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***Viscum album* L., a therapeutic option for neoplastic diseases in companion animals? A systematic review**
Ist *Viscum album* L. eine Behandlungsmöglichkeit für neoplastische Erkrankungen bei Haustieren? Ein systematisches Review

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Short Title: systematic review of Viscum album L. in cancer treatment of animals
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List of abbreviations

AE	adverse effects
AMSTAR	a measurement tool to assess systematic reviews
BCT	base line cancer treatment
CAM	complementary and alternative medicine
COX-2	cyclooxygenase-2
CNS	central nervous system
ES	equine sarcoid
FeLV	feline leukemia virus
GCP	good clinical practice
HLM	hematopoietic and lymphoid malignancies
HPT	human pancreatic tumor cell
MET	most efficient treatment protocol
LET	least efficient treatment protocol
sc_{max}	maximally attainable score

PICOS	population intervention comparison outcome study design
PRISMA	preferred reporting items for systematic reviews and meta-analyses
TVT	transmissible venereal tumor
QoL	quality of life
VAE	Viscum album extract

Abstract
Cancer is a common disease in humans and in companion animals and treatment is challenging. The aim of this systematic review was to identify and assess the potential use of *Viscum album* L. extracts (VAE) for treatment of neoplastic diseases in companion animals. Peer-reviewed animal, *in vivo* and *in vitro* studies were included, considering the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement and a measurement tool to assess systematic reviews (AMSTAR). Overall, 6,148 references were identified. Following a predefined protocol, 114 full-text references were assessed. Ultimately, 61 references were included for further assessment, 25 references included *in vitro* experiments, 26 included *in vivo* and clinical experiments and 10 references included both *in vitro* and *in vivo* experiments. These 61 references comprised data of 193 *in vitro* and 67 *in vivo* and clinical experiments. Most of the 67 *in vivo* and clinical experiments were conducted with mice (59), followed by rats (4), dogs (3) and horses (1). So far, oral melanomas, mammary tumors and sticker sarcomas in dogs, as well as sarcoids in horses, have been investigated in controlled clinical trials.
A scoring system was established to evaluate the outcomes of each study based on defined effect levels.
The efficacy of VAE treatment was most pronounced for melanomas, sarcomas, mammary carcinoma and equine sarcoids. The limited number and quality of published studies on VAE treatment in companion animals impedes to draw definitive conclusions regarding the efficacy of VAE in the treatment of cancer. Thus, further research is needed to elucidate the impact of VAE on the treatment of cancer in companion animals and possible underlying mechanisms.

Zusammenfassung
Krebserkrankungen sind sowohl bei Menschen als auch bei Haustieren weit verbreitet, und ihre Behandlung herausfordernd. Ziel dieser systematischen Übersichtsarbeit war es, die Anwendungsmöglichkeiten von *Viscum album* L.-Extrakten (VAE) zur Behandlung von neoplastischen Erkrankungen bei Haustieren zu ermitteln und zu bewerten.
Eingeschlossen wurden peer-reviewte klinische Tier-, *in vivo* und *in vitro* Studien unter Einhaltung der Vorgaben der PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) Kriterien und des AMSTAR (Measurement Tool to Assess Systematic Reviews) Bewertungstools.
Insgesamt konnten 6.148 Referenzen identifiziert werden. Nach einem festgelegten Protokoll wurden 114 Referenzen im Volltext bewertet. Letztlich wurden 61 Referenzen für die weitere Bewertung berücksichtigt. 25 Referenzen beinhalteten *in vitro* Experimente, 26 beinhalteten *in vivo* - oder klinische Experimente und 10 Referenzen beinhalteten sowohl *in vitro* - als auch *in vivo* Experimente. Diese 61 Referenzen umfassten Daten von 193 *in vitro* und 67 *in vivo* und klinischen Experimenten. Die Mehrzahl der 67 *in vivo* und klinischen Experimente wurde mit Mäusen (59) durchgeführt, gefolgt von Ratten (4), Hunden (3) und Pferden (1). Bisher wurden in kontrollierten klinischen Untersuchungen orale Melanome, Mamma Tumore und Sticker-Sarkome bei Hunden sowie Sarkoide bei Pferden untersucht.
Zur Bewertung der Ergebnisse der einzelnen Studien wurde ein Punktesystem auf der Grundlage definierter Effektstärken erstellt.
Am ausgeprägtesten war die Wirksamkeit der VAE-Behandlung bei Melanomen, Sarkomen, Mammakarzinomen und Equinen Sarkoiden. Die begrenzte Anzahl und Qualität der veröffentlichten Studien zur VAE-Behandlung bei Haustieren erschwerten es, endgültige Schlussfolgerungen über die Wirksamkeit von VAE bei der Behandlung von Krebs zu ziehen. Daher sind weitere Forschungsarbeiten erforderlich, um die Auswirkungen und die zugrunde liegenden Mechanismen von VAE in der Behandlung von Krebs bei Haustieren zu klären.

Introduction

Neoplastic diseases are common in companion animals, in particular in dogs, cats and horses, and treatment often presents tremendous challenges to veterinary practitioners. The most frequently occurring tumor types in dogs are mammary gland tumors. Other commonly found neoplasia in dogs include oral melanomas and mast cell tumors as shown in table 1.

Feline tumors have not been investigated extensively and published data is sparse [1]. In a Swiss feline cancer registry, based on patient records and pathological samples, about 80% of the tumors were malignant. Common tumor types were skin and soft tissue tumors, mammary gland tumors and a smaller number are lymphoid tumors [2]. In the oral cavity of cats, 80% of the tumors were reported to be squamous cell carcinomas [3] (Tab. 1).

