Adjuvant immunotherapy of feline injection-site sarcomas with the recombinant canarypox virus expressing feline interleukine-2 evaluated in a controlled monocentric clinical trial when used in association with surgery and brachytherapy

CrossMark

D. Jas [a], C. Soyer [c], P. De Fornel-Thibaud [b], F. Oberli [a], D. Vernes [a], P.-M. Guigal [a], H. Poulet [a,*], P. Devauchelle [b]

[a] Merial S.A.S., R&D, 254 rue Marcel Mérieux, 69007 Lyon, France
[b] Veterinary Oncology Centre, MICEN VET, 58 rue Auguste Perret, 94000 Créteil, France
[c] Royal Veterinary College, Hawkshead House, Hawkshead Ln, North Mymms, Hatfield, Hertfordshire AL9 7TA, United Kingdom

A B S T R A C T

The objective of this randomised, controlled, parallel-group monocentric clinical trial was to assess the efficacy (at low and high dose) and the safety (at high dose) of a recombinant canarypox virus (ALVAC®) expressing feline interleukin 2 (IL-2). ALVAC IL-2 was administered to cats as an adjunct treatment of feline fibrosarcoma in complement to surgery and brachytherapy (reference treatment). Seventy-one cats with a first occurrence of feline fibrosarcoma were referred to the Veterinary Oncology Centre for post-surgical radiotherapy. They were randomly assigned to three treatment groups: reference treatment group (23 cats), ALVAC IL-2 low dose group (25 cats) and ALVAC IL-2 high dose group (23 cats). Two dosages of ALVAC IL-2 were used to assess both safety (high dose) and efficacy (high and low doses). The treatment consisted of six consecutive doses of ALVAC IL-2 administered subcutaneously at the tumour site on Day 0 (one day before brachytherapy treatment), Day 7, Day 14, Day 21, Day 35 and Day 49. All cats were evaluated for relapse (i.e. local tumour recurrence and/or metastasis) every three months for at least one year (ALVAC IL-2 high dose group) or two years (reference treatment and ALVAC IL-2 low dose groups) by complete physical examination and regular CT scans. ALVAC IL-2 treatment was well tolerated and adverse effects were limited to mild local reactions. ALVAC IL-2 treatment resulted in a significant longer median time to relapse (>730 days in the ALVAC IL-2 low dose group) than in the reference treatment group (287 days), and a significant reduction of the risk of relapse by 56% at one year (ALVAC IL-2 treatment groups versus reference treatment group) and 65% at two years (ALVAC IL-2 low dose treatment group versus reference treatment group).

© 2014 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/3.0/).

1. Introduction

Feline injection-site sarcomas (FISS) are aggressive cutaneous mesenchymal tumours and represent 6–12% of all feline tumours [1]. Although the pathogenesis of this tumour is not fully understood, it is currently accepted that, in genetically predisposed cats, a traumatism causing inflammation in the subcutis (i.e. injection) may trigger the development of FISS. Aggressive and complete surgical excision was the treatment of choice [2–4]. However, because of the invasive nature of FISS, the recurrence rate is high ranging from 30% to 75%, with metastasis occurring in 6–25% of affected cats [3,5,6]. Adjunct treatments such as pre or post-operative radiation therapy and chemotherapy are used but their efficacy has not always been experimentally demonstrated [8]. Currently, the treatment of choice consists in an aggressive surgery combined with radiation therapy [6,9]. Despite this treatment, local recurrences are still observed in a high proportion of the patients. Any therapy that could further reduce the rate of relapse would therefore be useful for veterinary oncologists.

Immunotherapy has been investigated in two previous studies in cats with FISS.

Local IL-2 therapy has been shown to be efficacious against various tumours including FISS. When combined with surgery and

---

brachytherapy, repeated local injections of xenogenic Vero cells secreting human IL-2 reduced the rate of relapse and increased the median survival time [10]. Similar results were observed with repeated local injections of either the canarypox virus vector (ALVAC®) or the genetically attenuated vaccinia virus vector (NYVAC®) expressing feline IL-2 or human IL-2, respectively [11].

The first objective of this new study was to confirm the efficacy of an ALVAC-IL2 construct over a longer period (two-year follow-up). Second objective was to confirm its safety at high dose. ALVAC IL-2 was administered to cats as an adjunct treatment of FISS in combination with surgery and brachytherapy.

2. Materials and methods

2.1. Animals

The study was a randomized, controlled, parallel-group, monocentric safety and efficacy study. It took place at the Veterinary Oncology Centre of Maisons-Alfort, France from October 2006 to October 2012. Eligible patients were cats in good general condition with a first occurrence of FISS having undergone surgical excision and presented to the Centre for a brachytherapy treatment. Surgical excision was performed by several veterinary practitioners and there was no standardized surgical procedure. Tumour size had to be between 2 and 5 cm (size measured with a caliper by the pathologist on the fixed formalin sample). Histological analysis was performed or reviewed at the Veterinary Histo-Cytopathology Laboratory (France) to confirm the nature of the tumour and assess the status of the surgical margins. The margins were evaluated on four full thickness sections on the vertical plan (anterior, posterior and lateral margins) and one complete section of deep muscular layers on the horizontal plan (deep margin). Margin results were defined as clean (complete) or dirty (incomplete or close) margins. Only cats with no pulmonary metastasis or enlarged lymph nodes, no macroscopic disease and no wound healing complications were enrolled. The absence of previous adjunct treatment (radiotherapy, chemotherapy, immunotherapy or genetic therapy), of corticosteroids treatment within 1 month (long acting corticosteroid) or 1 week (short acting corticosteroid) prior to enrollment was another prerequisite.

At the time of enrolment, all cats underwent a full clinical examination including serum biochemistry, complete blood cell (CBC) count, urine density and screening for FeLV antigens and FIV antibodies and a computed tomography (CT) scan of the tumour area and thorax.

