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Treatment of Equine Sarcoid with the mistletoe extract ISCADOR® P (*viscum album austriacus*)

- a double-blind placebo controlled study

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Summary

Background:

The Equine Sarcoid (ES) is the most common skin tumour in horses. It is difficult to treat: recurrence rate is high and no single universally effective treatment for ES is known. Viscum album extracts (VAE) are used as adjunct in the treatment of some human cancers without known side effects.

Hypothesis:

We hypothesized that the long-term therapy with VAE (Iscador® P) is effective in Equine Sarcoids as a single treatment or as an adjunct treatment to selective surgical excision.

Material and methods:

Of 53 horses with ES, 42 were treated solely with VAE as monotherapy and 11 were treated after selective excision of ES. Horses were randomly assigned to the treatment group (VAE; n=32) or control group (Placebo; n=21). The horses received rising concentrations of Iscador® P extract from 0.1 mg per ml to 20 mg per ml or physiological NaCl solution 3 times a week over 105 days s.c. Number, localization, size and type of the ES were documented over 12 months. Horses were grouped in 3 classes: individuals with <3 ES, 3 to 9 ES and >9ES. Statistical analysis of effects was divided in horse level and sarcoid level analysis. In horse level analysis effects were assessed considering the development of all

tumours found in the horse. Outcome of this analysis was cure, improvement (decrease of tumour size by 50% or more in at least half of the tumours), unchanged status or deterioration, respectively. Sarcoid level assessment included analysis of 1 to 7 clinically relevant sarcoids per horse comparing parameters of size and quality of single tumours in both treatment groups. All analyses were performed using statistical package STATA ver.9.2. Significance level was set at $p<0.05$.

Results:

Horse level**:** In the VAE group, 13 horses (40.6%) showed an improvement. Of these, 9 patients showed complete remission (28.1%). In the control group only 3 cases (14%), all showing complete remission, were classified as improved. Significantly better results were observed after VAE treatment in horses with 3 to 9 ES compared to those with <3 ES or >9 ES. Sarcoid level: After one year observation time in the VAE group 27 ES showed complete remission (37.5%) and there was an improvement in 48 (66.7%) ES. In contrast, in the control group 9 ES with complete remission (13.2%; n.s.) and 17 ES (39.5%; p=0.005) with improvement were found. The only side effects observed after VAE were slight oedema at the injection site in 5 of 32 cases, which disappeared spontaneously within two to three days.

Conclusions:

VAE **(**Iscador® P) represent a safe and effective treatment for ES, particularly in cases with multiple sarcoids.

Key words: Equine sarcoid, horse, mistletoe therapy

Introduction

The Equine Sarcoid (ES) is the most common skin tumour in equids (Marti et al. 1993; Teifke 1994). While infiltrative growth and frequent recurrences are observed, there is no tendency for metastasis into other organs (Gerber 1994). Studies in Switzerland revealed prevalences for the Swiss Warmblood horse and the Freiberger horse breed of 0.7% and 0.4%, respectively in 1986 (Dubath 1986) compared to 11.5% (Studer et al. 2007) and 11.9% (Mele et al. 2007) in 2007. Younger horses aged between 2 and 5 years are more frequently affected than horses of other age classes (Reid et al. 1994; Torrontegui et al. 1994; Schnabel 2000). In older horses, an increase of tumour size is observed (Brostrom 1995). Some studies (Lepage et al. 1998) found no evidence for a predisposition according to breed, gender (Torrontegui et al. 1994) and coat colour (Brostrom 1995), while others found that Quarter Horse (Angelos et al. 1988), Appaloosa and Arabian breeds (Mohammed et al. 1992) as well as geldings (Mohammed et al. 1992; Reid et al. 1994) have a higher risk to suffer from ES. Furthermore, a recent study shows that chestnut horses are at an increased risk to develop ES (Mele et al. 2007).

Sarcoids are classified as occult, verrucous, nodular, fibroblastic or mixed types (Diehl et al. 1987; Martens et al. 2000). Additionally, a rare malign type has been described (Knottenbelt et al. 1995; Pascoe et al. 1999). The frequency of multiple appearance ranges between 33 (Brostrom 1995) und 76% (Torrontegui et al. 1994) of all cases (Brandt et al. 1996; Martens et al. 2001). ES are found more frequently on the head, abdomen, axillary and inguinal regions than in other areas of the body (Teifke 1994; Brandt et al. 1996). Spontaneous regression of ES is probably rare, but has been observed in young horses (Studer et al. 1997), in patients with low numbers of tumours (Brostrom 1995; Piscopo 1999) and those, which have not been treated (Germann 2006).

Knowledge of causes and pathogenesis is still incomplete. There is now good evidence that

Bovine Papilloma Virus Type 1 and 2 (BPV1, BPV2) represent the causal agents (Ragland et al. 1969; Lancaster et al. 1977; Chambers et al. 2003). Virus-DNA could be detected in tumour material by PCR (Trenfield et al. 1985; Carr et al. 2001; Martens et al. 2001) and the presence of the BPV protein E5 was found by Western blot in tumours too (Carr et al. 2001). A genetic disposition appears to play a prominent role in the growth of ES (Meredith et al. 1986; Brostrom et al. 1988; Gerber 1989). Occurrence of ES is highly associated with certain equine leucocytes antigens, which reflect the Major Histocompatibility Complex (MHC) of the individual (Lazary et al. 1985; Lazary et al. 1986; Lazary et al. 1994; Brostrom 1995). Tumour-induced immuno-suppression may also play a role in sarcoid development (Muller 1991; Jungi 2000; Chambers et al. 2003). Prior skin lesions as a causal factor and as possible entry site are discussed as part of the multifactorial sarcoid genesis (Voss 1969; Carr et al. 2001). Hypotheses are exist about virus transmission by biting flies (Knottenbelt et al. 1995; Chambers et al. 2003) and ES promoting factors associated with the environment (Brandt et al. 1996). The vicinity of cattle infected with BPV (Bogaert et al. 2005) and barn conditions (Mele et al. 2007) are discussed as risk factors.

A clinical diagnosis of ES is based on tumour morphology in association with localization, history and age (Lepage et al. 1998). However, in order to confirm the diagnosis, histological examination of tumour tissue is mandatory (Tarwid et al. 1985). When performing a diagnostic biopsy, one showed keep in mind that every invasive manipulation of tumours may result in more aggressive tumour growth (Tarwid et al. 1985; Lepage et al. 1998).

Various therapeutic measures have been described. Common surgical approaches are conventional surgical excision (Ragland et al. 1970; Diehl et al. 1987), cryosurgery using liquid nitrogen (Klein 1987) and laser surgery (Brandt et al. 1996; Carstanjen et al. 1997). Other physical treatment methods are thermotherapy (McConaghy et al. 1994), radioactive implants with Iridium-192 (Theon et al. 1995; Byam-Cook et al. 2006) and photodynamic therapy (Martens et al. 2000). Local chemotherapy is used in the form of solutions and

ointments containing for example cisplatin (Theon et al. 1993; Otten et al. 1994; Knottenbelt et al. 2000; Rols et al. 2002; Nogueira et al. 2006; Stewart et al. 2006). Immunotherapy in order to stimulate the auto-defensive mechanisms represents another therapeutic strategy (Rush et al. 2000). Veterinary immunomodulatory preparations include exogenous immunostimulants derived from bacterial (BCG-vaccine) (Wyman et al. 1977; Klein 1987; Owen et al. 1987; Steiner 1988; Vanselow et al. 1988; Martens et al. 2001), viral (Baypamun) (Studer et al. 1997), or plant (Rush et al. 2000) sources and endogenous immunostimulants as TNF (Otten et al. 1994), interleukin (Germann 2006), interferon or autogenous vaccine (Kinnunen et al. 1999; Hallamaa et al. 2005; Mattil-Fritz et al. 2008). Finally, complementary and alternative treatment methods, like homoeopathy (Wolter 1985; Schwierczena 1993) and phytotherapy (Von Felbert et al. 2005) complete the variety of therapeutics concepts. Drawbacks of some of these treatments include cost (Carstanjen et al. 1997), technical difficulty (Carstanjen et al. 1998) and the need of special equipment (Diehl et al. 1987; Carstanjen et al. 1998). Some therapies are useless in certain ES localisations (Owen et al. 1987; Marti et al. 1993; Carstanjen et al. 1998), are of low efficacy (Otten et al. 1994; Studer et al. 1997), are accompanied by frequent recurrences (Ragland et al. 1970; Diehl et al. 1987; Carstanjen et al. 1997), or generate undesirable side effects (Klein 1987; Vanselow et al. 1988; Knottenbelt et al. 2000; Nogueira et al. 2006). Overall, it can be concluded that none of these methods is universally effective (Marti et al. 1993). While ES is a virus-associated disease (Ragland et al. 1969; Trenfield et al. 1985; Carr et al. 2001), an immunosuppressive component has been shown (Muller 1991; Jungi 2000; Chambers et al. 2003). Therefore, stimulation of the immune system is the rationale for several therapeutic approaches.

Therefore, combinations of treatment modalities are often necessary in clinical practice. The decision, which single or combined therapy to use, depends on many factors such as tumour characteristic, history, clinical preference, practicability, side effect and costs (Diehl et al.

1987; Goodrich et al. 1998; Pascoe et al. 1999) and is important to increase remission rates (Bertone et al. 1990; Brandt et al. 1996).