In horses and other equids, skin-associated tumors are by far the most common form of cancer found in these species, with equine sarcoids (ES) accounting for an estimated 90% of all skin-associated neoplastic growths in equids [4, 5]. Although ES are usually not life-threatening, they can considerably compromise welfare, use and value of affected individuals [6, 7]. Other skin tumors commonly found in horses include squamous cell carcinomas and melanomas [8-10] (Tab. 1).

Surgical excision can be successful if complete removal of the tumor is feasible and if metastases are absent. Therefore, surgery plays a key role in cancer treatment of pets [11, 12]. Chemotherapy is also used in veterinary oncology and considered treatment of choice for selected tumor types such as lymphomas [13]. However, in a significant proportion of animals, standard protocols were described to be ineffective due to resistance to chemotherapeutic agents [11, 14]. Furthermore, adverse effects of chemotherapy and radiation are frequent and many pet owners reject these treatment options for various reasons [11, 15]. Although large-scale investigations that critically assess the impact of chemotherapeutic agents are not available for veterinary medicine, many chemotherapeutic agents used, e.g. cisplatin, are potentially hazardous compounds and human contact and environmental contamination must be avoided [6, 16]. Furthermore, treatment options based on complementary and alternative medicine (CAM) are available, and pet owners increasingly encourage the concurrent use of CAM. This also includes the treatment with Viscum album extracts (VAE) [17-19]. In veterinary oncology, the use of CAM has so far not been quantified. In human oncology, however, a European survey documented that more than one third of all cancer patients receive CAM [20]. Likewise, a similar demand for CAM may be anticipated for pet owners and for the treatment of their animals suffering from cancer [19-21]. European white berry mistletoe (*Viscum album* L.) is one of the noteworthy medications in human CAM cancer treatment [22-25]. In *in vitro* and preclinical research VAE was shown to reveal different anti-cancer effects including cytotoxicity, induction of apoptosis, cancer-related immunomodulation, as well as inhibition of angiogenesis [26-33]. It was also demonstrated that VAE exerts anti-inflammatory effects by selective cyclooxygenase 2 (COX-2) inhibition [34-36].

The aim of this systematic review was to identify, summarize and evaluate the role of VAE for treatment of neoplastic diseases in companion animals, while taking recent developments and requirements specific to veterinary oncology into account. Based on the available peer-reviewed *in vitro* and *in vivo* studies and clinical trials, the obtained data are meant to define future research on the use of VAE in the treatment of cancer in cats, dogs and horses.

Methods

The search of this review followed the strategy of preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement and a measurement tool to assess systematic reviews (AMSTAR) and was conducted in February 2019. In addition, the research question followed the PICOS scheme (population intervention comparison outcome study design) to develop the literature search strategy to answer the formulated questions [37-39]. This review focused on companion animals like cats, dogs and horses and their most important cancers. The intervention was defined by the administration of whole plant extracts of European *Viscum album* L.. These extracts were based on different standardized extraction procedures used for registered medicinal products (Lectinol®, Isorel®, Iscador®, Helixor®, Abnoba®), such like aqueous, fermented and non-fermented as well as

ethanolic extraction. Some other experiments were based on experimental mistletoe preparations. The mistletoe preparations were compared to baseline cancer treatments (BCT) including chemotherapy and radiation or placebo and untreated controls. References with different study designs, namely clinical and *in vivo* studies performed in animals, as well as *ex vivo* and *in vitro* studies were included. Various clinical and cytological parameters were evaluated.
A detailed protocol of the systematic review is provided in supplementary document 1. Database searches were conducted in: Medline via PubMed, (incl. PMC, NCIB bookshelf) Agricola, Cab Abstracts, Cochrane Library and Embase. The search terms 'viscum album' (scientific name) or 'mistletoe' (the common trivial name in English) were applied, for the German search portal (Livivo), the search term 'mistletoe' was replaced by 'Mistel' (the common trivial name of the plant in German). All peer-reviewed articles written in English or German language were considered for further evaluation to ensure contemporary scientific quality. Duplicates were removed in EndNote. Inclusion and exclusion criteria were predefined by the authors (see supplementary document 1). The first author screened the obtained references using a selective approach starting out with title and abstract and according to the PICOS scheme. References remained if the content was appropriate to the objective and the set criteria of this review. References that did not match the defined criteria were excluded. References were included if an abstract was provided, the publication was peer- reviewed, and if the investigation specifically referred to the whole plant extract of European *Viscum album* L. Veterinary clinical oncology publications based on *in vivo* experiments or experiments carried out in laboratory animals or *in vitro* on cell lines with matching controls or placebo groups were included. Studies based on *in vitro* and *ex vivo* experiments were included if at least one group treated with VAE was part of the study. Studies lacking a control group were excluded, and studies referring to experiments with other mistletoe species like Korean mistletoe (*Viscum coloratum* (KOMAR*)* NAKAI) or those using single phytochemicals of the plant, were also excluded. Proceedings and reviews were not considered.
All remaining references with clinical, *in vivo, ex vivo* and *in vitro* data were studied by the same person, and during this process, further references were excluded if they did not match the predefined selection criteria. One and the same reference could include one or several experiment(s). The experiments could represent the same or different methods with: (a) mammalians (*in vivo* and clinical) and (b) cell lines (*in vitro*), tissues or organs (*ex vivo*).
Data collected in *in vivo* and clinical experiments are presented in supplementary document 2. Data derived from *in vitro* and *ex vivo* studies are presented in supplementary document 3. For both study types, one experiment was defined as the comparison of several treatments for the same or several tumor types under the same conditions with the treatments being VAE, chemotherapy and/or a non-treated or placebo control. For the *in vivo* or clinical studies, a study was considered eligible when a combination of a standard therapy with VAE was used and compared with an untreated and/or placebo group.
References reporting of clinical and *in vivo* experiments were eligible if tumor bearing laboratory animals were kept under the same standardized conditions and companion animals were client owned. These references had to provide at least one of the following aspects: (a) number, morphology, volume or size and weight of the tumor, (b) cancer-related immunomodulation, (c) survival time, (d) mitigation of adverse effects due to BCT, (e) adverse effects of *Viscum album* L. treatment, and (f) further cancer-related examination parameters. References with *ex vivo* and *in vitro* experiments had to provide at least one of the following aspects: (a) cytotoxic or cytolytic effects, (b) apoptosis, (c) cell replication and proliferation, (d) mitochondrial activity, (e) cell count, (f) cancer-related immunomodulation (g) cell viability (h) tumor growth and (i) further examination parameters closely related to the cancer cell line used.
A scoring system as shown in table 2 was developed and implemented to evaluate significant differences between treatments: VAE (= verum) compared to placebo/untreated (two armed experiments) or both compared to chemotherapy (three armed experiments) for each parameter. Various VAE treatment designs and parameters were used in the studies included in this systematic review. As these differences were summarized in the *in vitro* experiments, and in contrast to the *in*