As tumour ablation completeness is considered a key prognostic factor [3,7,12], cats were randomly assigned to three treatment groups using permuted-block randomization stratified on the status of the surgical margins assessed by histological analysis: control group, ALVAC IL-2 low dose and ALVAC IL-2 high dose. For ethical reasons and to avoid any bias in the study, no placebo could be administered to the control animals. Indeed, these cats are highly sensitive to any injection or traumatism and administration of a placebo would likely increase the rate of relapse in the controls. Consequently, only the pet owners were maintained blind to the treatment group.

Written consent for entry to the trial was provided for each cat's owner. Cats were managed similarly and with due regard for their well being.

2.2. Products

Recombinant canarypox virus expressing feline interleukin 2 gene (ALVAC IL-2) was provided by Merial, France. The virus was grown in chicken embryo fibroblasts. Vaccine consisted of a clarified viral suspension, which was freeze-dried and kept at +4 °C until use. The lyophilisate containing between $10^{6.0}$ and $10^{8.3}$ ELISA Infectious Dose 50% ($EAID_{50}$)/dose was diluted in 1 mL of water for injection immediately before use.

2.3. Immunotherapy

The treatment protocol consisted in six consecutive doses of less than $10^{6.5}$ $EAID_{50}$ ALVAC IL-2/dose in low dose group or up to $10^{8.3}$ $EAID_{50}$ ALVAC IL-2/dose in high dose group over 7 weeks. Cats from treated groups received one dose once a week for 4 weeks followed by once every 2 weeks for the last two doses (i.e. on Days 0, 7, 14, 21, 35 and 49). The first dose was administered on the day before radiotherapy (i.e. about one month after surgery). Each dose of ALVAC IL-2 was administered subcutaneously around the tumour excision site (0.2 mL at the centre and 0.2 mL at each corner of a 5 × 5 cm square centered on the middle of the surgical scar (see Fig. 1) using a same 0.50 mm needle gauge. No anesthesia or tranquilization was required for the treatment administration. Cats were observed for approximately 15 min post treatment for any immediate local or general adverse reactions.

2.4. Radiotherapy

All cats received an iridium-based brachytherapy (high dose rate technique). Distribution of the dose was planned by 3D treatment planning software (Eclipse™, Varian). Four fractions of 6.5 Gy each were delivered at 6 mm from the radioactive source at Day 1, Day 1 + 8 h, Day 3 and Day 3 + 8 h, respectively, by using interstitial plastic flexible needles with mandrin (diameter 2 mm, length 113 mm, Varian) for a total equivalent dose of 60 Gy according to the linear quadratic model.

For each therapy session, cats were anesthetised with 4 mL/5 kg of propofol (Propofol Lipuro® 1%, B Braun Médical or Rapinovet®, Merck Animal Health) by intravenous route.

2.5. Follow-up

Cats treated with the high dose of ALVAC IL-2 were followed for only one year (because the main objective for this group was to evaluate the safety) and the low dose and the control groups were followed for two years.

The time to relapse was recorded for all cats (see definition below).
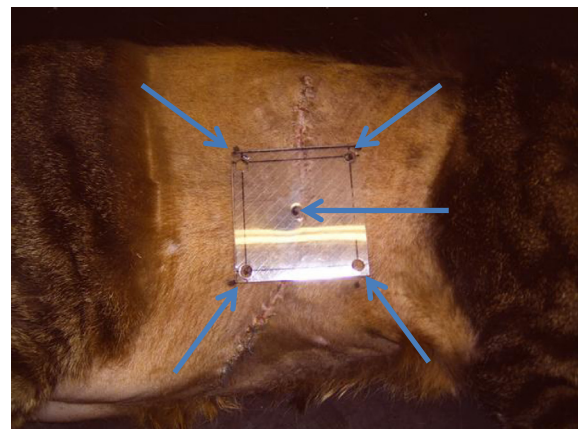
Fig. 1. Modalities of treatment administration. After reconstitution of the lyophilisate with the solvent, five injections (each approximately 0.2 mL – blue arrows) were administered subcutaneously around the tumour excision site: one injection at each corner and one injection at the centre of a 5 × 5 cm square centred on the middle of the surgical scar. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

For safety purpose, adverse events were recorded in all groups and local and systemic reactions were monitored in cats treated with the high dose ALVAC IL-2.

The follow-up consisted in a complete physical examination at the Oncology Centre every 3 months for at least 1 year (high dose ALVAC IL-2 treated cats) and 2 years (low dose ALVAC Il-2 treated cats and controls). In case of relapse notified by the client or the veterinarian, the cat was brought to the Oncology Centre for a full examination. A CT-scan of the excision site and the thorax was performed at 3, 6, 12 (all groups) and 24 (low dose treated and control groups) months after enrolment. Cats were anesthetised with 4 mL/5 kg of propofol (Propofol Lipuro® 1%, B Braun Médical or Rapinovet®, Merck Animal Health) for the CT-scan. CBC count and serum biochemistry were performed on Days 1, 21, 49 for the high dose treated group and on 3 months and 6 months post-enrolment for all cats.

2.6. Data analysis

Prior the efficacy analysis, the groups were checked for comparability on the baseline criteria: completeness of tumour ablation (χ^2) and interval between tumour ablation and enrolment (ANOVA).

The primary efficacy endpoint was the time until relapse (disease-free interval, DFI) defined by the time in days between Day 0 and relapse. A relapse was defined as a recurrence of the primary tumour (identified by palpation or by CT-scans) and/or pulmonary or regional lymph nodes metastasis (identified by CT-scans). The appearance of another FISS at a different site was not considered as a relapse. Recurrence of primary tumours were confirmed by the Veterinary Histo-Cytopathology Laboratory who was blinded to the treatment group. Time of relapse was the first day of suspicion (date of the clinical examination or day of the CT-scan). Cats withdrawn, dropped out or dead for any other reason than FISS were considered right-censored and the date of the last known observation was taken into account in the analysis.

