFN-ω follows the therapy protocol for human IFN-α.[27] Recombinant feline IFN-α is not commercially available, whereas rFeIFN-ω is the only IFN licensed for use in cats.

IFN-γ increases antigen presentation by upregulation of MHC expression on antigen presenting cells and by activation of natural killer cells.[21,28,29] Tumor cells often

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have a decreased expression of MHC class I molecules.[15,30–33] This so-called tumor escape mechanism prevents recognition of tumor antigens by cytotoxic T cells, because tumor antigens are presented by MHC class I molecules on the surface of malignant cells. Because IFN-ω and IFN-γ may be able to increase expression of MHC class I molecules and, therefore, antigen presentation, these cytokines may render tumor cells susceptible to attack by natural killer cells, cytotoxic T cells, activated macrophages, or antibodies.[32,34] To assess the biological effect of rFeIFN-ω on MHC class I and II expression on feline fibrosarcoma cells, flow cytometry from 5 unrelated fibrosarcoma cell lines was carried out. The effect of rFeIFN-ω was compared with that of rFeIFN-γ. RFeIFN-ω was administered to domestic cats before and after tumor excision. To evaluate the safety and the feasibility of this adjuvant treatment, an open-label prospective clinical trial was conducted in 20 cats.

Materials and Methods

Patients

The study was an open-label, single-arm, monocentric, prospective, safety and feasibility study. From January 2004 until August 2004, cats with fibrosarcoma were evaluated for inclusion in the study. Cats were enrolled in the study if they met defined inclusion criteria: resectable tumor (excision possible without opening a visceral cavity, amputation or partial resection of parts of the scapula or the spine), absence of previous immunomodulatory treatment within 6 weeks of the start of IFN-ω treatment, and life expectancy >1 year. Twenty cats with fibrosarcoma met the inclusion criteria: 18 Domestic Shorthair, 1 Domestic Longhair and 1 Persian cat. They ranged in age from 4 to 16 years (mean, 10 years), and there were 11 castrated male and 9 spayed female cats. Eleven cats had primary tumors, and 9 cats had tumors that had recurred after surgery.

The tumors were mainly located in the interscapular region (11 cats). Other sites were the thoracic wall (5 cats), the lumbar area (3 cats), and the shoulder region (1 cat). The tumors all were located at sites that are commonly used for administration of vaccines. However, it was not possible to determine whether vaccines had indeed been given at the tumor site. Nine cats had stage I tumors (<2 cm), 8 cats had stage II tumors (2–3 cm), and 3 cats had stage III tumors (>3 cm). Two cats had multiple tumors at 1 site. In both cats, 3 tumors were present at 1 site. In these cats, the diameters of the tumors were added (tumor staging according to Hirschberger and Kessler[35]). Nine cats could not be included in the study because of metastasis in the liver (1 cat), nonresectable tumor (4 cats), no clinical evidence of fibrosarcoma (3 cats), and 2 tumors at 2 different sites (1 cat).

Reagents and Products

Recombinant FeIFN-ω[a] was provided by Virbac, S.A., France. The 5×10^6 U powder fraction was diluted in 1 ml of isotonic saline solution immediately before use.

Immunotherapy with rFeIFN-ω

The treatment protocol consisted of 12 injections of 1×10^6 U/kg rFeIFN-ω[a] over 5 weeks. The dosage of 1×10^6/kg rFeIFN-ω has been established in cats and is recommended by the manufacturer.[36] RFeIFN-ω was administered intratumorally (1st through 4th injection) and then subcutaneously (SC) (5th through 12th injection) at the tumor excision site. In the first week of treatment, the cats received 1 intratumoral injection daily for 3 consecutive days. In the second week, the cats again received 1 injection daily for 3 consecutive days, but the 4th injection was administered intratumorally and the 5th and 6th injections were given SC at the excision site after surgical excision of the tumor. In the next 3 weeks, the cats received 2 injections of rFeIFN-ω SC per week.

Surgery

Wide surgical excision (ie, tumor excision en bloc with 3-cm margins to palpable evidence of disease) was carried out during the second week of treatment, after the 4th and before the 5th injection of rFeIFN-ω. All of the operations were performed by the same surgeon (RK). Cross-sectional imaging was not performed in any cat.