vivo experiments, it was required to report the results of the *in vitro* experiments twice for each parameter in each experiment regarding the most efficient treatment (MET) and once for the least efficient treatment (LET) protocol. The LET scoring was conducted to detect possible tumor promotive properties of VAE.
Effects of VAE treatment for all experiments were estimated by comparing the maximally attainable score (sc_{max}) and the score obtained for VAE treatment. The sc_{max} was calculated based on (a) the study design (two-armed or three-armed (Table 2), (b) the number of experiments, and (c) the number of parameters measured within the experiments. In order to weight and discuss the results, effect levels were defined as presented in table 3.

Results
In total, 6148 references were identified in the initial database search. Following the screening process, 61 eligible references were included in the final analysis. These 61 remaining references were divided into three reference types: i. *in vivo* and clinical references (n=26), ii. *in vitro* references (n=25), and a combination of both, *in vivo* and *in vitro* studies (n=10) (Fig. 1). The scientific content of the 61 references comprised data of 67 *in vivo* and clinical, and 193 *in vitro* experiments. In total, this also included 188 VAE treatment groups (Fig. 2) of which four VAE groups also received chemotherapy (see Tab. 4 and supplementary document 2). Overall, 59 experiments were conducted with 169 groups of mice treated with VAE, followed by four experiments conducted with 15 groups of rats treated with VAE, three experiments in dogs and one experiment in horses with each having one group treated with VAE. In total, four clinical and 63 *in vivo* experiments were conducted.
All *in vivo* experiments and one clinical experiment were prospective investigations. Thirteen experiments, with 55 VAE groups, were conducted in a randomized manner. The experiments included a minimum of five and a maximum of 39 animals per group, with an average group size between eight and 15 animals in most experiments.
Carcinoma was the predominantly investigated cancer type (n=68 VAE groups and 23 experiments) when compared with other cancers like sarcoma (n=51 VAE groups within a total of 19 experiments), followed by, hematological and lymphoid malignancies (n=33 VAE groups and 10 experiments). Four clinical studies on VAE treatment were conducted in cohorts of dogs with naturally occurring sticker sarcoma, oral melanomas and mammary tumors, and in a cohort of horses with ES. In experimental studies comprising 172 experimental VAE treatment groups, tumors were predominantly induced by subcutaneous injection of tumor cells (n=99) and injection of tumor initiators and/or promotors such as 20-methylcholanthrene and methyl-nitrosurea (n=8).
VAE from different host trees were used, mostly from apple (*Malus domestica* BORKH.), pine (*Pinus ssp*.) or fir (*Abies ssp*.). A total of 69 untreated control or placebo groups and 17 positive control groups served for comparison, mainly consisting of chemotherapy.
Observation periods ranged from seven days to more than six months in *in vivo* experiments with rats and mice. In clinical experiments with dogs and horses, the observation period ranged from one year to lifelong observations.
The following routes of administration for VAE were used: intraperitoneal (n=80), subcutaneous (n=43), intralesional (n=15), intravenous (n=10), oral (n=1) and intravesical (n=1). In four experiments (n=4), VAE administration methods were combined. In 19 experiments, no information was provided regarding the route of application of VAE. The occurrence of adverse effects to VAE treatment were not reported in 140 VAE groups. In 48 groups VAE treatment was reportedly well tolerated. In a clinical experiment with 18 dogs treated with VAE, self-limiting adverse effects were described in 2 cases.
Sarcoma were studied in 19 experiments with a total of 51 groups treated with VAE. Based on the design of the studies, a total sc_{max} of 96 could have been reached if the effect of VAE was significantly superior to placebo or untreated (in two-armed experiments) and significantly superior to chemotherapy (in three-armed experiments) when all investigated parameters were considered as shown in table 4. Evaluations of all studies on sarcoma resulted in a score of 56 out of 96 sc_{max} for