A Proportional Hazards Cox regression model was fitted to DFI data to assess the effect of ALVAC IL-2 treatment while controlling for the type of surgical margin by computing the Hazard Ratio (HR) and its 95% confidence interval for the group. The result is interpreted in terms of reduction (in percent) of the hazard of relapse in treated cats and calculated as $[1 - HR] \times 100$. The Kaplan–Meier Product-Limit method was used to plot the survival curves and to compare the median DFI (Log-rank test).

As a secondary efficacy endpoint, we also assess the ALVAC IL-2 treatment on the relapse rate calculated at final time-point (1 or 2 years) over completers as cats who had a recurrence or a metastasis among cats who completed the study with or without relapse. The treatment efficacy was estimated while controlling for the type of surgical margin using a Cochran–Mantel–Haenszel test.

The efficacy analyses were performed on a intent-to treat basis and were conducted over the one- and the two-year follow-up periods independently. For the one-year follow-up period the two treated groups (i.e. high and low doses) were combined after checking their comparability.

The absence of toxic side effect was checked by comparing descriptively for each cat the post-treatment values for CBC count and serum biochemistry to those obtained at the time of enrolment.

All analyses were two-tailed and conducted with SAS 9.2 (SAS Institute Inc, Cary, NC). The type I error was set to α = 5%.

3. Results

Seventy-one cats (67 European, 1 Persian crossed, 1 Burmese, 1 Russian Blue cat and 1 Siamese – 39 neutered males, 31 spayed females and 1 female) aged between 4 and 17 years (mean, 10 years) met all the inclusion criteria and were enrolled. The tumours ranged from 2 to 5 cm and were usually located in the interscapular region (34). Other sites were the thoracic (10), abdominal (16), shoulder (6), lumbar (4) or the thigh region (1). The histological analysis showed that 42 cats had dirty surgical margins (incomplete tumour resection) and 29 had clean surgical margins. A CT-scan of the tumour area and thorax confirmed that all cats were free of metastasis before inclusion. Cats were randomly allocated to the control group (23 cats), ALVAC IL-2 low dose (25 cats) and ALVAC Il-2 high dose (23 cats). Characteristics of the cats are shown in Table 1.

Groups were comparable on the baseline criteria (Table 2).

Eight cats were right-censored for the DFI analysis because of death for fibrosarcoma unrelated causes (4 cats), lost to follow-

Table 1
Characteristics of the cats and individual results.

Sex	Age	Breed	Tumour localization	Surgical margins	Estimated time between surgery and Day 0	DFI	Relapse within 1 year	Relapse within 2 years	
Control group									
F*	4.8	EUROPEAN	Interscapular	Clean	39	182	Yes	Yes	
M*	9.9	EUROPEAN	Flank	Clean	53	778	No	No	
M*	9.2	EUROPEAN	Shoulder	Clean	24	602	No	Yes	
M*	6.6	EUROPEAN	Interscapular	Clean	41	197	Yes	Yes	
M*	12.5	EUROPEAN	Thorax	Clean	20	190	Yes	Yes	
F*	9.9	EUROPEAN	Interscapular	Clean	20	774	No	No	
M*	9.4	EUROPEAN	Interscapular	Clean	19	196	Yes	Yes	
M*	14.4	EUROPEAN	Interscapular	Clean	54	287	Yes	Yes	
F*	4.0	EUROPEAN	Shoulder	Clean	25	637	No	Withdraw	(Lost to follow-up)
M*	9.2	EUROPEAN	Thorax	Dirty	13	721	No	No	
M*	11.9	EUROPEAN	Shoulder	Dirty	28	7	Yes	Yes	
M*	6.9	EUROPEAN	Thorax	Dirty	31	756	No	No	
M*	8.3	EUROPEAN	Flank	Dirty	18	98	Yes	Yes	
F*	14.1	EUROPEAN	Interscapular	Dirty	20	175	Yes	Yes	
M*	10.5	PERSIAN CROSSED	Lumbar	Dirty	62	739	No	No	
M*	7.6	EUROPEAN	Interscapular	Dirty	49	735	No	No	
F*	9.3	EUROPEAN	Thorax	Dirty	52	732	No	No	
M*	6.9	EUROPEAN	Flank	Dirty	27	35	Yes	Yes	
M*	6.6	EUROPEAN	Flank	Dirty	38	49	Yes	Yes	
F*	13.8	EUROPEAN	Thorax	Dirty	13	273	Yes	Yes	

(continued on next page)

Table 1 (continued)