Pain Relief and Antibiotic Treatment

For perioperative analgesia, buprenorphine[b] was given at a dosage of 0.01 mg/kg on the day of surgery. For postoperative analgesia, all cats received meloxicam[c] for 5 days (1st day after surgery: 0.3 mg/kg; 2nd through 5th days after surgery: 0.1 mg/kg). Furthermore, the cats were treated with amoxicillin-clavulanic acid[d] at a dosage of 25 mg/kg per day for 5 days.

Follow-up Period

The cats were reevaluated for tumor recurrence on days 28, 42, 90, 180, 270, and 360 after surgery or on an ad hoc basis if the owner reported regrowth of the tumor. At each reevaluation, the cats underwent a clinical examination, which included weighing, as well as evaluation of wound healing, potential adverse effects, potential recurrence, and metastasis of the tumor. On days 28, 42, 180, and 360, CBC count and serum biochemistry were performed. The final evaluation on day 360 also included thoracic (left and right lateral and ventrodorsal views) and abdominal (lateral view) radiographs and abdominal ultrasonography.

Common Terminology Criteria for Adverse Events

All data were recorded in detail in case report forms and were analyzed to determine the toxicity of the treatment. To provide a basis for the evaluation of toxicity, the ''Veterinary Co-operative Oncology Group—Common terminology criteria for adverse events following chemotherapy or biological antineoplastics therapy in dogs and cats'' V 1.0 was used.[37]

Cell Culture

Five unrelated feline fibrosarcoma cell lines (fibrosarcomas from control animals of another study) were cultured at the Institute for Experimental Oncology, TU Munich, Munich, Germany, by standard methods: cells were mechanically and enzymatically dissociated and cultured in Dulbecco's Modified Eagle Medium[e] supplemented with 10% fetal calf serum,[f] 1% glutamine,[g] 100 U/mL penicillin,[h] and 100 μg/mL streptomycin[i] at 37°C in a 5% CO_2 atmosphere.

Incubation with rFeIFN-ω and rFeIFN-γ

Fibrosarcoma cells lines (1.75×10^6 cells each) were incubated in vitro for 48 hours with 50 μL isotonic saline solution (negative control), 1,000 ng rFeIFN-γ,[j] or 25,000 U rFeIFN-ω.[a]

Staining of the Cells

The flow cytometry protocol described by Schwarz[28] was used. For each tumor cell line, 13 FACS tubes[k] were prepared ($3–4 \times 10^5$ cells each): 3 for negative control, 5 for each rFeIFN-ω and rFeIFN-γ. The monoclonal antibodies used were mouse anti-MHC class I ω6/32[l] and mouse anti-feMHC class II 42.3.[m] The antibodies were added as follows: (a) no antibody (negative control), (b) 10 μL immunogloublin (Ig) G1,[n] and (c) 10 μL IgG2a[o] (isotype controls), (d) 60 μL MHC I antibody (ω6/32), and (e) 10 μL MHC II antibody (42.3). The cells were washed twice and incubated with 10 μL fluorescein isothiocyanate-conjugated rabbit anti-mouse IgG antibody[p] for 20 minutes in the dark and resuspended afterward.

Flow Cytometric Analysis

Expression of MHC class I and II on the cell surface was evaluated by performing flow cytometric analysis with FACS-Vantage.[q] Negative and isotype controls were used as previously described.[28] The Cell Quest Program[r] was used for data analysis.

Statistical Analysis

Kaplan-Meier analysis of recurrence was carried out by using SPSS v.13.O[s]. The number of events (3 in the group of cats without previous recurrence, 6 in the group of cats with at least 1 previous recurrence) was too small for additional statistical analyses. A paired Student's *t*-test was used to evaluate differences between the serum results and blood counts of days –7 (before 1st treatment) and 0 (day before surgery) and to test for change of expression of MCH class I and II. In all statistical analyses, a P value of $<.05$ was considered significant.