VAE-treated animals also shown in Table 4. Scores for parameters related to tumor size, number or tumors for all tumor types and survival are given in table 4.
The effectiveness of VAE treatment for carcinoma (68 VAE groups) was comparable to that of sarcoma with a total score of 83 out of a sc_{max} of 156 when all study parameters and all experiments were considered.
VAE treatment of hematopoietic and lymphoid malignancies (HLM) (33 VAE groups) showed lower scores when compared to all other tumor classes, reaching a score of 39 out of 103 sc_{max} for all parameters and all experiments.
VAE treatment in animals with melanomas was investigated in 29 groups. A total score of 52 out 71 sc_{max} was reached when all experiments and study parameters were considered.
Four clinical studies (ES, canine oral melanoma, canine mammary carcinoma, canine sticker sarcoma) were included in this systematic review, consisting of a total of eight VAE groups, resulting in a score of six out of eight sc_{max}. VAE treatment was investigated in 52 groups of animals with skin cancers, resulting in a score of 68 out of 100 sc_{max}.
In tumor bearing animals, the reduction of the number of tumors or metastases was investigated in 53 VAE groups and a score of 37 out of 53 sc_{max} was obtained. Outcomes for other tumor-related parameters are given in Table 4. Prolongation of survival time was investigated in 56 VAE groups and a score of 28 out of 81 sc_{max} was obtained. For other tumor-related parameters (62 VAE groups), a score of 45 out of 92 sc_{max} was reached.
Negative effects of VAE treatment were observed in two experiments of CBA/HZgr mice that were treated for skin fibrosarcoma and a negative score and therefore, a worsening effect was found for tumor-related immunomodulation when compared to placebo-treated controls. In all other experiments (i.e. 186 VAE treatment groups) and all other assessed parameters, effects were either beneficial or absent in comparison to placebo-treated control groups.
The overall sc_{max}, calculated by including all parameters and cancer types, in the 188 VAE groups was 444, and VAE treatment resulted in a score of 228 as shown in table 4.
In the 35 *in vitro* studies included, a total of 193 experiments were conducted in a 147 different cell lines, as shown in table 5 and supplementary document 3. These studies included 19 experiments with 16 sarcoma cell lines, mainly originating from bone and muscle tissues. Furthermore, experiments (n=93) involving 78 carcinoma cell lines derived from lung tissue, mammary gland and colon were included. In addition, 63 experiments with 39 cell lines of hematological and lymphoid malignancies, mainly lymphoma and leukemia, were identified and assessed. Another 16 experiments including 14 melanoma cell lines, predominantly from skin samples, and two experiments with brain tumor cells and one uterus cancer cell line were conducted. Most experiments were performed with human cell lines (n=182), followed by experiments with murine cell lines (n=10) and one rat cell line. A total of 193 *in vitro* experiments was included. Untreated controls were implemented in 169 experiments, placebo controls in 15 experiments, and chemotherapy as alternative treatment was used in nine experiments.
The most frequently administered VAE were obtained from the following host trees: *Populus* L. (56), *Quercus* L, *Malus domestica* BORK. and *Pinus ssp.* (38), *Quercus* and *Malus* (19) and solely *Malus* (24) or *Fraxinus excelsior* L. (10), also *Abietis ssp Tilia* L., *Acer* L. and *Robinia pseudoaccacia* L.
One dosage of VAE was administered in 11 experiments, two dosages were given in three experiments, 3-5 different dosages were administered in 172 experiments, and 6-8 different dosages were given in five experiments.
Effects of VAE were investigated in 19 experiments using sarcoma cell lines. Based on the design of the experiments, a sc_{max} of 44 could have been reached if the effect of VAE was superior to placebo (in two-armed experiments) and predominantly better than chemotherapy (in three-armed experiments) considering all parameters investigated. Evaluations of all experiments on sarcoma resulted in a score of 28 for VAE treatment as shown in Table 5, which was the best response of VAE treatment in all investigated tumor classes in experiments with sarcoma cell lines. VAE treatment induced mainly apoptosis and reduced cell proliferation in sarcoma cell lines.

Carcinoma cell lines were treated with VAE in a total of 93 experiments. A score of 60 out of 143 sc_{max} was obtained for carcinoma cell lines. The best results for the VAE treatment were obtained for apoptosis (25 experiments), followed by reduction of cancer cell proliferation and cytotoxicity in a total of 30 experiments. The respective scores are given in Table 5.
In 63 experiments with HLM cell lines, a score of 60 out of 122 sc_{max} was obtained after VAE treatment. Scores relative to the sc_{max} for cellular proliferation, cytotoxicity, apoptosis, and reduction of cell viability can be found in Table 4.
Treatment of melanoma cell lines with VAE resulted in a score of 13 out of 24 sc_{max} when all parameters of the 16 experiments were considered, reaching about half of the sc_{max} for related parameters such as reduction of cancer cell proliferation (15 experiments), cytotoxicity (4 experiments) and apoptosis, whereas the sc_{max} was reached for reduction of cell viability of the melanoma cells and cell viability, respectively. However, only one or two experiments were conducted (Table 5).
Overall, when all cell types were considered, VAE treatment was most efficient in inducing apoptosis and in reducing cell viability with scores reaching more than half the sc_{max}, followed by a reduction of cell proliferation, cytotoxicity and other cancer-related parameters with scores of nearly half the sc_{max} (Table 5).
In most cases, the score of the LET for all parameters was zero and, in a few cases, even positive, whereas a negative effect was documented for VAE treatment in five experiments when compared to untreated or placebo controls as shown in table 5. Negative scores were obtained for cell viability in the cervical carcinoma cell line HeLa and for other cancer related effects, the lymphoma T-cell line CEM, and the chemotherapy-resistant leukemia (chronic myelogenous) cell line, K 562. Negative scores were also obtained for cell proliferation in the chemotherapy-resistant lymphoma (Burkitt's) cell line, Raji and the lung carcinoma cell line, MR65. A sc_{max} of 335 was obtained when all parameters and cancer types in 193 experiments were considered and for VAE treatment in all MET protocols, the score was nearly half of the sc_{max} (Table 5).