Sex	Age	Breed	Tumour localization	Surgical margins	Estimated time between surgery and Day 0	DFI	Relapse within 1 year	Relapse within 2 years	
F*	11.9	EUROPEAN	Interscapular	Dirty	49	728	No	No	
F*	6.6	EUROPEAN	Shoulder	Dirty	46	175	Yes	Yes	
M*	7.4	EUROPEAN	Interscapular	Dirty	33	792	No	No	
Low dose ALVAC IL2 group									
M*	11.7	EUROPEAN	Flank	Clean	12	22	Withdraw	Withdraw	(Other localization of FISS)
F*	11.1	EUROPEAN	Interscapular	Clean	38	763	No	No	
F*	9.3	EUROPEAN	Flank	Clean	25	760	No	No	
M*	4.5	EUROPEAN	Thorax	Clean	35	742	No	No	
F*	12.6	EUROPEAN	Flank	Clean	35	724	No	No	
M*	7.2	EUROPEAN	Interscapular	Clean	76	26	Withdraw	withdraw	(Death by strangulation)
M*	10.7	EUROPEAN	Thigh	Clean	55	749	No	No	
F*	7.9	EUROPEAN	Interscapular	Clean	41	161	Yes	Yes	
F*	10.7	EUROPEAN	Flank	Clean	59	756	No	No	
M*	14.2	EUROPEAN	Thorax	Clean	39	204	Withdraw	Withdraw	(Euthanasia – diabetes complication)
F*	8.5	EUROPEAN	Interscapular	Clean	60	408	No	Withdraw	(Lost to follow-up)
M*	7.0	EUROPEAN	Interscapular	Dirty	12	729	No	No	
F*	5.2	EUROPEAN	Flank	Dirty	34	91	Yes	Yes	
M*	13.3	EUROPEAN	Interscapular	Dirty	45	112	Yes	Yes	
M*	8.8	EUROPEAN	Interscapular	Dirty	33	749	No	No	
M*	5.6	EUROPEAN	Interscapular	Dirty	14	21	Yes	Yes	
M*	5.2	EUROPEAN	Interscapular	Dirty	45	735	No	No	
M*	10.1	EUROPEAN	Interscapular	Dirty	24	732	No	No	
M*	13.3	EUROPEAN	Flank	Dirty	68	99	Withdraw	Withdraw	(dead from liver tumour)
F	9.0	EUROPEAN	Flank	Dirty	55	182	Yes	Yes	
F*	6.8	EUROPEAN	Shoulder	Dirty	34	770	No	No	
M*	9.0	BURMESE	Lumbar	Dirty	32	735	No	No	
F*	12.5	EUROPEAN	Interscapular	Dirty	21	763	No	No	
F*	8.2	SIAMESE	Interscapular	Dirty	82	98	Withdraw	Withdraw	(Other localization of FISS)
F*	17.0	EUROPEAN	Lumbar	Dirty	31	555	No	Withdraw	(Euthanasia – anal carcinoma)
High dose ALVAC IL2 group									
M*	12.1	EUROPEAN	Interscapular	Clean	13	379	No	NA	
M*	6.6	RUSSIAN BLUE CAT	Thorax	Dirty	27	364	No	NA	
M*	13.3	EUROPEAN	Interscapular	Clean	27	275	Yes	NA	
F*	13.6	EUROPEAN	Interscapular	Clean	32	372	No	NA	
F*	10.7	EUROPEAN	Flank	Clean	44	370	No	NA	
M*	10.6	EUROPEAN	Interscapular	Clean	47	364	No	NA	
M*	12.1	EUROPEAN	Thorax	Clean	41	364	No	NA	
M*	11.1	EUROPEAN	Interscapular	Clean	10	368	No	NA	
F*	11.9	EUROPEAN	Interscapular	Clean	24	385	No	NA	
M*	8.8	EUROPEAN	Shoulder	Clean	48	350	No	NA	
F*	9.4	EUROPEAN	Interscapular	Dirty	26	182	Yes	NA	
F*	13.0	EUROPEAN	Thorax	Dirty	24	189	Yes	NA	
F*	9.4	EUROPEAN	Interscapular	Dirty	27	368	No	NA	
M*	7.1	EUROPEAN	Flank	Dirty	32	366	No	NA	
F*	13.0	EUROPEAN	Interscapular	Dirty	42	98	Yes	NA	
F*	9.0	EUROPEAN	Interscapular	Dirty	40	91	Yes	NA	
F*	10.4	EUROPEAN	Flank	Dirty	49	379	No	NA	
M*	11.5	EUROPEAN	Interscapular	Dirty	46	91	Yes	NA	
F*	10.6	EUROPEAN	Flank	Dirty	45	364	No	NA	
M*	7.7	EUROPEAN	Lumbar	Dirty	45	182	Yes	NA	
F*	9.3	EUROPEAN	Interscapular	Dirty	49	364	No	NA	
F*	7.3	EUROPEAN	Flank	Dirty	31	368	No	NA	
M*	7.0	EUROPEAN	Interscapular	Dirty	46	378	No	NA	

* Castrated or spayed.

Table 2
Groups comparability before treatment.

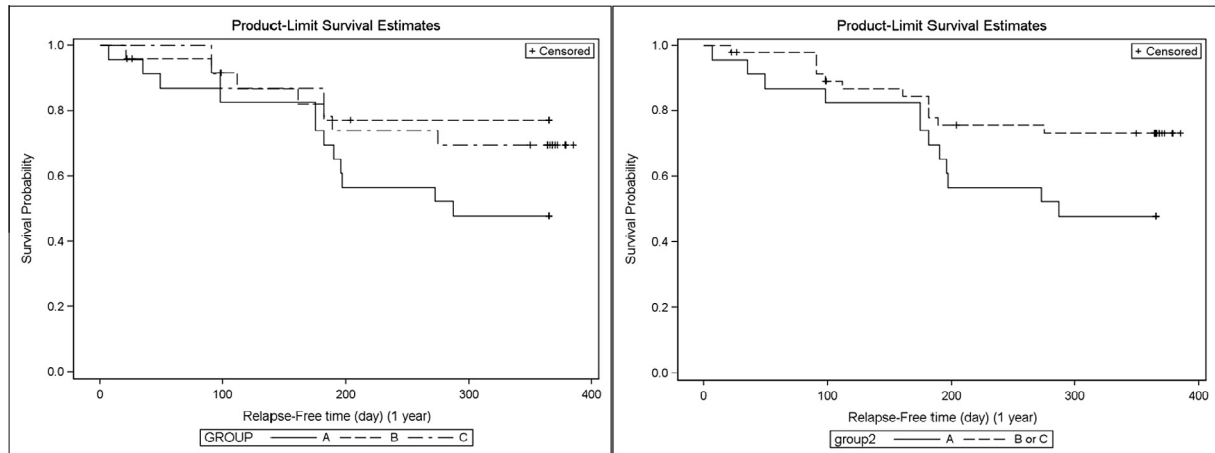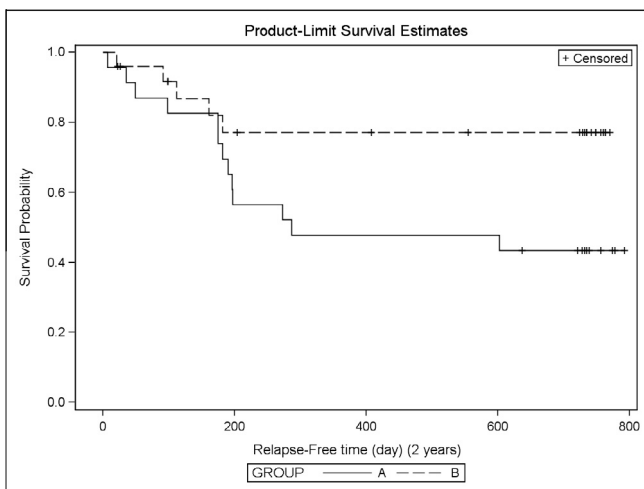|	Control group	ALVAC IL-2 low dose group	ALVAC IL-2 high dose group	Groups comparability
Delay between tumour ablation and post-operative radiotherapy (in days): range (mean/median)	13–62 (34/31)	12–82 (40/35)	10–49 (35/40)	$p = 0.320$[a]
Numbers of cats with dirty margins/total cats (% of dirty margins)	14/23 (61%)	14/25 (56%)	14/23 (61%)	$p = 0.924$[b]