Results

Common Terminology Criteria for Adverse Events

The Common Terminology Criteria for Adverse Events (CTCAE) analysis produced the results shown in Table 1. The statistical analysis of CBC count, serum biochemistry results, and body weight on days –7 (before treatment) and 0 (after 1 week of treatment but before surgery) produced the following results: the parameters neutropenia ($P = .021$), eosinophilia ($P = .025$), and weight loss ($P < .001$) showed significance and were positively correlated with the rFeIFN-ω treatment.

Clinical Findings

The tumor recurred locally in 9 of 20 cats (45%); 1 other cat developed pulmonary metastases and died on day 258. The tumor had not recurred at the original site. The disease-free interval of this cat was censored at day 258, but it was included in the statistical analysis. At the final evaluation, on day 360, 10 cats were disease free. To investigate the influence of previous recurrences on recurrence-free time, the study population was divided into 2 groups: a group without previous recurrence (10 cats) and a second group with at least 1 previous recurrence (10 cats). The Kaplan-Meier curves for both groups are shown in Fig 1.

Modulation of MHC Molecules on Fibrosarcoma Cells by rFeIFN-ω and rFeIFN-γ

Expression of MHC class I and II antigens on feline fibrosarcoma cell lines was evaluated by using flow cytometry (see Fig 2). A 1-fold increase of expression of MHC class I molecules was observed in fibrosarcoma cell lines in response to in vitro incubation with 25,000 U rFeIFN-ω ($P = .026$). The expression of MHC class II molecules also was influenced by rFeIFN-ω, but this effect was not statistically significant ($P = .22$). The incubation of feline tumor cell lines with 1,000 ng rFeIFN-γ caused a 1.5-fold increase of expression of MCH class I ($P = .010$) and a 3-fold increase of expression of MHC class II molecules ($P = .020$). This observation means that rFeIFN-ω is able to increase the expression of MHC class I molecules on feline fibrosarcoma cells, whereas rFeIFN-γ can modulate the expression of MHC class I and II molecules.

Discussion

Immunotherapy has been investigated in several studies in cats with fibrosarcoma. King et al[38] reported that the immunostimulant acemannan may be an effective adjunct to surgery and radiation therapy in the treatment of fibrosarcoma. Acemannan enhances macrophage release of interleukin-1, interleukin-6, tumor necrosis factor-α, and IFN-γ. Jourdier et al[39] studied local immunotherapy of spontaneous feline fibrosarcomas by using recombinant poxviruses expressing interleukin-2. This study demonstrated a decrease in tumor recurrence rates from 61% (11 of 18 cats) in control animals (treated with surgery and iridium-based radiotherapy) to 39% (7 of 18) and 28% (5 of 18) in cats who received human or feline interleukin-2 in addition to surgery and iridium-based radiotherapy.

Recombinant feline IFN-ω has antiviral[40,41] and antiproliferative effects,[42,43] and has been used in the treatment of feline calicivirus infection[44]; feline herpetic keratitis[45]; feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) infection[46]; feline infectious peritonitis[47]; gingivitis, stomatitis, and oropharyngitis[48]; and canine parvovirus infection.[49] De Mari et al[46] tested rFeIFN-ω in 81 cats with FeLV and with FeLV and FIV-coinfection. In this placebo-controlled trial, the cats were treated with 1×10^6 U/kg rFeIFN-ω for 5 consecutive days in 3 series on day 0, day 14, and day 60. The investigators observed systemic adverse events in 5 of 81 of the cats, including lethargy, transient vomiting, and diarrhea, but with no statistically significant differences between the IFN-treated group and the placebo group.