Mistletoe extracts have been used in human cancer treatment for over 80 years: European mistletoe, *Viscum album L.*, aqueous extracts (VAE) were introduced for the treatment of cancer by Steiner and Wegmann in the early 1900s (Kienle et al. 2003). Mistletoe products contain a variety of biologically active compounds: lectins, viscotoxins, flavonoids, phenylpropanoids, phytosterols, triterpenes, mono- and polysaccharides (Franz et al. 1981; Kienle et al. 2003). Mistletoe grows on host trees and the tree species significantly influences the chemical composition of the VAE (Ochocka J.R. 2002). The most important host trees for commercial use of VAE are: oak tree (Quercus=Qu), apple tree (Malus=M) and pine tree (Pinus=P) and the most common Iscador® preparations, Iscador® Qu, Iscador® M and Iscador® P, differ in there concentrations of lectins and viscotoxins, the main active ingredients (Overstolz 2005). Iscador® P, manufactured by extracts of pine mistletoes (*viscum album ssp. austriacus*) contains less viscotoxin and lectines than the other preparations (Urech et al. 2006) and is recommended for the treatment of human skin tumours ("Fachinformation Iscador®" 2007).

The anti-tumoural properties of the VAE are mainly attributed to mistletoe lectins and viscotoxins acting at high concentrations by a direct cytotoxic inhibition of tumour growth, but other components may also contribute to these effects (Maldacker 2006; Eggenschwiler et al. 2007) and at low dosages (Thies et al. 2007) as immunostimulation (Heinzerling et al. 2006). Mistletoe extracts have cytotoxic and growth-inhibiting effects on a variety of human tumour cell lines, lymphocytes and fibroblasts in vitro (Kovacs et al. 2006). The cell death of tumour cells is a result of a dose dependent apoptosis (Janssen et al. 1993; Lavastre et al. 2002) (induced by lectins) or necrosis (induced by viscotoxins) (Bussing et al. 1999; Mossalayi et al. 2006). At low dosages (Thies et al. 2007), VAE modulate the function of a number of immunological effectors cells (Beuth et al. 1994; Huber et al. 2006). In vitro and in

vivo studies have demonstrated activation of macrophages, natural killer cells (Tabiasco et al. 2002), granulocytes and B- and T-lymphocytes (Fischer et al. 1997; Stein et al. 1997), which is associated with the release of interleukins, tumour necrosis factor-α and interferon γ (Eggenschwiler et al. 2006). So the mistletoe extract have induced an activation of both specific and non-specific components of the immune system (Gorter et al. 1998). Mistletoe extracts possess antimutagenic effects, DNA stabilizing properties (Kovacs et al. 1991; Bussing et al. 1995) and inhibit in vivo angiogenesis, which plays an essential role in tumour progression and metastasis (Elluru et al. 2006).

In human medicine, VAE are used as an adjunct therapy in addition to chemotherapy or radiotherapy rather than as monotherapy (Bock et al. 2004; Augustin et al. 2005). A systematic review of prospective and controlled clinical trials conducted with mistletoe extracts reported a benefit for survival, quality of life and a reduction of side effects of surgery, chemotherapy and radiation but related tumour remissions in certain cases too (Kienle et al. 2007). In laboratory animals, VAE show potent anti-tumoural effects when administered either directly into the tumour, systemically, subcutaneously or by the oral route (Beuth et al. 2006; Pryme et al. 2006). Results of in vitro trials and experiences in longitudinal observations of immunological parameters suggest that individual dosage and host tree of viscum preparation are of high importance (Beuth et al. 1994; Harmsma et al. 2004; Eggenschwiler et al. 2006).

Anecdotal reports from practitioners in Switzerland and Germany have indicated good results with mistletoe therapy, but scientific data on VAE therapy in ES are not available. The goal of the present study was therefore to evaluate the effects of a viscum album preparation (Iscador® P, Weleda Inc. Arlesheim, CH) on ES of horses. A placebo-controlled double-blind study design was chosen to test the hypothesis that long-term VAE therapy is an effective treatment of ES, as monotherapy or as an adjunct treatment to selective surgical excision.

Material & Methods

Horses

Fifty-three horses with ES entered the study. The patients were recruited in Western Switzerland and represented different breeds and age classes (3 to 17 years, mean 7.2±3.9) **(table 1)**.

Tab.1: Sex, age, breed, coat colour and number of tumours per horse in the study population.

Group		VAE			Control			Total	
	Level	N	%		N	%		N	%
Sex	Mare (23)	14	43.8		9	42.9		23	43.4
	Stallion (5)	1	3.1		4	19.0		5	9.4
	Gelding (25)	17	53.1		8	38.1		25	47.2
Age	1 to 5 years	14	43.7		8	38.1		22	41.5
	6 to 9 years	10	31.2		10	47.6		20	37.7
	Over 9 years	8	25.0		3	14.3		11	20.7
Breed	Freiberger (11)	6	18.8		5	23.8		11	20.8
	Swiss Warmblood (26)	15	46.9		11	52.4		26	49.1
	Thorough-bred (6)	4	12.5		2	9.5		6	11.3
	Other (10)	7	21.9		3	14.3		10	18.9
Colour	Chestnut (15)	9	28.1		6	28.6		15	28.3
	Bay (29)	16	50.0		13	61.9		29	54.7
	Other (9)	7	21.9		2	9.5		9	17.0
ES freq.	1 to 2 per horse	7	21.9		6	28.6		13	24.5
	3 to 9 per horse	12	37.5		13	61.9		25	47.2
	Over 9 per horse	13	**40.6**[a]		2	**9.5**[b]		15	28.3
Total		32			21			53	
Values with different superscripts (a,b) are significantly different with $p<0.05$ (Fisher Exact Test)									

Before the start of the therapeutic protocol, all horses were examined clinically. Data concerning signalement, history, and general condition were recorded. Blood samples were taken for haematology and blood-chemistry and only horses without significant changes in blood cell or chemical parameter were included in the study. Forty-two horses were treated solely with the trial preparations, VAE or placebo, as monotherapy, whereas in 11 patients the

VAE or placebo treatment protocol was started after selective surgical excision. Based on the decision of the clinician and the owner, in these horses a total of 1 to 9 sarcoids were selected for excision. A total of 3 to 45 ES remained as clinically apparent tumours. The decision for tumour excision depended on size, localisation, clinical and pathologic type (i.e verrucous, nodular, fibroblastic, us.) as well as aesthetic criteria. VAE or placebo treatment was started two weeks after surgery. In seven horses only one tumour was excised. In the other 4 patients 2, 3, 8 and 9 tumours, respectively, were excised. Horses after complete excision of all sarcoids were not included. Furthermore, horses with other ES related therapies within 8 weeks before treatment or with other diseases were excluded.

Equine Sarcoids

In all patients the number of sarcoids, localisation (head, neck, chest, axillary region, abdomen, prepuce and udder, inner hind leg and distal leg), demarcation, type (occult, verrucous, nodular, fibroblastic, mixed) and status (dry, ulcerated, sanguineous, infected) was recorded before the start of the treatment. Degree of severity was categorized in 3 classes: Horses with more than nine tumours were classified as severely affected, the others as moderately (3-9 tumours per horse) or mildly affected (1-2 tumours per horse). Tumours were documented by digital photography. In 42 cases a histological examination was performed on the excised tumours or biopsy.

In order to analyse the development of single tumours under treatment (sarcoid level), selected tumours of foremost clinical importance according to morphology and/or size (1 to 7 per horse; n=163) were observed and evaluated in detail (see above). Additionally the largest width, length and height were recorded. The sarcoid volume was then calculated based on these three measures using the formula (1) (Steel 1997):

$$TV_i = a * b * h * \pi / 6 \quad (1)$$

Where TVi is the estimated volume (mm^3) of single tumour, a and b are the length of two maximum perpendicular diameters and h the maximal height over skin level.

For assessment of therapy effectiveness on horse level, all clinically apparent tumours were considered (1 to 45 per horse; n=444) concerning qualitative development and remission.

Treatment groups and protocol

The horses were randomly assigned to the treatment (VAE) or control group treated by placebo. In order to limit the size of placebo group, randomization was conducted with a ratio of 2:1 throwing a cube for every case where the numbers 1 to 4 were assigned to the VAE group and 5 and 6 to the control group. The distribution was not significantly different to the expected distribution (p=0.55). Trial preparations were coded; the investigator (OC) was blinded to VAE or placebo injection. Thirty-two horses received the VAE formulation and twenty-one the placebo. All horses were treated for 15 weeks. They received 3 subcutaneous injections per week (Monday, Wednesday and Friday) each consisting of 1 ml of the formulation. The VAE group received an aqueous extract of fermented *viscum album austriacus* juice, mixed of juices harvested in summer and winter (Iscador® P, Weleda, Arlesheim) (Overstolz 2005). The basic extract was diluted with sterile saline water to concentrations between 0.01 mg/ml to 20 mg/ml.

The dosage protocol is shown in **table 2**. The treatment protocol and the choice of pine mistletoe was based on human treatment schedules applied in skin cancer patients ("Fachinformation Iscador®" 2007), adapted to the body weight of horses and pre-evaluated for undesired side effects. The preparations used in the therapy scheme were identical to the commercial packages of Iscador® P Series I (week 1 to 5) and Series II (week 6 to 10). Series packages consist of 14 dosages with increasing VAE concentrations as shown in table 2. After

dosage escalation by series packages, the maintenance dosage of 20mg/ml was administered during week 11 to 15. The maximum dose of 20 mg/ml extract was evaluated for undesired side effects in a previous tolerance test in 7 horses. Horses of the control group were injected 1 ml of 0.9% saline solution with the same frequency as described for the VAE group. All injections were administered subcutaneously in the pectoral region.

Tab.2: Treatment protocol of the VAE group; administered concentrations.