[a] ANOVA.
[b] χ^2 test.

up (2 cats) or secondary FISS at a different site (2 cats). One cat presented a second FISS starting 7 days after Day 0 at a distant site (right axilla) from the primary tumour (right flank). The other cat presented on Day 0, a nodular lesion, visible on the CT-scan. As this lesion was not organized (only an heterogeneity in the left trape-

zius muscle thickness), it was not suspected to be a fibrosarcoma. Three months later this lesion had evolved and was tinted by the contrast medium at CT-scan. This mass was confirmed to be a FISS. This lesion was neither the initial tumour which was not entirely removed, nor a recurrence, as the locations of the tumours were

Table 3
Relapse rate.

		Type of relapse	Rate of relapse (%)	p value[a]
1-Year analysis	Control (n = 23)	9 Recurrence, 3 metastasis	52.2	0.052
	Treated (low and high dose ALVAC IL-2) (n = 43)	7 Recurrence, 3 metastasis, 2 recurrence & metastasis[b]	27.9	
2-Year analysis	Control (n = 22)	10 Recurrence, 3 metastasis	59.1	0.053
	Treated (low dose ALVAC IL-2) (n = 18)	3 Recurrence, 2 metastasis	27.8	

[a] Cochran–Mantel–Haenzel test.
[b] Low dose ALVAC IL-2 (3 recurrence, 2 metastasis) and high dose ALVAC IL-2 group (4 recurrence, 1 metastasis, 2 recurrence & metastasis).

Fig. 2. Survival curves per group at one-year (group A = control group; group B = low dose ALVAC IL-2 treated group; group C = high dose ALVAC IL-2 treated group – left: groups A, B and C; right: groups A and B + C).

Fig. 3. Survival curves per group at two-years (group A = control group; group B = low dose ALVAC IL-2 treated group).

distant (by around 4 cm) and there was no macroscopic lesion between the initial tumour and the new lesion. All other cats were followed-up until relapse for one (high dose ALVAC Il-2) or two years (control and low dose ALVAC IL-2) after enrolment.

The treatment was well tolerated even after administration of a high dose of ALVAC IL-2. No immediate general reaction and a single immediate local reaction attributable to the product were reported (pain on palpation in one cat). Delayed general reactions that were not attributed to another cause were apathy (with no other associated signs) in two cats at one or two occasions, vomiting in one cat and diarrhoea in two cats. Delayed local reactions were limited to a moderate pain in one cat and minor events such as crusts, scratching, alopecia or papule in 6 cats receiving the ALVAC IL-2 at high dose.

There was no significant variation in the biochemical and hematological parameters of treated cats.

ALVAC IL-2 tended to reduce significantly the frequency of relapse at one year post-treatment (52.2% of relapse in the controls versus 27.9% in treated cats, p = 0.052) and at two years post-treatment (59.1% of relapse in the controls versus 27.8% in treated cats, p = 0.053) (Table 3). At one year post-treatment, the performance of ALVAC IL-2 was similar in the low-dose and in the

Table 4
DFI analysis.

		Median DFI (p value[a])	HR [95% HR confidence limits] (p value[b])
1-Year analysis	Control (n = 23)	287 days versus > 365 days (0.048)	0.44 [0.197–0.981] (0.0447)
	Treated (low and high dose ALVAC IL-2) (n = 48)		
2-Year analysis	Control (n = 23)	287 days versus > 730 days (0.046)	0.353 [0.126–0.992] (0.0483)
	Treated (low dose ALVAC IL-2) (n = 25)		

[a] Log-rank test.
[b] Cox model.

high-dose groups (rate of relapse of 25% and 30.4%, respectively), and the median DFI was not reached whichever the treatment (Fig. 2). In the absence of significant difference between the low-dose and high-dose treatments, the comparison after one year of treatment was made between all treated cats and controls.

The median DFI was 287 days for the control group and higher than 365 days (one-year analysis) or higher than 730 days (two-years analysis) for treated cats (Figs. 2 and 3).

Our results show that the ALVAC IL-2 treatment is efficacious and reduced the risk of relapse over one year by 56% and over two-years by 65% in treated cats compared to controls (HR = 0.44, $p = 0.045$ for one-year analysis and HR = 0.35, $p = 0.048$ for two-years analysis, respectively) (Table 4).

4. Discussion

The efficacy of ALVAC IL-2 as an adjunct therapy for FISS has been demonstrated over a one-year follow-up [11]. However, it was important to confirm the efficacy over a longer period of time. Indeed, different recurrence rates at one and two years post-treatment have been reported with some studies [6]. Evaluating the dose effect on both the efficacy and safety of the product was the second objective. Since the safety of ALVAC is related to the vector viral titre, the absence of negative impact of the administration of a high dose to cats that are reported to be very sensitive to any local inflammation was checked.

In addition, after the initial study, the method of administration of the ALVAC IL-2 was optimized. The local expression of IL-2 in the subcutaneous tissue of a 5×5 cm area was measured by quantitative RT-PCR after administering one dose (1 mL) of ALVAC IL-2 in the centre of the square or after splitting it in five points (0.2 mL injected in the centre and in the four corners). Overall, local IL-2 expression was higher with the 5 injections points procedure (data not shown). This method of administration was then applied in our efficacy and safety trial. To standardize the treatment and avoid any difficulty in the interpretation of the results, ALVAC IL-2 was administered according to the same distribution in all cats. We may expect a distribution fitting more with the shape of the tumour bed to be potentially more efficacious.