Discussion

Companion animals often suffer from cancer, and the established treatment approaches in veterinary practice comprise surgical excision, radiation- and chemotherapy [40-43]. However, especially chemo- and radiation-therapy are often rejected by pet owners because of fear of possible adverse effects and the stress associated with treatment [11, 15, 44, 45]. This is also reflected by the fact that QoL was rarely assessed in studies of cancer treatment in cats and dogs, which likely leads to an overestimation of treatment benefits while neglecting adverse effects of therapy [11, 46]. Furthermore, when applying chemotherapeutic drugs, strict measures to prevent direct contact are part of good clinical practice (GCP) and associated risks for veterinary professionals, pet owners and companion animals living in the same household or stable need to be considered [16, 47-49]. In addition, contaminated excrements, deposited in the environment also pose a risk [50]. VAE, on the other hand, is well tolerated by the treated pets, their owners and attending veterinarian.
In human oncology, VAE is a frequently used adjuvant treatment in cancer therapy in German-speaking countries [51, 52]. Several systematic reviews and meta-analyses on VEA treatment in cancer patients are available also referring to the parameter 'quality of life' [22, 24, 53-64]. Likewise, the present study aimed to critically assess the therapeutic potential of VAE for cancer treatment specifically in companion animals and horses. This systematic review is based on data from clinical trials provided in peer-reviewed publications and includes evidence derived from *in vivo* as well as *in vitro* experiments conducted in animals.
As proposed in recent publications defining the methodological requirements for systematic reviews [38, 65, 66], careful attention was given to disclose all single steps of the analyses in the processing of the available data (supplementary document 1). Moreover, all extracted information is provided in the supplementary documents 2 und 3 in detail. Source selection bias was minimized by considering several online databases. Furthermore, no limitation of the publication date was set. To ensure adequate quality, exclusively experiments that included a placebo, or non-treated, or a

chemotherapy control, respectively, were considered in this review. In order to provide a comprehensive summary of the results of a broad spectrum of studies, a semi-quantitative scoring system to uniformly assess predefined outcome parameters was developed and implemented. Ultimately, the proposed scoring-system aimed at estimating the therapeutic potential based on the treatment effect categories low, moderate, good and high.

Particularly in studies with plant extracts, bias cannot be excluded due to the variance between batches and the complex phytochemical composition of VAE [67, 68]. Arguably, this bias is not significant, because most of the VAE products used in both, in *in vitro* and in *in vivo* experiments were registered medicinal products approved by control authorities.

The majority of references in this review, did not investigate dose-response effects, the majority of studies that tested different dose-responses, gave evidence, that higher dosages were more effective. Unfortunately, these studies are not comparable with one another, as they involved different preparations and different dosages [69-78].

Finally, only few studies were available on VAE treatment in companion animals per tumor class, causing an evaluation bias regarding the value of VAE in cancer diseases in these species. This was also the case in previously published reviews [79, 80].

Carcinoma

Nearly half of the *in vivo* and one-third of the *in vitro* experiments were conducted in carcinoma-bearing animals or carcinoma cell lines, reflecting the high frequency of carcinoma in humans [81] as well as in companion animals [2, 82]. The therapeutic potential of VAE was found to be moderate based on *in vivo* (50% of sc_{max}) and *in vitro* (41% of sc_{max}) experiments. A high *in vivo* effect was found for VAE in various tumor measures. Data from *in vitro* studies support the good effect of VAE in the investigated parameters. VAE was demonstrated to be efficient regarding the severity of symptoms and it was reported to increase the overall survival times in human patients with carcinoma [31, 83-85]. In contrast, a low effect of VAE in was found for survival time in companion animals. However, this parameter was only considered in one out of five studies included in our systematic review.

Mammary carcinoma

About half of the *in vivo* and approximately one-fifth of the *in vitro* experiments were carried out in mammary carcinomas and mammary carcinoma cell lines. Mammary carcinomas occur frequently in dogs, less frequently but more aggressively in cats, and rarely in horses [82, 86]. The assessment of the effect of VAE treatment against mammary carcinomas showed good *in vivo* (63 % of sc_{max}) and moderate *in vitro* results (50% of sc_{max}). While VAE did not prolong survival, tumor numbers and tumor weight were reduced and led to an amelioration of tumor morphology, and a decrease in histopathological features of malignancy was found. These findings are supported by a moderate *in vitro* effect in related parameters. In contrast, VAE treatment in human breast cancer patients revealed an increase in survival time, health-related QoL, but in agreement with the results of this systematic review, a reduction of the remission rate, and alleviation of adverse effects has been reported [60, 83, 87-93]. The different findings might be explained by study size and number of available studies in human versus veterinary medicine.

Only one clinical trial was conducted on canine mammary tumors. The investigators of this study reported a trend ($p = 0.07$) towards a decrease of the hazard ratio of tumor-related death risk (HR 0,251, 95%-KI 0,056–1,122) while maintaining stable QoL [47]. No indication of worsening of the mammary cancer disease state or growth promotion of the mammary cancer cells was found. Overall, outcomes of this review, data from human clinical trials, and limited data available for conventional postoperative adjuvant therapies [86, 94, 95] indicate that VAE treatment might be a promising candidate for postsurgical adjuvant treatment of mammary cancer in dogs.