	MONDAY	WEDNESDAY	FRIDAY
Iscador® P SERIE I			
Week 1	0.1 mg/ml	0.1 mg/ml	0.1 mg/ml
Week 2	0.1 mg/ml	1.0 mg/ml	1.0 mg/ml
Week 3	1.0 mg/ml	1.0 mg/ml	10.0 mg/ml
Week 4	10.0 mg/ml	10.0 mg/ml	10.0 mg/ml
Week 5	10.0 mg/ml	10.0 mg/ml	-
Iscador® P SERIE II			
Week 6	1.0 mg/ml	1.0 mg/ml	1.0 mg/ml
Week 7	1.0 mg/ml	10.0 mg/ml	10.0 mg/ml
Week 8	10.0 mg/ml	10.0 mg/ml	20.0 mg/ml
Week 9	20.0 mg/ml	20.0 mg/ml	20.0 mg/ml
Week 10	20.0 mg/ml	20.0 mg/ml	-
Maintenance Dosage: Iscador® P 20mg/ml			
Week 11-15	20.0 mg/ml	20.0 mg/ml	20.0 mg/ml

Follow up

During the treatment period a clinical examination focussing on side effects and local oedema from the former treatment was performed before any injection. During the treatment period every 5 weeks and during post-treatment observation time every 3 months, a detailed examination of the tumours was performed, as described above. Because of the non-linear growth dynamics of ES, the calculated volume was logarithmically transformed and then the relative tumour size compared to the base-line value (=1) calculated using formula (2):

$$RTV_t = \log(TV_t) / \log(TV_0) \quad (2)$$

Where RTVt is the relative tumour volume after time t, TVt is the tumour volume after time t and TV0 the base-line tumour volume before the start of the treatment.

The evaluation of tumour state was conducted every 3 months. The control points after start (t0) were defined as t1 (3 months after therapy start), t2 (6 months), t3 (9 months) and t4 (1 year), respectively. During these 4 control points the outcome at horse level was assessed as well as the findings at sarcoid level. In case of decision of an early study abandon by the owner or the veterinarian, the evaluation at the last control point was considered as the Final Assessment (FA). The FA of the other horses was represented by the findings at t4. While 47 horses finalized the entire observation course, 16 horses had to be assessed ahead of observation time **(table 3)**.

Horse No.	Trial group	Break Reason	Last Status	Final Assessment (FA)
52	Control	surgery	T2	Deteriorated
29	Control	Deterioration	T2	Deteriorated
39	VAE	surgery	T2	Deteriorated
64	VAE	surgery	T2	Unchanged
21	Control	surgery	T3	Deteriorated
35	Control	sold	T3	Deteriorated
62	Control	surgery	T3	Deteriorated
27	Control	Deterioration	T3	Deteriorated
32	Control	death	T3	Unchanged
24	VAE	surgery	T3	Deteriorated
70	VAE	Deterioration	T3	Deteriorated
59	VAE	surgery	T3	Deteriorated
41	VAE	death	T3	Improved
50	VAE	local therapy	T3	Unchanged
34	VAE	local therapy	T3	Improved
8	VAE	local therapy	T3	Deteriorated

Tab. 3: Horses with early observation breaks before final status evaluation and overall assessment.

Final assessment

In order to assess the effects between the treatment groups, the outcome on horse level was defined by distinguishing between 4 effect levels **(table 4)**: horses were classified as "TUMOUR FREE" when all sarcoids had disappeared. Patients were assessed as "IMPROVED" if they showed a decrease of tumour size by 50% or more in at least half of the tumours. They were assigned as "UNCHANGED" if the tumour size changes less than 50% or size reduction of a minority of tumours. All horses, in which tumour growth exceeded these thresholds, were classified as "DETERIORATED" at the respective control point.

Tab.4: Definitions of therapy efficacy assessment on horse and sarcoid level.

Assessment	**Cured**	**Improved**	**Unchanged**	**Deteriorated**
Outcome	**Non-deteriorated rate (NDR) ---------->**			
	Improvement rate (IR) ---- >			
	Cure rate (CR)			
Criteria horse level	Tumours completely disappeared; no recurrences	Tumour size reduction to 50% or more of the base-line size in at least half of the ES	Tumour size changes less than 50% or size reduction of a minority of tumours	Tumour size increase by at least 50%, or new tumours
Criteria sarcoid level	Tumour completely disappeared	Tumour size reduction by at least 50%	Tumour size changes less than 50%	Tumour size increase by at least 50%

In order to calculate graded effect rates for group comparison, the Cure Rate (CR) including only tumour free horses, the Improvement Rate (IR) including tumour free and improved horses, and the Non-deterioration rate (NDR) including all tumour free, improved and not

deteriorated horses, were assessed as binary outcomes. In analogy, for single tumours the Cure rate (complete regression of tumours), Improvement rate (tumour size decreased by 50% or more) and Non-deterioration rate was calculated (**table 4)**. The described outcomes (CR, IR, NDR) were calculated for the 4 control points (t1-4) and for the final assessment (FA).

Statistical methods

Standard descriptive methods were used for summarizing statistical data. Effects between treatment groups (horse level, tumour level) were compared according to the outcome rates (CR, IR, NDR) at the 4 control points (T1-4) and the final assessment (FA). Differences were tested by using Chi-square and Fisher Exact tests.

For the evaluation of factors influencing therapy effects, on horse level and tumour level a mono-factorial analysis of association was performed using Chi-square / Fisher Exact Test methods. This analysis was used only for T4 and FA. On horse level, the variables SEX (mare, stallion, gelding), AGECLASS (1 to 5 years, 5 to 9 years, 10 and more years), BREED (Swiss Warmblood, Freiberger, other), COLOUR (chestnut, brown, other), SARCOIDCOUNT (1 to 2 ES/horse, 3 to 9 ES/horse, 10 and more ES/horse), THERAPY (monotherapy, selective surgery) and HUSBANDRY (box, free, other) were evaluated. On sarcoid level, the independent variables BIOPSY (yes, no), SARCOIDTYPE (verrucous, occult, nodular, fibroblastic, mixed), SARCOIDSTATUS (ulcerated, dry, other), DEMARCATION (demarcated, diffuse) and VOLUME (<10 mm^3, 11-100 mm^3, 101-1000 mm^3, >1000 mm^3) were tested. If monofactorial analysis showed a $p<0.20$, the variable was included in a multivariate logistic regression model for each of the outcome variables (CR, IR, NDR) with treatment group as a fixed variable. The logistic regression model was optimized stepwise by omitting the variable with the highest p-value when $p>0.05$. When all model variables showed significant effects, the final model was calculated. In order to allow for horse effects, in tumour level models the independent tumour variables have been

clustered within the corresponding horse (variable horse identity (HorseID)) using the function parameters "…,cl(horseID) r" in the LOGISTIC procedure of the statistical software package.

Relative tumour growth was compared after logarithmic transformation as described above. Since data was not normally distributed, comparison between treatment groups was performed using non-parametric rank-sum-test (Wilcoxon). All analyses were performed using statistical package STATA ver. 9.2. Significance level was set at $p<0.05$.

Results

Horse level

A total of 53 horses entered the study. Of these, 32 were assigned to the VAE group (Iscador®) and 21 to the control group (placebo). The distribution of gender, breed, coat colour and number of ES per horse is shown in table 1. The average age of the horses was 7.2 (±3.9) with a range from 3 to 17 and a median of 6 years. In the VAE group the horses were on average 7.4 years (±4.2) old compared to 6.8 (±3.4) in the control group, both groups were in the same range of 3 to 17 years. A significant difference between treatment (10.4 ±10.2) and control groups (5.2 ±3.2) was found for the initial number of ES per horse (p=0.028) **(table 1, p.10)**. A total number of 444 sarcoids were identified in 53 horses (1 to 45 sarcoids per horse, mean 8.4 +/-8.5). Three horses (5.7%) showed only one tumour, ten horses suffered from two (18.9%) and 40 (75.5%) had more than two sarcoids. 15 horses were classified as severely affected (28.3%), 25 as moderately affected (47.2%) and 13 as mildly affected (24.5%). For detailed tumour observation 163 sarcoids were selected following the inclusion criteria described above (3.20 ±1.40 per horse).

Most frequently affected were the abdominal region (43 horses; 81.1%), the axillary region (24 horses; 45.3%) and the inner hind leg (29; 54.7%). Furthermore, sarcoids were found on the head (18; 34.0%), neck (18; 34.0%), chest (17; 32.1%), prepuce and udder (13; 24.5%) and distal leg regions (3; 5.7%). Except for the malignant form of equine sarcoid, all clinical tumour types were encountered. Horses with multiple ES showed different types: The most common types of ES were verrucous (48 horses; 90.6%) and occult (44; 83.0%) followed by mixed forms (14; 26.4%), nodular (9; 17.0%) and fibroblastic sarcoids (9; 17.0%). While all horses had some dry sarcoids, in 35% of the cases ulcerous and in 7.5% bleeding or infected tumours were also identified.

16 horses (9 in VAE, 7 in control group) could not be observed until the end of trial period due to other therapies (10 ; 7 VAE, 3 control group), ES deterioration (3 ; 1 VAE, 2 control group) and euthanasia due to COPD (1 VAE) and leg fracture (1 control group). One horse (1 control group) was sold and less to the follow up. Two of these 16 censored horses were assessed as improved at their last control point t3. Both were treated with VAE. The clinical status of the other 14 horses was unchanged or deteriorated at the break point (**table 3, p.14**).

The Iscador® P therapy was well tolerated by the horses. None of the horses reacted with fever, anorexia or other systemic signs. In 5 horses (15.6%) treated by Iscador® P, a slight oedema in the injection area was observed after a VAE concentration of 10 mg/ml (n=3) or 20 mg/ml (n=2). This oedema disappeared after 2 to 3 days without treatment. Two mares of the VAE group were pregnant for more than 3 months. Both were treated using the identical protocol and had a normal pregnancy and subsequent partum.

At the end of the trial in the VAE group, 9 horses were classified as “tumour free” (28.1%). Adding 4 horses with significant tumour reduction, 13 horses (40.6%) showed an improvement. Furthermore, twelve cases showed no worsening of the status. So, a total of 25 horses were stable, improved or clinically cured during the observation period (78.1%). In the control group, only 3 cases (14.3%), all showing complete remission, were classified as improved. A further 5 cases were stable (23,8%). Differences in improvement rate (p=0.039) and non-deterioration rate (p=0.004) between groups were significant **(table 5 and fig.1)**.

Tab. 5: Outcome on horse level assessed within the 4 quarterly status evaluation points (T1-4) and final assessment at break point (FA).