Anti-tumour responses have been shown to be stronger when IL-2 is administered locally several times [13]. To achieve a sustained local expression of IL-2, administrations of ALVAC IL-2 were repeated over time. This regimen was based on the local persistence of the virus at the site of administration (about 4 days) and, consecutively, the transient expression of the transgene for several days (canarypox virus is not replicative in mammals) [14].

The selection criteria for the enrollment of the patients were restrictive to avoid any bias in the analysis and conclusions of the study. Only cats with a primary tumour within the same limited size range and without metastasis or lymph node enlargement were enrolled. Since the quality of the surgery is a key factor of success for the treatment [3,7,12], the status of the surgical margin assessed by histological examination with the same pathologist was taken into account in the design of the trial (through the randomization scheme) and the statistical analysis. Importantly as well, the interval between surgery and initiation of radiotherapy was similar between the treatment groups. Indeed, a longer interval between surgery and radiotherapy has been reported to have a negative effect on the outcome of the treatment [6]. Overall, the cohort of patients enrolled in this study was representative of the feline population likely to develop FISS.

ALVAC IL-2 treatment resulted in a longer median time to relapse (>730 days) than in controls (287 days) and a reduction of the risk of relapse by 56% after one year and 65% after 2 years compared to controls. No ALVAC IL-2 dose effect was observed

which is consistent with the absence of clear dose–response of local IL-2 reported by others [15,16]. IL-2 is efficacious locally at relatively low doses, equivalent to 10^3 to 10^5 IU IL-2 [16]. On the basis of *in vitro* expression in feline cells, and consistently with other estimations [17], ALVAC IL-2 dosage (between 10^6 and 10^7 $EAID_{50}$) corresponds to a local expression estimated between 10^3 and 10^4 IU IL-2.

Importantly, our study results were consistent with Jourdier's. In that study, the cats were treated according to the same regimen at a dose of $10^{6.7}$ $CCID_{50}$ ALVAC IL-2/dose (1 $CCID_{50}$ # 1 $EAID_{50}$). The rates of relapse at one year in the controls and treated animals were very comparable in the two studies (61% and 52% in Jourdier' and our controls respectively, 28% in treated animals in both studies). It should be noted that only cats with primary tumours were enrolled in our study and this could explain the slightly lower relapse rate observed in controls. The similarity of the results between the two studies demonstrated the robustness of ALVAC IL-2 treatment over a wide range of ALVAC titres.

DFI in control cats was lower than DFI reported in other studies investigating surgery and post-surgery radiotherapy [5, 6]. In our study, surgical excision was done by the referring practitioner for all cats, whereas a fraction of the cats in Bregazzi and Cohen's studies underwent surgery at the university (60% and 37%, respectively). This may have a strong impact on the DFI [3]. In Cohen's study, cats that had conservative surgery started radiotherapy within 1 or 2 days after surgery. Time between surgery and radiotherapy has been shown to be an important parameter and this may also explain the higher DFI in that study.

The DFI in cats treated with surgery/brachytherapy/ALVAC IL-2 was >730 days. This value is one of the highest reported so far in FISS [4]. It is combined with the absence of serious adverse effects such as those occasionally reported after chemotherapy or external radiotherapy.

Control group did not receive any placebo. Cats with FISS are at risk of developing tumours after traumatisms or injections causing inflammation in the subcutis. Injecting a placebo was therefore not acceptable from an ethical standpoint and could have biased the final results. This phenomenon was reported in the efficacy trial with Vero-huIL-2 in cats [10]. Cats receiving Vero cells not expressing IL-2 relapsed within 3 months.

It may then be speculated that the anti-tumour efficacy of ALVAC IL-2 was mediated by the vector itself. ALVAC is indeed able to stimulate innate immunity and induce the production of pro-inflammatory cytokines with potential anti-tumour activity [18,19]. However, treatment of human patients with metastatic melanoma or cutaneous metastases of other cancers with ALVAC expressing either IL-2, IL-12 or GM-CSF resulted in different clinical outcomes and immunological effects [17,20]. In particular, tumour cellular infiltrates and local production of IFNγ depended upon the expressed transgene. Interestingly, tumour regression was more frequent with ALVAC-IL-2 than with the other candidates.

In addition, the efficacy of local IL-2 therapy against FISS has been shown with other delivery systems like Vero cells [10] and vaccinia vector [11].

Overall, this strongly suggests that the anti-tumour activity is determined by the expression of IL-2.

Many cancers are associated with active immune suppression and therefore immunostimulating cytokines are a potentially attractive therapy. As a known cell growth and activation factor for T cells and Natural Killer (NK) cells, IL-2 is one of the candidate cytokines used in cancer immunotherapy. It was the first cytokine licensed by FDA for the treatment of renal cell carcinoma and malignant melanoma. Efficacy of IL-2 is however limited by the toxic effects of the large doses required for effective therapy. Adverse effects of systemic treatment may be overcome by local

delivery using adapted technologies like plasmid-based delivery systems or viral vectors (eg. adenovirus, poxvirus). It has been well established in murine models that local delivery of IL-2 in tumours (intra-tumoural injection or administration in the tumour bed after surgery) may be an effective alternative [21,22]. Local IL-2 therapy has been shown to work against various tumours (fibrosarcoma, melanoma, mastocytoma, …) and in several species (cat, dog, cattle, human) [16]. Anti-tumour effect of local IL-2 was usually associated with the stimulation of CD4+ T cells, CD8+ T cells or NK cells, and production of IFNγ [17,20,23–26]. Since ALVAC IL-2 was administered after surgical excision of the tumour in client-owned cats, it was not possible to assess the local immunological effects of the treatment. Intra-tumoural administration of ALVAC-IL-2 in human patients with melanoma metastases resulted in a peritumoural infiltration with CD3, CD4 and CD8-positive T cells [17].