Squamous cell carcinoma (SCC)

These carcinomas are highly relevant in all companion animals as well as in humans [96-98]. SCC represent approximately half of all malignant canine digital lesions [99, 100], but successful therapies in cats and dogs are still missing [101-104]. There are only two *in vitro* experiments with human cell lines of SCC of the tongue that showed high effect of VAE treatment [105], so it might be a treatment option in veterinary medicine.

Sarcoma

The effectiveness of VAE treatment against sarcomas was investigated in about one quarter of all *in vivo* and one tenth of all *in vitro* experiments comprised in this study, reflecting the high frequency of sarcomas in humans (RKI 2019) as well as in companion animals [2, 82]. Overall, a good effect of VAE treatment was found in *in vivo* (58% sc_{max}) and *in vitro* (62% of sc_{max}) studies. VAE treatment showed moderate effects in prolonging survival time. VAE treatment was also efficient in reducing the various tumor-related measures. Interestingly, the *in vitro* effect differed widely depending on the measured parameter. These inconsistent findings may explain some of the contradictory results of reviews on VAE treatment in clinical studies in human sarcoma patients [53, 54, 59, 106].

Only one single clinical study on VAE treatment in dogs with sarcomas was considered in the analyses of this review. Compared to dogs receiving only chemotherapy, the use of VAE as adjuvant resulted in a significant reduction of the duration of the chemotherapy and total amount of vincristine used. Likewise, the immunosuppressive adverse effects (neutropenia) of chemotherapy directed against sticker sarcoma could be alleviated [107]. So far, no studies have been published concerning other important sarcomas found in companion animals, including mast cell tumors, hemangiosarcoma, soft tissue sarcomas, and histiocytic sarcomas.

Sarcoma in bones

Although osteosarcomas are rare in dogs (<1%), and even more so in cats and horses, they represent a major challenge for pet owners and veterinary oncologists due to their high tendency to metastasize and the painful nature of the disease [2, 82, 108-110]. Based on the studies considered in the present review, VAE treatment has a good *in vivo* effect, specifically in decreasing tumor volume in Ewing sarcoma, and these findings were supported by the results of *in vitro* studies. A moderate treatment effect of VAE was found in three experiments conducted in osteosarcoma cell lines [111, 112]. Human data on osteosarcoma treatment using VAE for adjuvant demonstrated a prolongation of survival time from four to 39 months compared to chemotherapy alone [53, 113]. The radical therapeutic measures recommended to address osteosarcoma in dogs and cats (radiation and amputation) have drastic implications on the QoL [108, 109] and are therefore often not supported by pet owners. So far, VAE treatment has not been investigated in this specific context.

Fibrosarcoma

Feline Fibrosarcoma, also so-called 'injection site sarcoma', are challenging to treat and associated with notoriously high recurrence rates even following radical excision surgery [114-118]. Based on findings from one *in vivo* study, adjuvant VAE therapy has a good effect (85% of sc_{max}), especially for the decrease in the number of tumors or metastases, survival time of about 50%. However, the small number of studies prevents a causal conclusion. A prospective case series assessed the treatment effect of postsurgical oral VAE treatment in 44 cats with fibrosarcoma [119] compared to existing literature data. In this study, the disease-free survival of cats receiving the VAE adjunctive treatment was reported to be comparable (438 days) when to cats receiving chemotherapy (365–475 day) and longer when cats treated by surgery alone (120–261 days). These results need to be verified in prospective controlled trials.

Hematopoietic and lymphatic malignancies

Hematopoietic and lymphoid malignancies are common cancers in companion animals and chemotherapy is the first-choice therapy [120]. Various subtypes exist for lymphomas and leukemia [1, 121, 122]. Only one sixth of all *in vivo,* but about one third of all *in vitro* experiments considered in this review, were conducted on HLM. Overall, VAE showed a moderate *in vivo* (38% of sc_{max}) and *in vitro* (49% of sc_{max}) effect. The *in vivo* effect was high for various tumor measures [70, 72, 78, 123], which was supported by the *in vitro* data. In contrary, improved cell viability was found in three experiments conducted in three cell lines, namely the B-cell leukemia cell line (CEM), and the two chemotherapy-resistant cell lines, K652 and Raji.

Leukemia

Infection with feline leukemia virus (FeLV) is usually fatal in cats [124]. Its therapy is difficult and almost never curative. The overall *in vivo* effect of adjuvant VAE treatment was low, based on the outcome of this review. In contrast, the *in vitro* effect was good for the investigated parameters.

Only a few preclinical studies are available, assessing effects of VAE treatment and cytotoxic effects were shown in pediatric leukemia cell lines [125-127] and *in vivo* trials [128]. Chemotherapy is the first-choice treatment in human medicine, but the frequently encountered resistance to doxorubicin negatively impacts therapy success rates. VAE treatment resulted in a sensitization of leukemia cells to doxorubicin in human patients, but no studies were available for veterinary oncology. Moreover, a good *in vivo* effect was demonstrated for a combination of VAE and chemotherapy [129]. Hence, there is some evidence that VAE modulates the immune response, but this needs to be studied in cats with leukemia.