		Proportion of Outcome assessment at Status evaluation time T1-T4				
Outcome	**Group**	**T1[1)] n (%)**	**T2[1)] n (%)**	**T3[1)] n (%)**	**T4[1)] n (%)**	**FA n (%)**
Cure Rate (CR)	VAE	1 (3.1)	2 (6.5)	2 (6.7)	6 (26.1)	9 (28.1)
	Control	-	2 (10.0)	3 (15.8)	3 (21.4)	3 (14.3)
	P[2)]	0.604	0.514	0.288	0.749	0.202
Improvement Rate (IR)	VAE	7 (21.9)	11 (35.5)	12 (40.0)	12 (52.2)	**13 (40.6)**
(Improvement / cure)	Control	3 (14.3)	3 (15.0)	3 (15.8)	3 (21.4)	**3 (14.3)**
	P[2)]	0.376	0.099	0.068	0.065	**0.039**
Non-deterioration rate (NDR)	VAE	26 (81.3)	24 (77.4)	24 (80.0)	**22 (95.7)**	**25 (78.1)**
(Unchanged state / Improvement / cure)	Control	15 (71.4)	11 (55.0)	11 (57.9)	**7 (50.0)**	**8 (38.1)**
	P[2)]	0.306	0.085	0.090	**0.002**	**0.004**
Missing	VAE	-	1 (3.1)	2 (6.3)	9 (28.1)	-
	Control	-	1 (4.8)	2 (9.5)	7 (33.3)	-

[1)] percentages and group comparison within the remaining study population excluding dropouts
[2)] 1-sided Fisher exact test

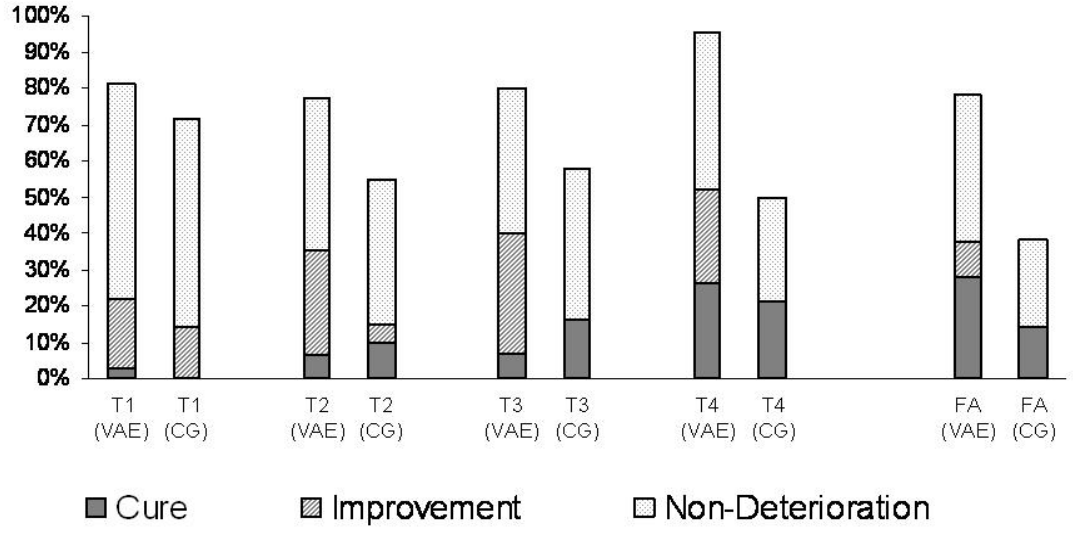


Fig. 1:Outcome on horse level assessed within the 4 quarterly status evaluation points (T1-4) and final assessment at break point (FA) by treatment (VAE) and control group (CG).

The univariate analysis of the above described variables showed no significant effects on dependent variables **(table 6, p.22)** except for an increased CR in horses with 3 to 9 tumours per patient (p=0.010) in the VAE group, 58,3% compared to 0% in horses with 1 to 2 ES and 15,4% in horses with more as 9 ES. In the VAE group a not significant increase of CR (p=0.098) and IR (p=0.122) was found for younger horses up to 5 years 42,9% (CR), 57,1% (IR) compared to 30,0% (CR) and 40.0% (IR) in 6 to 9 year old individuals and 0% (CR) and 12.5% (IR) in older horses. Although not significant due to generally small CR and IR, this age gradient could be observed in the control group, too. The final logistic regression models for IR and NDR showed odds ratios of 4.11 for improvement and 5.80 for stabilization after VAE treatment compared to the control group **(tables 7 and 8)**. Due to the small number of cured horses in the placebo group, the logistic regression model for CR could not be calculated.

Tab. 7: Final logistic regression model including significant variables (p<0.05) and trial group as obligate variable. OR represents the effect chance of the respective level on **Improvement rate (IR)** of the horses (n=53).

Variable	**Level**	**OR**	**CI 95%**			**p**
Trial group	VAE	4.11	1.00	-	16.84	0.050
	Control	1				
Log likelihood = -28.787436						

Tab. 8: Final logistic regression model including significant variables (p<0.05) and trial group as obligate variable. OR represents the effect chance of the respective level on **Non-deteriorated rate (NDR)** of the horses (n=53).

Variable	**Level**	**OR**	**CI 95%**			**p**
Trial group	VAE	5.80	1.72	-	19.57	0.005
	Control	1				
Log likelihood = -30.227309						

Tab.6: Univariate analysis of factors influencing therapy effects concerning Cure Rate (CR), Improvement Rate (IR) and Non-deterioration rate (NDR). Significance test performed by using univariate logistic regression.

		VAE					Control				Total			
Variable	**Level**	**Tot**	**CR N (%)**	**IR N (%)**	**NDR N (%)**	**Tot.**	**CR N (%)**	**IR N (%)**	**NDR N (%)**	**Tot.**	**CR N (%)**	**IR N (%)**	**NDR N (%)**
Sex	Mare (23)	14	5 (35.7)	7 (50.0)	12 (85.7)	9	1 (11.1)	1 (11.1)	5 (55.6)	23	6 (26.1)	8 (34.8)	17 (73.9)
	Stallion (5)	1	0	1 (100)	1 (100)	4	0	0	1 (25.0)	5	0	1 (20.0)	2 (40.0)
	Gelding (25)	17	4 (23.5)	5 (29.4)	12 (70.6)	8	2 (25.0)	2 (25.0)	2 (25.0)	25	6 (24.0)	7 (28.0)	14 (56.0)
Age	1 to 5 years (22)	14	6 (42.9)	8 (57.1)	11 (78.6)	8	2 (25.0)	2 (25.0)	4 (50.0)	22	8 (36.4)	10 (45.5)	15 (68.2)
	6 to 9 years (20)	10	3 (30.0)	4 (40.0)	9 (90.0)	10	1 (10.0)	1 (10.0)	3 (30.0)	20	4 (20.0)	5 (25.0)	12 (60.0)
	Over 9 years (11)	8	0	1 (12.5)	5 (62.5)	3	0	0	1 (33.3)	11	0	1 (9.1)	6 (54.6)
Breed	Freiberger (11)	6	2 (33.3)	4 (66.7)	5 (83.3)	5	0	0	2 (40.0)	11	2 (18.2)	4 (36.4)	7 (63.6)
	Swiss Warmblood (26)	15	5 (33.3)	7 (46.7)	12 (80.0)	11	2 (18.2)	2 (18.2)	4 (36.4)	26	7 (26.9)	9 (34.6)	16 (61.5)
	Thorough bred (6)	4	1 (25.0)	1 (25.0)	2 (50.0)	2	1 (50.0)	1 (50.0)	1 (50.0)	6	2 (33.3)	2 (33.3)	3 (50.0)
	Other (10)	7	1 (14.3)	1 (14.3)	6 (85.7)	3	0	0	1 (33.3)	10	1 (10.0)	1 (10.0)	7 (70.0)
Colour	Chestnut (15)	9	4 (44.4)	5 (55.6)	7 (77.8)	6	2 (33.3)	2 (33.3)	4 (66.7)	15	6 (40.0)	7 (46.7)	11 (73.3)
	Bay (29)	16	4 (25.0)	7 (43.8)	13 (81.3)	13	1 (7.7)	1 (7.7)	4 (30.8)	29	5 (17.2)	8 (27.6)	17 (58.6)
	Other (9)	7	1 (14.3)	1 (14.3)	5 (71.4)	2	0	0	0	9	1 (11.1)	1 (11.1)	5 (55.6)
ES freq	1 to 2 per horse (13)	7	**0**	1 (14.3)	6 (85.7)	6	2 (33.3)	2 (33.3)	3 (50.0)	13	2 (15.4)	3 (23.1)	9 (69.2)
	3 to 9 per horse (25)	12	**7 (58.3)**	9 (75.0)	11 (91.7)	13	1 (7.7)	1 (7.7)	5 (38.5)	25	8 (32.0)	10 (40.0)	16 (64.0)
	Over 9 per horse (15)	13	**2 (15.4)**	3 (23.1)	8 (61.5)	2	0	0	0	15	2 (13.3)	3 (20.0)	8 (53.3)
Strategy	Mono-therapy (42)	24	7 (29.2)	10 (41.6)	20 (83.3)	18	3 (16.7)	3 (16.7)	8 (44.4)	42	10 (23.8)	13 (31.0)	28 (66.7)
	Selective Surgery (11)	8	2 (25.0)	3 (37.5)	5 (62.5)	3	0	0	1(33.3)	11	2 (18.2)	3 (27.3)	6 (54.5)
Barn	Free stall (9)	5	0	0	2 (40.0)	4	1 (25.0)	1 (25.0)	2 (50.0)	9	1 (11.1)	1 (11.1)	4 (44.4)
	Box (41)	24	8 (33.3)	12 (50.0)	20 (83.3)	17	2 (11.8)	2 (11.8)	6 (35.3)	41	10 (24.4)	14 (34.2)	26 (63.4)
	Other (3)	3	1 (33.3)	1 (33.3)	3 (100)	0	-	-	-	3	1 (33.3)	1 (33.3)	3 (100)
Total		32	9 (28.1)	13 (40.6)	25 (78.1)	21	3 (14.3)	3 (14.3)	8 (38.1)	55	12 (22.6)	16 (30.2)	33 (62.3)

Sarcoid level

Of 163 closely observed sarcoids (Iscador n= 95, Control n=68) the most frequently affected body areas were the ventral (54 ES; 33.1%), the axillary and the thoracic regions (each 23 ES; 14.1%), followed by head and neck (each 20; 12.3%), prepuce and inner hind leg (each 10; 6.13%) and the rest of the extremities (3 ES; 1.84%). 81 sarcoids were of the verrucous (49.7%), 55 of the occult (33.7%), 14 of the mixed (8.6%), 8 of the fibroblastic (4.9%), and 5 of the nodular type (3.1%). While 134 (82.2%) of all sarcoids were dry, twenty-six (16.0%) were ulcerated and three (1.8%) were sanguineous. Demarcation was observed in 46% of all tumours.