ALVAC IL-2 treatment was well tolerated even at the highest dose tested. The mild adverse reactions recorded in treated cats were consistent with the safety profile of ALVAC-based vaccines in cats. In those patients, however, it cannot be ruled out that some of these reactions were caused by the brachytherapy treatment and the stress associated with it. As the result of the localized expression of low doses of IL-2, no general reaction which could be associated with the toxicity of this cytokine was observed and the hematological and biochemical parameters remained unchanged. Safety is often a concern with anti-cancer therapies. The good tolerance of ALVAC IL-2 is therefore a key parameter in the benefit/risk balance of the product.

The efficacy of local IL-2 as a single therapy has been demonstrated in several tumour models [21,22,24,25,27–29] and confirmed in cancer patients [17,20,30–33]. Local IL-2 induced 63% of complete regression in cattle with bovine ocular squamous cell carcinomas [15,32] and had clear therapeutic effect in dogs with unresectable mastocytoma [34]. Intra-tumoural administration of ALVAC-IL-2 in human patients with cutaneous metastases of melanoma or leiomyosarcoma resulted in partial regression in 3 out of 8 patients [17]. In face of highly aggressive tumours like FISS, surgical excision remains the primary treatment of choice and IL-2 alone is not expected to be sufficient.

5. Conclusion

Despite aggressive surgery and radiotherapy, FISS remains a therapeutic challenge. The high rate of relapse usually leads to the euthanasia of the cat. As an adjunct therapy to surgery and brachytherapy, ALVAC IL-2[1] was shown to be both efficacious and safe.

This study confirmed that local IL-2 therapy may be efficacious against accessible tumours. Its efficacy as an adjunct therapy would deserve further investigations in other cancers.

Disclaimer

This document is provided for scientific purposes only. Any reference to a brand or a trademark herein is for international purposes only and is not intended for a commercial purpose or to dilute the rights of the respective owner(s) of the brand(s) or trademark(s).

Conflict of interest statement

---

[1] A European marketing authorization has been granted for Oncept IL-2. Oncept IL-2 is registered trademark of Merial. All others marks are the property of their respective owners.

References

[1] Rounak Nande, Gene transfer in a newly established spontaneous feline fibrosarcoma cell line (Theses, Dissertations and Capstones), Paper 429, 2013.
[2] K.M. Davis, E.M. Hardie, F.R. Martin, J. Zhu, C. Brownie, Correlation between perioperative factors and successful outcome in fibrosarcoma resection in cats, Vet. Rec. 161 (2007) 199–200.
[3] A.E. Hershey, K.U. Sorenmo, M.J. Hendrick, F.S. Schofer, D.M. Vail, Prognosis for presumed feline vaccine associated sarcoma after excision: 61 cases (1986–1996), J. Am. Vet. Med. Assoc. 216 (2000) 58–61.
[4] M. Martano, E. Morello, P. Buracco, Feline injection-site sarcoma: past, present and future perspectives, Vet. J. 188 (2011) 136–141.
[5] V.S. Bregazzi, S.M. LaRue, E. McNiel, D.W. Macy, W.S. Dernell, B.E. Powers, et al., Treatment with a combination of doxorubicin, surgery, and radiation versus surgery and radiation alone for cats with vaccine-associated sarcomas: 25 cases (1995–2000), J. Am. Vet. Med. Assoc. 218 (2001) 547–550.
[6] M. Cohen, J. Wright, W.R. Brawner, A. Smith, R. Henderson, E. Behrend, Use of surgery and electron beam irradiation, with or without chemotherapy, for treatment of vaccine-associated sarcomas in cats: 78 cases (1996–2000), J. Am. Vet. Med. Assoc. 219 (2001) 1582–1589.
[7] T. Kobayashi, M. Hauck, R. Dodge, R.L. Page, G.S. Price, L.E. Williams, et al., Preoperative radiotherapy for vaccine associated sarcoma in 92 cats, Vet. Radiol. Ultrasound 43 (2002) 473–479.
[8] M.N. Mayer, P.L. Treuil, S.M. LaRue, Radiotherapy and surgery for feline soft tissue sarcoma, Vet. Radiol. Ultrasound 50 (2009) 669–672.
[9] C. Eckstein, F. Guscetti, M. Roos, J. Martin de las Mulas, B. Kaser-Hotz, C. Rohrer Bley, A retrospective analysis of radiation therapy for the treatment of feline vaccine-associated sarcoma, Vet. Comp. Oncol. 7 (2009) 54–68.
[10] F. Quintin-Colonna, P. Devauchelle, D. Fradelizi, B. Mourot, T. Faure, P. Kourilsky, et al., Gene therapy of spontaneous canine melanoma and feline fibrosarcoma by intratumoural administration of histoincompatible cells expressing human interleukin-2, Gene Ther. 3 (1996) 1104–1112.
[11] T.M. Jourdier, C. Moste, M.C. Bonnet, F. Delisle, J.P. Tafani, P. Devauchelle, et al., Local immunotherapy of spontaneous feline fibrosarcomas using recombinant poxviruses expressing interleukin 2 (IL2), Gene Ther. 10 (2003) 2126–2132.
[12] K. Cronin, R.L. Page, G. Spodnick, R. Dodge, E.N. Hardie, G.S. Price, et al., Radiation therapy and surgery for fibrosarcoma in 33 cats, Vet. Radiol. Ultrasound 39 (1998) 51–56.
[13] W. Den Otter, J.W. De Groot, M.R. Bernsen, A.P. Heintz, R. Maas, G.J. Hordijk, et al., Optimal regimes for local IL-2 tumour therapy, Int. J. Cancer 66 (1996) 400–403.
[14] C. Zanotto, E. Pozzi, S. Pacchioni, L. Volonté, C. De Giuli Morghen, A. Radaelli, Canarypox and fowlpox viruses as recombinant vaccine vectors: a biological and immunological comparison, Antiviral Res. 88 (2010) 53–63.
[15] R.J. Stewart, F.W. Hill, A. Masztalerz, J.J. Jacobs, J.W. Koten, W. Den Otter, Treatment of ocular squamous cell carcinomas in cattle with interleukin-2, Vet. Rec. 159 (2006) 668–672.
[16] W. Den Otter, J.J. Jacobs, J.J. Battermann, G.J. Hordijk, Z. Krastev, E.V. Moiseeva, et al., Local therapy of cancer with free IL-2, Cancer Immunol. Immunother. 57 (2008) 931–950.
[17] G.F.L. Hofbauer, T. Baur, M.C. Bonnet, E. Tartour, G. Burg, N.L. Berinstein, et al., Clinical phase I intratumoural administration of two recombinant ALVAC canarypox viruses expressing human granulocyte-macrophage colony-stimulating factor or interleukin-2: the transgene determines the composition of the inflammatory infiltrate, Melanoma Res. 18 (2008) 104–111.
[18] R. Ignatius, M. Marovich, E. Mehlhop, L. Villamide, K. Mahnke, W.I. Cox, et al., Canarypox virus-induced maturation of dendritic cells is mediated by apoptotic cell death and tumour necrosis factor alpha secretion, J. Virol. 74 (2000) 11329–11338.
[19] E.J. Ryan, A. Harenberg, N. Burdin, The Canarypox-virus vaccine vector ALVAC triggers the release of IFN-gamma by Natural Killer (NK) cells enhancing Th1 polarization, Vaccine 25 (2007) 3380–3390.
[20] J. Tartaglia, M.C. Bonnet, N. Berinstein, B. Barber, M. Klein, P. Moingeon, Therapeutic vaccines against melanoma and colorectal cancer, Vaccine 19 (2001) 2571–2575.
[21] L. Cordier, M.T. Duffour, J.C. Sabourin, M.G. Lee, J. Cabannes, T. Ragot, et al., Complete recovery of mice from a pre-established tumour by direct intratumoural delivery of an adenovirus vector harboring the murine IL-2 gene, Gene Ther. 2 (1995) 16–21.
[22] J.P. Levraud, M.T. Duffour, L. Cordier, M. Perricaudet, H. Haddada, P. Kourilsky, IL-2 gene delivery within an established murine tumour causes its regression without proliferation of preexisting antitumour-specific CTL, J. Immunol. 158 (3335) (1997) 3343.
[23] E. Tartour, M. Mehtali, X. Sastre-Garau, I. Joyeux, C. Mathiot, J.M. Pleau, et al., Phase I clinical trial with IL-2 transfected xenogeneic cells administered in