In another clinical trial,[48] 20 cats with chronic gingivitis, stomatitis, or oropharyngitis were treated by using subgingival and SC injections of 1×10^6 U/kg or 2.5×10^6 U/kg rFeIFN-ω. Mihaljevic[48] found transient lethargy, increased body temperature, and anorexia in some cats. These adverse events were mainly observed in cats treated at a dosage of 2.5×10^6 U/kg. In the present study, cats were treated at a dosage of 1×10^6 U/kg, as

Table 1. Number of adverse events during the study according to the Veterinary Co-operative Oncology Group grading scale.[a]

	Time of Diagnosis (28 adverse events)				Neoadjuvant Treatment (53 adverse events)				Surgery and Adjuvant Treatment (101 adverse events)				Adjuvant Treatment (68 adverse events)				Follow-up (76 adverse events)			
Adverse Event	Day 7 (before 1st treatment)				Day 6 to Day 0 (1st treatment until surgery)				Day 1 until Day 14 after surgery				Day 16 until Day 28 (last treatment on day 23)				Day 42 until Day 360			
	Grade				Grade				Grade				Grade				Grade			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Erythrocytopenia									2											
Hemoglobin ↓									4	1			3							
Hematocrit ↓	1								7	1			3							
Leukocytopenia	1	1			5	1			3	3			6	2			7	5		
Thrombocytopenia	2				3	1			2	1			2				6			
Thrombocytosis	1								2				1				1			
Lymphocytopenia	2	2			2	1			3	1	2		2	1			4	1		
Lymphocytosis	1				1				1				2				4			
Segmented neutropenia	3				2				1		1		4				9			
Eosinophilia	5				2				3				2				7			
AST ↑			2		6	1	1		3	2	1		2	3	1		6		2	
Bilirubin ↑					1				4	1			1	1			2			
Creatinin ↑	1				3				3				3				5			
Phosphorus ↓	2								1								2	1		
Sodium ↓									1								2			
Potassium ↓					1							1					1			
Potassium ↑	1				3				1	1			3				2	1		
Lethargy/fatigue					5				8		1		3	1						
Anorexia					4				4	1			3	1			1			
Body temperature	1	2			5				11				1							
Dyspnea												1								
Diarrhea									2											
Vomitus									1				3							
Constipation													1							
Wound healing disorder									3	1										
Weight loss					4	1			10	1			8	5			4	3		

↑, increased parameter; ↓, decreased parameter.
[a] From Ref. 37.

recommended by the manufacturer[36] to avoid the above-mentioned adverse events of the higher dosage.

The results of the CTCAE analysis of the study correspond with the adverse effects specified by the manufacturer.[36] These included hyperthermia; vomiting; diarrhea; mild decrease in the total number of leukocytes, thrombocytes and erythrocytes; increased activity of serum aspartate-amino-transferase; and transient lethargy. However, these changes generally were minor grade and self limiting. The statistical analysis of blood test results and body weight on days –7 (before treatment) and 0 (after 1 week of treatment) showed significant differences in neutropenia, eosinophilia, and weight loss, which indicated a correlation to the treatment with rFeIFN-ω. The manufacturer[36] describes neutropenia during treatment with rFeIFN-ω. Mild eosinophilia may be induced by rFeIFN-ω. Weight loss showed a clear statistical significance ($P < .001$), which could be correlated to the therapy or stress caused by frequent visits in the clinic. Weight loss was not observed in the above-mentioned studies.[46,48]

In the present study, there was a recurrence rate of 45% (9 of 20 cats); metastases were found in 1 cat. The current study design was not able to a demonstrate decreased recurrence rate, because of a lack of a control group and a lack of surgical margin assessment after

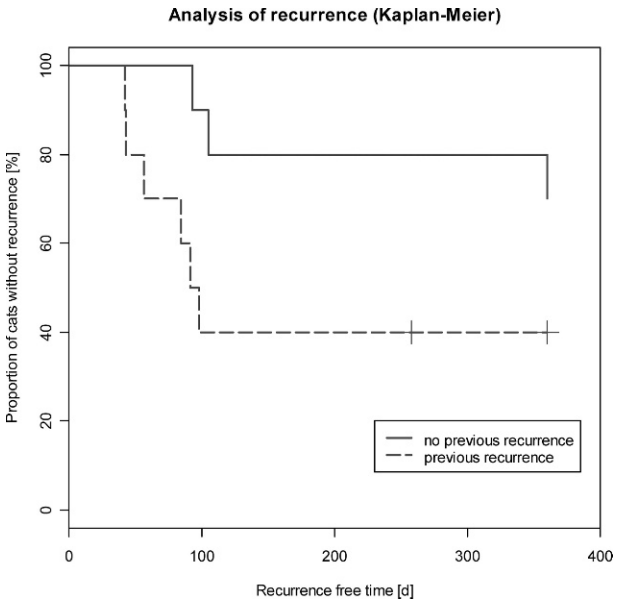

Fig 1. Analysis of recurrence (Kaplan-Meier) of cats without previous recurrences (n = 10) and cats with at least 1 previous recurrence (n = 10).

surgery. Histologic grading of the surgical specimens as described previously was not performed.