After one year of observation time (T4) in the VAE group, 27 ES showed a complete regression (37.5%) and 48 an improvement (66.7%) compared to 9 ES with complete regression (13.2%; n.s.) and 17 (39.5%; p=0.005) with improvement in the control group (**table 9**).

Tab. 9: Outcome on sarcoid level assessed within the 4 quarterly status evaluation points (T1-4). VAE group n=95, Control group n=68

		Proportion of Outcome assessment at Status evaluation time T1-T4			
Outcome	**Group**	**T1[1)] n (%)**	**T2[1)] n (%)**	**T3[1)] n (%)**	**T4[1)] n (%)**
Cure rate	VAE	6 (6.32)	14 (15.4)	16 (17.6)	27 (37.5)
	Control	3 (4.69)	6 (8.8)	9 (16.7)	9 (20.9)
	P[2)]	0.663	0.217	0.888	0.064
Improvement rate	VAE	27 (28.4)	38 (41.8)	41 (45.1)	**48 (66.7)**
	Control	14 (21.9)	20 (29.4)	22 (40.7)	**17 (39.5)**
	P[2)]	0.355	0.110	0.612	**0.005**
Non-deterioration rate	VAE	**79 (83.2)**	42 (46.2)	**69 (75.8)**	**59 (81.9)**
	Control	**43 (67.2)**	27 (39.7)	**31 (57.4)**	**24 (55.8)**
	P[2)]	**0.019**	0.417	**0.020**	**0.002**
Missing	VAE	-	4 (4.2)	4 (4.2)	23 (24.2)
	Control	4 (5.9)	-	14 (20.6)	25 (36.8)

Non-deterioration rates of 83.2% (control 67.2%; p=0.019), 75.8% (57.4%, p=0.0209) and 81.9% (55.8%; p=0.002) were found on T1, 3 and 4, respectively. There was no significant difference on T2 (46.2% vs. 39.7%; p=0.417). On T1 to T3 there was no significant difference between VAE and control group concerning CR and IR, while the IR and CR in the VAE group on T4 was 66.7% and 37.5% compared to 39.5% (p=0.005) and 20.9% (p=0.064) in the control group **(figure 2)**.

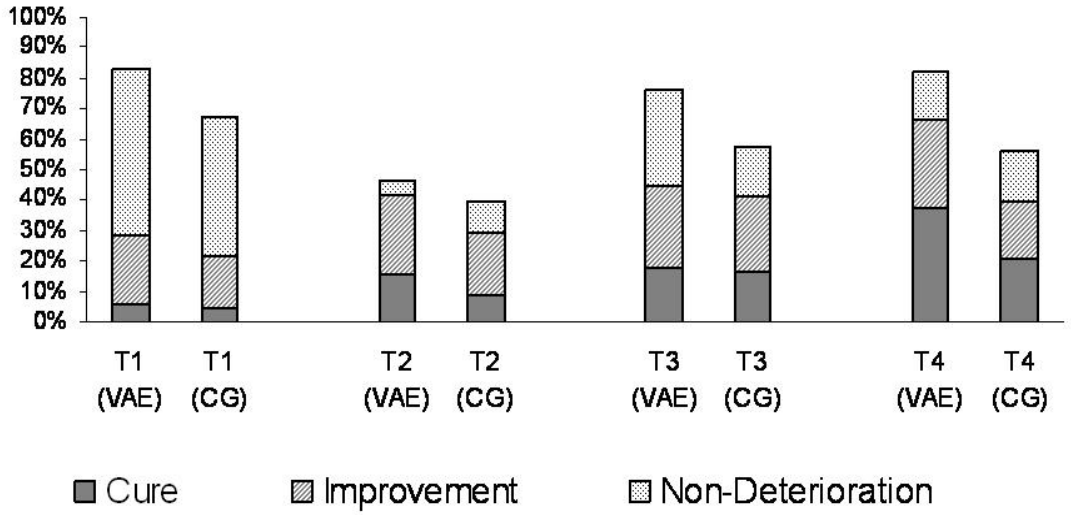
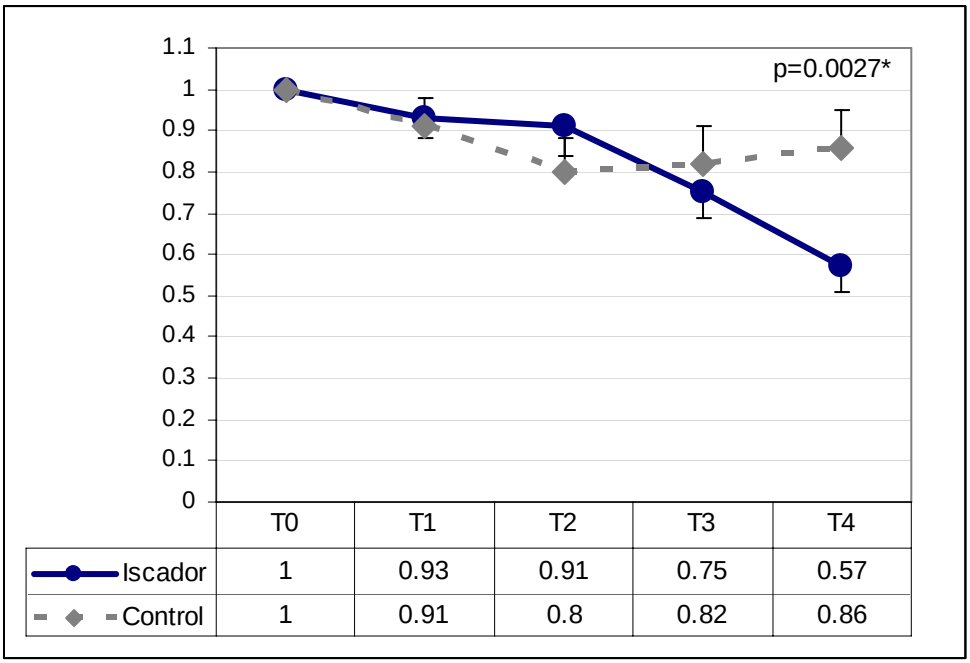


Fig. 2: Outcome on sarcoid level assessed within the 4 quarterly status evaluation points (T1-4). Treatment group (VAE) n=95, Control (CG) n=68

The growth development was similar in both treatment groups and decreased slightly until T3 (9 months). After one year, however, the sarcoid volumes in the VAE group were

significantly smaller. The relative tumour size at this time was only 57% compared to 86% in the control group ($p<0.01$; **figure 3**).



	T0	T1	T2	T3	T4
Iscador	1	0.93	0.91	0.75	0.57
Control	1	0.91	0.8	0.82	0.86

Fig. 3: Mean and SEM of Relative Tumour-Volume (T0=1) during observation time regarding focus sarcoids of horses remaining in the study at T4; 1 additional omission of an outliner due to measure problems (VAE: n=71, control n=43); Significance test in T4 performed by using Wilcoxon Rank sum Test.

Furthermore, potential factors influencing treatment effects on ES were investigated **(table 10)**: Tumour volume had no significant effect on tumour development. Diagnostic biopsy of a single tumour had a negative effect on complete tumour regression (21.1% compared to 33.3% in tumours with no biopsy, p=0.297), improvement (26.3% vs. 62.5%, p=0.006) and non-deterioration rate (47.4% vs. 77.1%, p=0.008). Verrucous sarcoids showed higher cure rates compared to the other types (for group proportions see table 10). Demarcation showed a positive effect on tumour remission (41.9% compared to 21.7% in diffuse tumours, p=0.022) and improvement (67.3% vs. 47.7%, p=0.027).

Tab.10: Univariate analysis of factors influencing T4 monotherapy effects on sarcoids concerning complete remission, improvement & complete remission and non-deterioration, improvement & complete remission, respectively. Significance test performed by using univariate logistic regression. N=115 (excluding sarcoids of dropout horses)

Variable	Level	Cured N (%)	P	Improved/ cured N (%)	p	Stable / improved/ cured N (%)	p
Biopsy	No (96)	32 (33.3)	0.297	**60 (62.5)**	**0.006**	**74 (77.1)**	**0.008**
	Yes (19)	4 (21.1)	Ref.	**5 (26.3)**	**Ref.**	**9 (47.4)**	**Ref.**
Type	Occult (39)	**9 (23.1)**	**Ref.**	19 (48.7)	Ref.	26 (66.7)	Ref.
	Verrucous (59)	**24 (40.7)**	**0.028**	38 (64.4)	0.081	45 (76.3)	0.316
	Nodular (5)	**1 (20.0)**	**Ref.**	2 (40.0)	Ref.	3 (60.0)	Ref.
	Fibroblastic (2)	**2 (100)**	**Ref.**	2 (100)	Ref.	2 (100)	Ref.
	Mixed (10)	**0**	**Ref.**	4 (40.0)	Ref.	7 (70.0)	Ref.
Status	Dry (100)	30 (30.0)	Ref.	54 (54.0)	Ref.	70 (70.0)	Ref.
	Ulcerous (14)	6 (42.9)	0.324	11 (78.6)	0.088	13 (92.9)	0.099
	Other (1)	0	Ref.	0	Ref.	0	Ref.
Demarcation	Demarcated (55)	**23 (41.9)**	**0.022**	**37 (67.3)**	**0.027**	44 (80.0)	0.076
	Diffuse (60)	**13 (21.7)**	**Ref.**	**28 (46.7)**	**Ref.**	39 (65.0)	Ref.
Volume	<10 cmm^3 (39)	15 (38.5)	0.114	23 (59.0)	0.368	26 (66.7)	0.736
	11-100 cmm^3 (39)	13 (33.3)	0.257	24 (61.5)	0.260	31 (79.5)	0.356
	101-1000 cmm^3 (29)	7 (24.1)	Ref.	15 (51.7)	Ref.	20 (69.0)	Ref.
	>1000 cmm^3 (8)	1 (12.5)	Ref.	3 (37.5)	Ref.	6 (75.0)	Ref.