subcutaneous metastatic tumours: clinical and immunological findings, Br. J. Cancer 83 (2000) 1454–1461.

[24] P. Slos, M. De Meyer, P. Leroy, C. Rousseau, B. Acres, Immunotherapy of established tumours in mice by intratumoural injection of an adenovirus vector harboring the human IL-2 cDNA: induction of CD8(+) T-cell immunity and NK activity, Cancer Gene Ther. 8 (2001) 321–332.

[25] C. Jackaman, C.S. Bundell, B.F. Kinnear, A.M. Smith, P. Filion, D. van Hagen, et al., IL-2 intratumoural immunotherapy enhances CD8+ T cells that mediate destruction of tumour cells and tumour-associated vasculature: a novel mechanism for IL-2, J. Immunol. 171 (2003) 5051–5063.

[26] L. Yao, S. Ng, H. Long-Fei, B.K.C. Chow, Z. Shen, M. Yang, et al., Effective melanoma immunotherapy with Interleukin-2 delivered by a novel polymeric nanoparticle, Mol. Cancer Ther. 10 (2011) 1082–1092.

[27] J.C. Becker, J.D. Pancook, S.D. Gillies, K. Furukawa, R.A. Reisfeld, T cell-mediated eradication of murine metastatic melanoma induced by targeted interleukin 2 therapy, J. Exp. Med. 183 (1996) 2361–2366.

[28] D.C. Saffran, H.M. Horton, M.A. Yankauckas, D. Anderson, K.M. Barnhart, A.M. Abai, et al., Immunotherapy of established tumours in mice by intratumoural injection of interleukin-2 plasmid DNA: induction of CD8+ T-cell immunity, Cancer Gene Ther. 5 (1998) 321–330.

[29] Y. Horiguchi, W.A. Larchian, R. Kaplinsky, W.R. Fair, W.D. Heston, Intravesical liposome-mediated interleukin-2 gene therapy in orthotopic murine bladder cancer model, Gene Ther. 7 (2000) 844–851.

[30] E. Galanis, P.A. Burch, R.L. Richardson, B. Lewis, H.C. Pitot, S. Frytak, et al., Intratumoural administration of a 1,2-dimyristyloxypropyl-3-dimethylhydr oxyethyl ammonium bromide/dioleoylphosphatidylethanolamine formulation of the human interleukin-2 gene in the treatment of metastatic renal cell carcinoma, Cancer 101 (2004) 2557–2566.

[31] S. Dow, R. Elmslie, I. Kurzman, G. MacEwen, F. Pericle, D. Liggitt, et al., Phase I study of liposome-DNA complexes encoding the interleukin-2 gene in dogs with osteosarcoma lung metastases, Hum. Gene Ther. 16 (2005) 937–946.

[32] R.J. Stewart, A. Masztalerz, J.J. Jacobs, W. Den Otter, Local interleukin-2 and interleukin-12 therapy of bovine ocular squamous cell carcinomas, Vet. Immunol. Immunopathol. 106 (2005) 277–284.

[33] B. Weide, T.K. Eigentler, A. Pflugfelder, U. Leiter, F. Meier, J. Bauer, et al., Survival after intratumoural interleukin-2 treatment of 72 melanoma patients and response upon the first chemotherapy during follow-up, Cancer Immunol. Immunother. 60 (2011) 487–493.

[34] P.G. Ziekman, W.D. Otter, J.F. Tan, E. Teske, J. Kirpensteijn, J.W. Koten, J.J. Jacobs, Intratumoural interleukin-2 therapy can induce regression of non-resectable mastocytoma in dogs, Anticancer Res. 33 (2013) 161–165.