Tumors in the study described here all were located at vaccination sites. However, it was not possible to determine whether vaccines had indeed been given at the tumor site. Hendrick et al[50] reported that 39% of cats with vaccination site sarcomas had tumor sizes of 2.0–3.9 cm and 33% of the cats had tumor sizes of 4.0–5.0 cm. However, 33% of cats with nonvaccination site sarcomas had tumor sizes of 2.0–3.9 cm. In the present study, cats were enrolled in the trial as they were presented in the clinic, therefore, 17 cats randomly had tumor sizes of <3 cm. This observation can be attributed to the awareness of the referring veterinarians about fibrosarcomas.

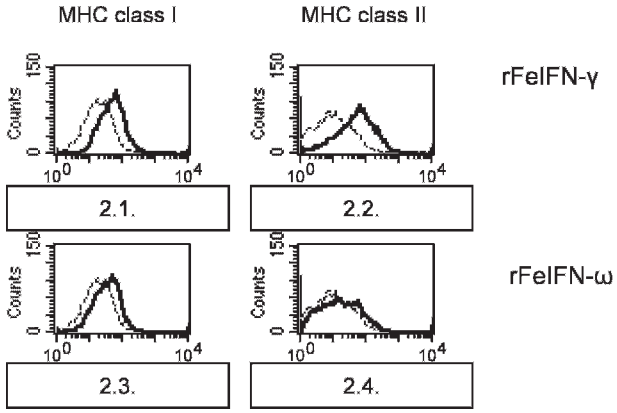

Fig 2. Results of flow cytometry of feline fibrosarcoma cell lines incubated in vitro with 25,000 U (rFeIFN-ω) and 1,000 ng rFeIFN-γ for 48 hours. The figure shows 1 of 5 experiments representative of all 5 fibrosarcoma cell lines tested. The *y*-axis shows the counts, and the *x*-axis shows the intensity of the fluorescence of fluorescein isothiocyanate-conjugated rabbit anti-mouse immunogloublin (Ig) G antibody (FITC). 2.1.: solid line: major histocompatibility complex (MHC) class I after stimulation with rFeIFN-γ, dotted line: negative control; 2.2.: solid line: MHC class II after stimulation with rFeIFN-γ; 2.3.: solid line: MHC class I after stimulation with rFeIFN-ω; 2.4.: solid line: MHC class II after stimulation with rFeIFN-ω.

The cats had to meet defined inclusion criteria: 4 cats were not included in the study, because they had nonresectable tumors. In these cats, tumor masses were so large that tumor excision was not possible without opening a visceral cavity or performing amputation or partial resection of parts of the scapula or the spine.

Cats with 2 tumors at 2 different sites (eg, thoracic wall and lumbar area) could not be included in the study. It cannot be assumed that 2 tumors at 2 different sites originated from the same tumor cells. Two different fibrosarcomas might not have the same biological behavior and potentially may have different antigenic properties. The cats received recombinant feline IFN-ω intratumorally or SC at the excision site, and for this reason only 1 tumor could be treated. Consequently, 1 cat with tumors at different sites was excluded from the study. Two cats had multiple tumors at 1 site. In both cats, 3 tumors were present at 1 site, for 1 cat, it was the first recurrence and, for the other cat, it was the second recurrence. This finding was thought to represent a recurrence of 1 fibrosarcoma as multiple small nodules at the surgical site.

Tumors were resected en bloc with 3-cm margins to palpable evidence of disease, and tumor excisions always were carried out by the same experienced surgeon. Cohen et al[4] stated that the location and the size of the primary tumor and the ability to remove it are important prognostic factors. The recurrence rate in the present study could have been influenced by the fact that most of the cats had small tumors and that the surgical procedure was performed by an experienced surgeon.