Lymphoma

B cell lymphomas are the most common lymphatic cancers in dogs with an incidence approaching 0.1%, and a total of 20–100 cases per 100,000 individuals [130]. The gastrointestinal lymphoma is the most common lymphatic neoplasm in cats, and the reported incidence is higher than in any other species [131]. Only a small number of case reports on VAE treatment in human lymphoma patients is available, reporting tumor reduction and even complete remission with VAE treatment [132-135]. Overall, a high *in vivo* effect was found after VAE treatment of lymphomas, especially for survival time, tumor volume and immunomodulation and the effect for a reduction in the number of tumors and metastasis was good. Even if the effect of VAE was low with regards to reducing cell proliferation in *in vitro* experiments, its effect was moderate; thus, supporting the *in vivo* findings.

Melanoma

Whereas in humans most melanomas are found in the skin [136, 137], in dogs, this tumor most frequently occurs in the oral cavity [106]. In this review, the overall *in vivo* effect of VAE treatment directed against melanomas was found to be good (73% of sc_{max}), especially for prolonged survival, and various tumor measures, but it was only moderately effective in reducing tumor volume or weight. Corroborating these data, the *in vitro* effect was good or moderate depending on the investigated parameter. No positive impact on survival, but a significant decrease in tumor progression was found in VAE-treated human melanoma patients [138, 139]. One clinical study was conducted with adjuvant VAE treatment after radiation of canine oral melanomas, and a marked prolongation of survival time was found. Considering all available *in vivo*, *in vitro* and veterinary clinical data, VAE treatment applied as adjunct in combination with radiation therapy may be a valid option to treat melanomas in companion animals, specifically oral melanomas in dogs.

Equine sarcoids

The treatment of ES can be challenging, not only because of the notoriously high propensity of recurrence, but also because large surface areas of the integument can be affected, or tumors are located in anatomical regions that impede surgical excision [110]. This dilemma is also reflected in the seemingly endless list of therapy options, suggesting that each treatment method is associated with specific advantages and disadvantages, and the sobering conclusion that ultimately none of the treatment methods is consistently successful [140]. To the authors' best knowledge, only one randomized blinded placebo-controlled clinical trial using VAE as a stand-alone treatment against ES in horses is available [141]. This study did not only demonstrate a high effect in reducing tumor volume and number of tumors per animal, but also an amelioration of the tumor morphology. Notably, these improvements were shown to have a sustainable, long-lasting effect over a five-year follow-up period [141]. The observed partial and complete regression of ES tumors (67%) following VAE treatment was similar to reported outcome of other ES treatments, ranging between 28 to 95% [142]. In this context, however, it is important to emphasize the tremendous potential mediated by the systemic, immunomodulatory effects of VAE. Whereas most other established treatment forms are directed against selected ES tumors, subcutaneously administered VAE is expected to produce systemic effects directed against all tumors present on the integument of the treated subject. As mentioned above, this is a considerable advantage, particularly when treating equids with multiple ES lesions, affecting large surface areas and in cases where excisional surgery is not an option and tissue-sparing treatment options are needed.

Conclusions and clinical relevance

Based on the findings of this systematic review, effects of VAE are most pronounced in the following selected tumor types: melanoma, sarcoma, mammary carcinoma and ES. This finding is mainly corroborated by *in vitro* data. However, the limited number and quality of published clinical studies on VAE treatment in companion animals does not allow causal conclusions about the efficacy of VAE treatment in clinical practice. So far, the available evidence is merely suggestive of positive effects of VAE treatment when used as an adjuvant with conventional therapy or as stand-alone therapy against selected tumor types in companion animals. Further research is needed to assess the efficacy of VAE treatment against cancer in companion animals and their underlying mechanisms of action.

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M.M., M.W., U.B., had the idea and conceived the study. Si.S. and K.R. were instrumental in compiling the list of major cancers in veterinary medicine and all related small animal issues. C.K. and O.C. substantially contributed to the writing on VAE in horses. U.B., H.A., M.W. and M.M. developed the PICOS scheme for this review. U.B., M.M. and M.W. developed the scoring system. U.B wrote the final manuscript and was mainly supported by C.K., M.M. and M.W. with the thorough review, revision and editing of the paper.