It was remarkable that the two peri-ocular tumours (2 horses) of the VAE group reacted earlier to the treatment. During the 4 observation dates (T1-4), the relative tumour volume of eye sited ES was 3%, 2%, 2%, 0% and 45%, 2%, 1%, 1% for case 17 and 53, respectively. The mean relative tumour volume for the other 3 tumours of case 17 was 108%, 150%, 168% and 29%, respectively. In case 53 the second tumour showed RTV of 93%, 167%, 1% and 1% for T1 to T4, respectively.

In multivariate analysis only BIOPSY remained as a significant variable in the final models for IR and NDR. Diagnostic biopsy reduced the improvement chance by 0.24 (p=0.016) and the stabilization chance by 0.30 (p=0.037) compared to ES with no manipulation. VAE treatment leads to significantly higher tumour stabilization (OR 3.32, p=0.012: **compare tables 11)**.

Tab. 11: Final logistic regression models including significant variables (p<0.05) and trial group as obligate variables after omission of initial model variables "Type_verrucous", "Demarcation" and "Volume". OR represents the effect chance of the respective level on **Cure rate (CR), Improvement rate (IR) and Non-deteriorated rate (NDR)** of the sarcoids (n=115).

Variable	**Level**	**OR**	**CI 95%**			**p**
CURE RATE						
Trial group	VAE	2.67	0.50	-	10.31	0.290
	Control	1				
Prob > chi2=0.2897; Log pseudolikelihood = -69.692879						
IMPROVEMENT RATE						
Trial group	VAE	2.83	0.83	-	9.61	0.096
	Control	1				
Biopsy	Yes	0.24	0.07	-	0.77	0.016
	No	1				
Prob > chi2=0.0170; Log pseudolikelihood = -71.222172						
NON-DETERIORETED RATE						
Trial group	VAE	3.32	1.30	-	8.42	0.012
	Control	1				
Biopsy	Yes	0.30	0.10	-	0.93	0.037
	No	1				
Prob > chi2=0.0013; Log pseudolikelihood = -61.111741						

Discussion

Colour and breed distribution of the study population reflected approximatively the distribution of affected horses in Switzerland (Studer et al. 1997). ES can affect horses of any age (Brostrom 1995), but an increased incidence in younger horses (3 to 6 years of age) is observed (Marti et al. 1993; Reid et al. 1994; Torrontegui et al. 1994). Again, this was reflected in our study population. The majority of horses were aged 3 to 9 years, only 20% were 10 years of age and older.

Possibly because individuals were entered in the study by their owners because of their large tumour load and therefore had a poor prognosis for complete remission by surgical therapy (Diehl et al. 1987; Brostrom 1995; Brandt et al. 1996; Carstanjen et al. 1997), the number of ES per horse (8.4/horse, 94% of the horses with more than one tumour) was higher compared with data from literature (Torrontegui et al. 1994; Brostrom 1995; Studer et al. 1997; Martens et al. 2001). This means that the study population was more severely affected compared with other affected groups described in the literature. A similar number of tumour burden within a study population is reported only by Steiner, who diagnosed a mean of 8 ES per horse in the population of a Swiss veterinary hospital in 1988 (Steiner 1988).

The localisation pattern of tumours in the present study agrees with distributions described in other investigations conducted in central Europe (Teifke 1994; Brandt et al. 1996). In contrast, the type of tumours in this study is different from the distribution in referral horse populations of veterinary hospitals (Teifke 1994; Carstanjen et al. 1997; Goodrich et al. 1998), likely because fibroblastic tumours are more frequently treated by surgery and both fibroblastic and mixed types are often observed as recurring lesions after primary therapy (Brostrom 1995; Pascoe et al. 1999; Martens et al. 2001). In the present study, in contrast, most of the horses (69.8%) were treated for the first time. In accordance to findings in other

field studies (Studer et al. 1997), sarcoids of occult and verrucous type were predominant in this investigation.

This randomized placebo-controlled double-blind study showed efficacy of viscum album extracts (VAE). The horses were treated solely with VAE or treated with VAE after selective excision of a certain number of tumours. After one year of observation time, subcutaneous application of mistletoe extract Iscador® P as monotherapy, three times a week during 15 weeks resulted in a significantly increased CR, IR and NDR in horses compared to the control group.

The results on horse level in the VAE group with 28% cure rate and 41% improvement are nearly the same as found by Steiner (1988), who used the immuno-therapeutic Nomagen (BCG-Vaccine) for treatment compared to cryosurgery (Steiner 1988). In the VAE group, 25 horses (78.1%) were cured, improved or stabilized. The owner, as a partial success, often perceives the stabilization of the state of the sarcoids of a horse. The efficiency of the treatment could be underlined by the fact that in the control group, the majority of the horses showed deterioration. The spontaneous tumour regression data in the placebo control group are in accordance to the results of other studies (Brostrom 1995; Studer et al. 1997; Piscopo 1999; Germann 2006). Spontaneous complete regression was observed only in horses aged between 3 to 7 years and predominantly in horses with 1 to 2 ES except for one case (3 years old) with 6 small tumours. In contrast, complete remission in the VAE group was observed in mildly, moderate and severely affected individuals of all ages.

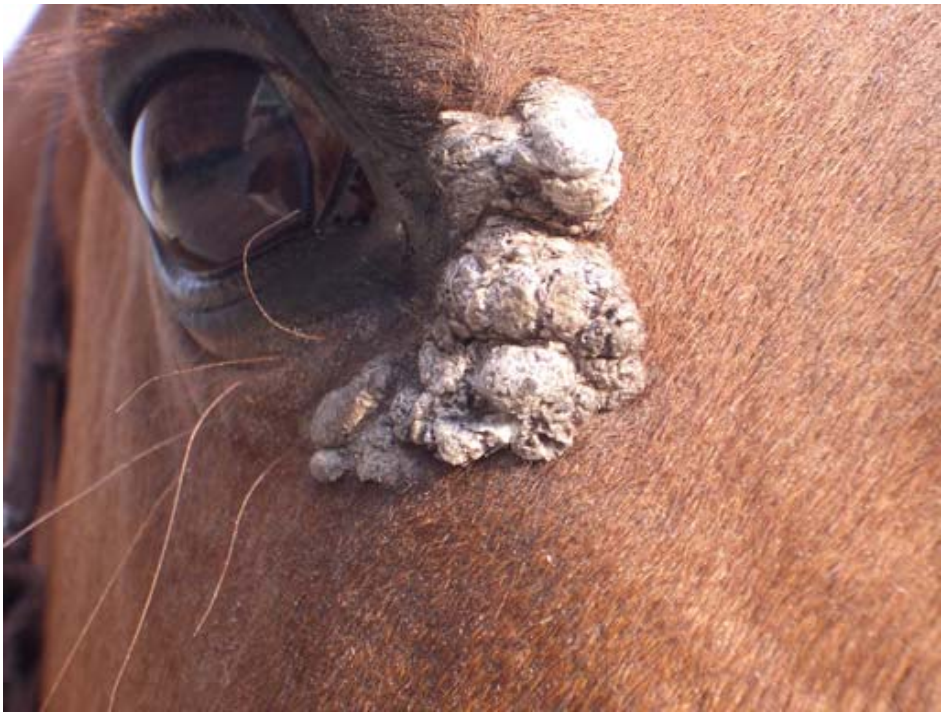
The development of all tumours per horse has to be taken into account since the owner judges the therapy success on horse level even single tumours are disappearing. However, the assessment of efficacy at the individual tumour level (sarcoid level) allowed more detailed results concerning tumour growth and regression after treatment. The improvement rate on sarcoid level after VAE (66.7%) corresponds with reported effects of intratumoural

administration of BCG-Vaccine, which showed partial or complete regression in 57.5% (Steiner 1988), 70% (Martens et al. 2001), 77% (Vanselow et al. 1988) of the sarcoids. On the other hand, the percentage of completely resolved tumours (37.5%) was lower than the rates reported for local CO_2 laser surgery (62%) (Carstanjen et al. 1997), (71%) (Martens et al. 2001), cryosurgery (79%) (Steiner 1988; Martens et al. 2001) and radioactive implants (86.7%) (Byam-Cook et al. 2006). The advantage of the Iscador® P therapy is its efficiency in horses with multiple tumours, which was shown in this study by an increase of the CR in horses with 3 to 9 tumours per patient.