Cohen et al[4] also reported that the prognosis of recurrent fibrosarcoma treated by surgical excision is worse than that of excision of primary fibrosarcoma. In the present study, the number of tumor recurrences before rFeIFN-ω treatment also may have had an impact on the outcome. Seven cats that had tumor relapses (6) or metastases (1) had a minimum of 1 fibrosarcoma removed surgically before inclusion in the study. Of these 7 cats, 4 had 1 previous fibrosarcoma and 3 had 2 previous tumors. In contrast, only 3 cats with a primary tumor had tumor recurrence in this study. Of the 10 cats that were tumor free at the end of the study, 7 had a primary tumor and 3 had had 1 previous fibrosarcoma at the time of admission. Visual inspection of the Kaplan-Meier curve (Fig 1) indicated an influence of previous recurrences on recurrence-free time, but, presumably because of small sample sizes, this effect did not achieve statistical significance.

All of the cats received meloxicam for postoperative pain once daily for 5 days, as described in other studies.[51,52] Nonsteroidal anti-inflammatory drugs also have been beneficial in the treatment of transitional-cell carcinoma in dogs.[53] These substances not only decrease the inflammatory reaction in the tumor tissue but also inhibit tumor growth. It seems unlikely that meloxicam

retarded tumor growth in this study, because it was administered for only 5 days.

The expression of MHC class I and II antigens on feline fibrosarcoma cell lines was evaluated by using flow cytometry. An increase in expression of MHC class I molecules was observed in all tested fibrosarcoma cell lines after in vitro incubation with recombinant feline IFN-ω. The expression of MHC class II molecules was affected by rFeIFN-ω to a lesser extent. The incubation of feline tumor cell lines with rFeIFN-γ resulted in increased cellular expression of MCH class I and II molecules. IFN-ω is a type I IFN and is able to increase the expression of MHC class I molecules.[19,20] In contrast, IFN-γ is able to modulate the expression of MHC class I and II antigens. This effect has been described in different tumor cell lines in vitro.[21,28,29] An increase in the expression of MHC class I antigens, and, therefore, an increase in antigen presentation, render tumor cells susceptible to attack by the immune system.[32,34] Recently, Murgia et al[33] reported down-modulation of MHC antigen expression in a canine transmissible venereal tumor as a tumor escape mechanism. Therefore, by increasing cellular expression of MHC I, recombinant feline IFN-ω may promote tumor-cell destruction by the immune system. Immunotherapy with recombinant feline IFN-ω in cats with fibrosarcoma is safe and well tolerated.

The treatment is straightforward and can be performed easily in clinical practice. The results of the present study encourage placebo-controlled clinical trials to evaluate the efficacy of this treatment.

Footnotes

[a] Virbagen Omega, Batch no OU4M,
[b] Temgesic, Essex Pharma, Munich, Germany
[c] Metacam, Boehringer Ingelheim, Ingelheim, Germany
[d] Synulox, Pfizer, Karlsruhe, Germany
[e] DMEM, Biochrom, Berlin, Germany
[f] FKS, Biotech, Aidenbach, Germany
[g] Glutamine, Biochrom, Berlin, Germany
[h] Penicillin, Biochrom, Berlin, Germany
[i] Streptomycin, Biochrom, Berlin, Germany
[j] Recombinant Feline Interferon-y, R&D Systems, Wiesbaden, Germany
[k] FACS tubes, BD, Pharmingen, Germany
[l] wb/32, Abcam, Cambridge, United Kingdom
[m] 42.3, Serotec, Oxford, UK
[n] IgG1, BD, Pharmingen, Germany
[o] IgG2a, BD, Pharmingen, Germany
[p] FITC, DAKO Diagnostica, Hamburg, Germany
[q] FACS-Vantage, Becton-Dickinson, San Jose, CA
[r] Cell Quest Program, Becton-Dickinson, San Jose, CA
[s] SPSS, Chicago, Illinois

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