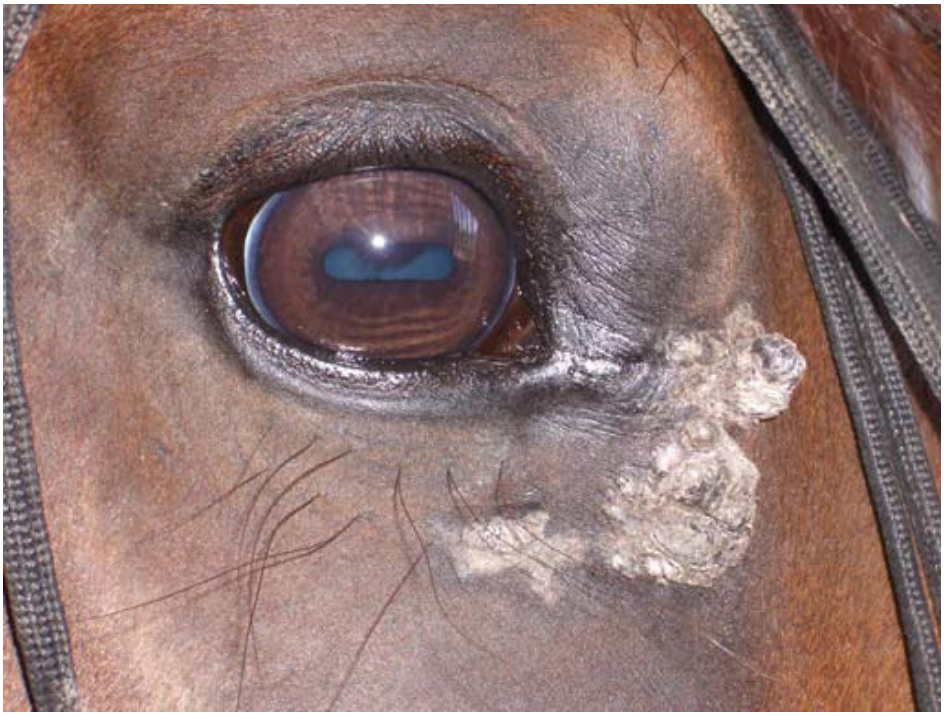
The curative effects on tumours were independent of localisation, type, size and state of the ES. Interestingly, therapeutics effects became apparent only after finishing therapy protocol. Only 6% were cured within the treatment period whereas nearly 38% had disappeared within one year **(see figure 4a-g)**, which is in accordance with observations after other immunotherapy protocols (e.g. auto-vaccination, BCG vaccination, Nomagen) (Klein 1987; Steiner 1988; Vanselow et al. 1988). The observation that eye-sited sarcoids in horses with multiple sarcoids seem to respond earlier than other tumours could not be confirmed statistically. But it may indicate that particularly these tumours may be treated by Iscador® P. As an alternative in this localisation, radiotherapy is a very effective method with nearly 100% success (Byam-Cook et al. 2006), but is reserved to special accredited veterinary clinic units and often not accepted by the owner. In other studies, periocular sarcoids are the most uniformly responsive to immunotherapy, too (Murphy et al. 1979; Steiner 1988; Vanselow et al. 1988). Steiner obtained 83.5% remission of periocular sarcoids, but only 48.5% remission from all other body regions (Steiner 1988).

Figure 4: Swiss warmblood horse, mare, 1997, seven occult, verrucous and mixed sarcoids on head, neck, lower abdomen and inner side of hind leg. Primary therapy with VAE (Iscador® P) from 04 April 2005 to 15 July 2005.

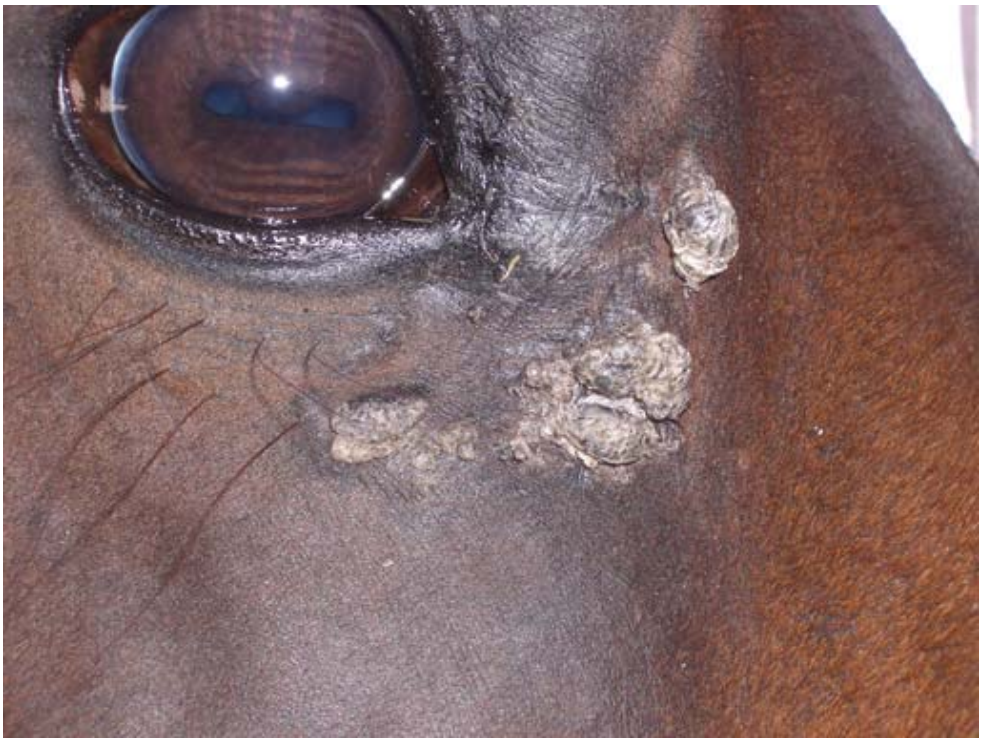
4 a) 4/4/2005: verrucous periocular sarcoid **before** VAE therapy (day 0)



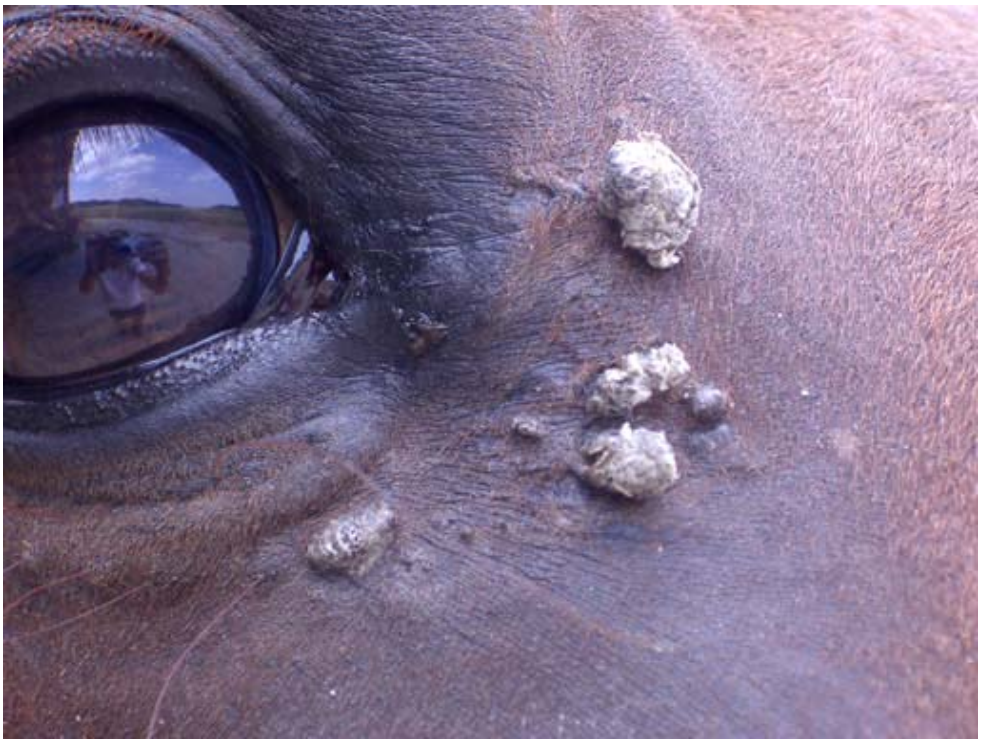
4 b) 5/25/2005: day 51 after therapy start



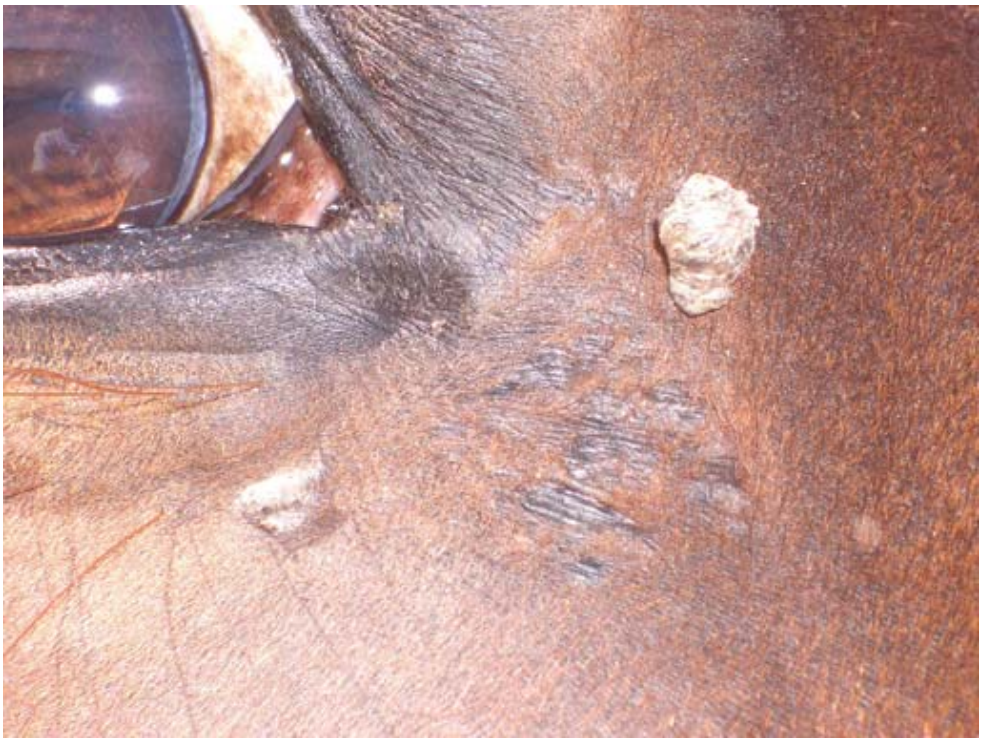
4 c) 6/17/2005: day 74 after therapy start



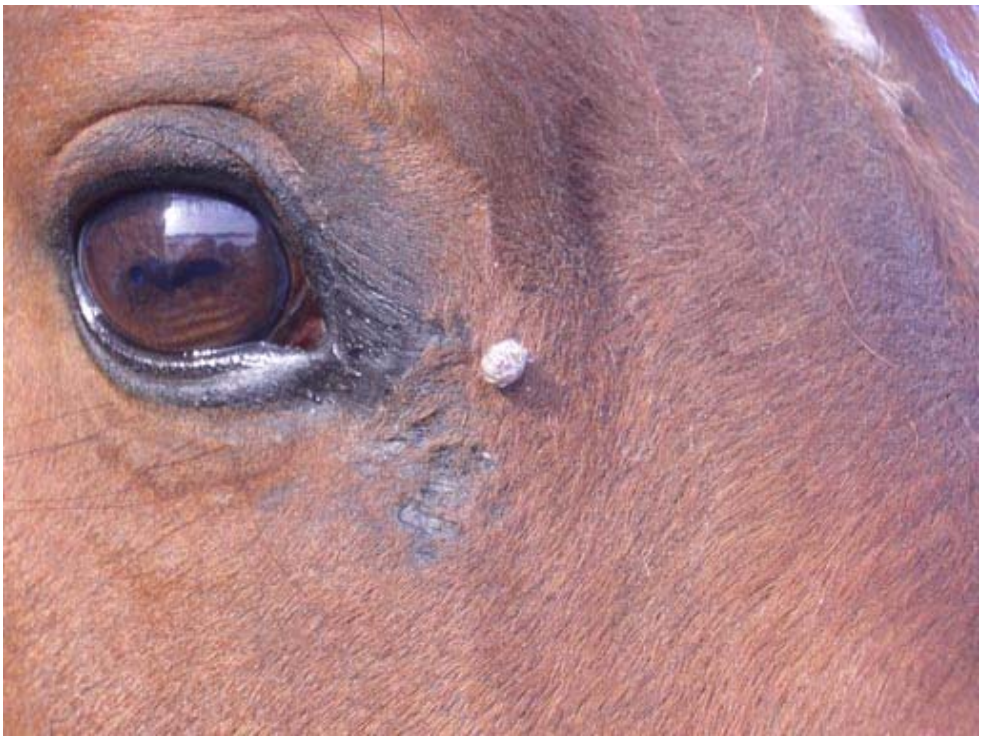
4 d) 7/15/2005: day 102 after therapy start, end of VAE therapy, beginning of post-therapy observation time



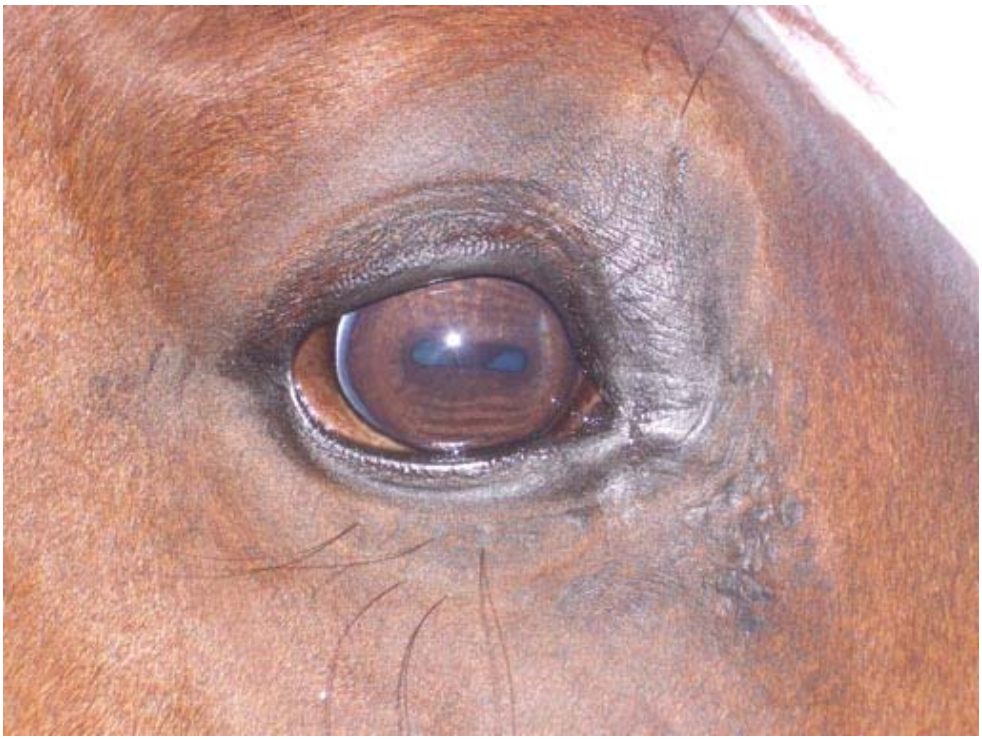
4 e) 9/7/2005: day 156 after therapy start (observation time)



4 e) 3/6/2006: day 336 after therapy start (observation time)



4 f) 5/4/2006: day 395 after therapy start (observation time)



Diagnostic biopsy had a significant negative effect on regression of the treated tumour. A total of 70% of biopsied tumours had increased in size afterwards, independently of therapy. It has to be taken into account that every invasive manipulation of former inactive tumours may result in an activation of the ES followed by more aggressive tumour growth (Tarwid et al. 1985; Lepage et al. 1998).

Compliance of the owners with the long observation time was difficult to maintain, particularly when owners wanted to sell the younger horses. Thus, the maximal observation time of one year was a compromise. The analysis of effects beyond the study observation period of one year in 22 horses was not part of the calculated results above, but should give an impression of the epicrisis about the ongoing dynamics in the patients. Of the 9 tumour free horses of the VAE group, seven showed no recurrences or newly grown ES, while two horses could not be assessed due to abandon of follow-up for other reasons. The four horses

primarily assessed as improved developed a complete tumour remission, while one horse was euthanasied due to COPD. Of the twelve unchanged horses during study period, four showed complete remission and three an improvement in terms of the study definition. Two horses remained unchanged according to tumour status, and two were deteriorated. Finally, one horse was lost for follow-up in this category.

Considering these additional data, the total Cure Rate in the VAE group exceeded 50.0% and the Improvement Rate 62.5%. Hence, it can be stated that the cure in these responders is sustainable over a long time (Klein 1987). The very low recurrence rate after administration of Iscador® P likely underlines the observation that successful immunotherapy is associated with long-term effects on ES development (Kinnunen et al. 1999). The mechanism of action (Heinzerling et al. 2006) of the mistletoe extract are thought to be based on mistletoes lectins and viscotoxins acting at high concentrations by direct cytotoxic inhibition of the tumour growth (Kovacs et al. 2006) and at low (Thies et al. 2007) dosages as immunostimulation (Beuth et al. 1994; Huber et al. 2006). Iscador® P contains less viscotoxin and lectines than the other preparations (Urech et al. 2006), so in this study the mistletoe extract was applied at low dosage as immunotherapy. One may speculate that the immune system, once stimulated, can continue to resist the virus (Karagoz et al. 2003) over a prolonged period. This raises the question of a tumour prophylaxis therapy even after or in combination with surgery of ES, in order to reduce the risk of recurrence.

A 15-weeks protocol was chosen based on human cancer treatment protocols. Possibly a prolongation of the treatment as conducted in human patients could improve results. However, the owner’s compliance is likely to decrease after continuation over unlimited periods. Also non-responders have to be taken into account. Possibly, these horses mount an antibodies response against the VAE contents, as shown in some human cancer patients (Stein et al. 1997; Klein et al. 2002). Potential non-responders can presently not be identified

prior to therapy start. Recent recommendations in human oncology consider the peri-tumoural or intra-tumoural application of Iscador® in order to achieve higher concentrations in the affected tissue. This procedure is difficult to standardize and was therefore not tested because of the variability of tumour number per horse. The sarcoid number variability would have lead to varying total dosages per horse. The altered skin texture around the ES would inhibit appropriate injections and the injection acceptance of local administration in critical body areas like prepuce, eye etc. would have been limited (Germann 2006). Finally, the multiple injections per horse in case of multiple ES would have been a limiting factor of compliance by the horse and the owner.

The absence of severe local reactions, as seen after local chemotherapy (Knottenbelt et al. 2000; Nogueira et al. 2006), BCG-vaccination techniques (Klein 1987; Vanselow et al. 1988) or radiotherapy (Knottenbelt et al. 2000) or the necessity of post-treatment care due to major intervention after certain surgical excisions (Martens et al. 2001) indicate that the therapy protocol is an appropriate alternative to common methods with less side-effects and applicable under practice conditions.

Therapy costs do not exceed those of surgical or other therapies, disregarding comparison of therapy efficacy and the fact of the long-term character of the protocol. This should be considered in a detailed cost-benefit analysis. The costs for the 15-week protocol are about 300 SFR, which is less than most of the common treatment protocols, by experience.

In conclusion, the therapy protocol using viscum album extract Iscador® P proved as effective for treatment of Equine Sarcoid as a monotherapy if excision is not indicated as the primary therapy due to clinical, pathological or esthetical reasons. Furthermore it can be recommended in cases of multiple ES where complete surgery can not be conducted. Particular good results can be expected in younger horses and in moderately or severely affected individuals.

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