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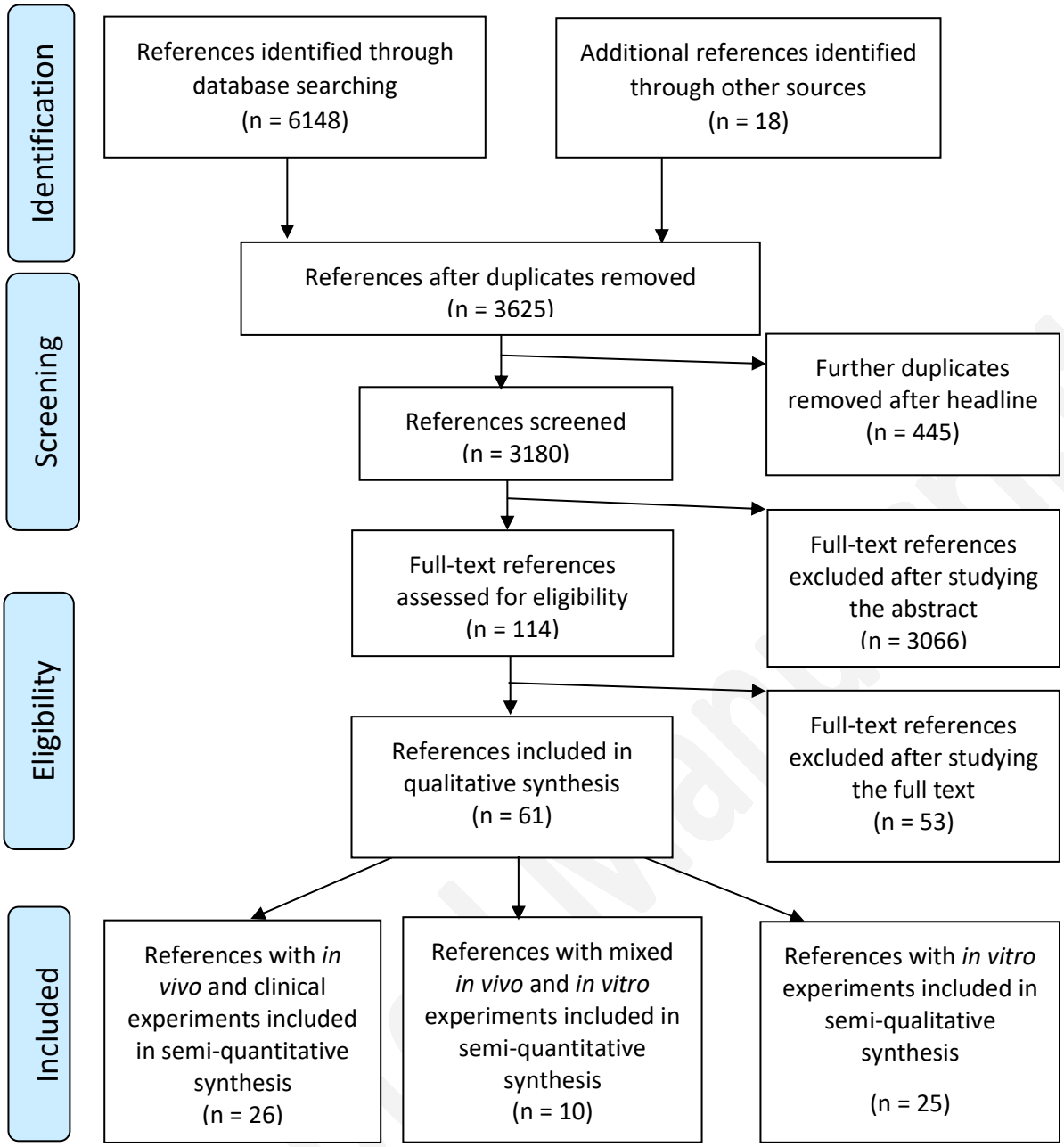
Figure Legends

Fig. 1. Flowchart of the systematic review

Fig. 2. Flow chart showing the included references and finally assessed experiments



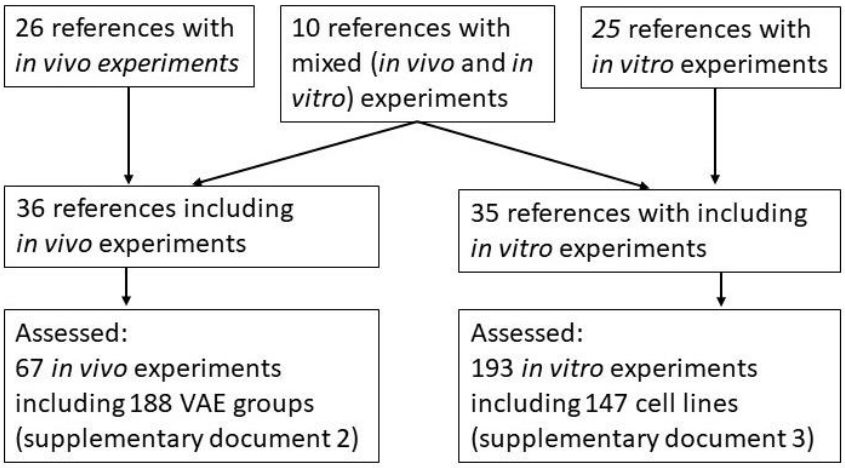


Table 1: Common cancer diseases in cats, dogs and horses

Species	Organ System	Tumor type	Percentage of tumor	References
Cat	Skin and sub-cutaneous tissue		25-43 % of cancers	[1] [2]
		Basal cell tumor	23	
		Mast cell tumor	16.5	
		Soft Tissue Sarcoma	17.9	
		Squamous Cell Carcinoma	10.4	
		Sebaceous Adenoma/Hyperplasia	3.4	
	Oral cavity	Squamous cell carcinoma	80	[1]
		Fibrosarcoma	15	
	GI tract	Gastric lymphoma	120 cats within 11 years [118]	[1] [118]
		Hepatobiliary Carcinoma	1-2.9% of all tumors	
		Intestinal Lymphoma	30% of all tumor	
		Intestinal Carcinoma	5% of all tumor	
	Mammary gland	Mammary tumor, various	25.4/100'000, 16-25% of all tumors in female cats	[1] [130]
	Respiratory tract	Lung Cancer	<1% of all tumor	[1]
	Hematopoietic system	Lymphoma	200/100'000 3 – 38%	[1] [130] [131]
Dog	Skin and sub-cutaneous tissue		33% of tumors	[1] [132, 133]

		Mast Cell Tumor	16.8	[134]
		Soft Tissue Sarcoma	9.7	
		Lipoma	8.5	
		Histiocytoma	8.4	
		Hepatoid (perianal) Adenoma/Hyperplasia	7.8	
		Sebaceous Adenoma/Hyperplasia	6.5	
		Squamous cell carcinoma	6.0	
		Melanoma	5.6	
		Basal Cell Tumor	5.0	
		Hemangiopericytoma	4.4	
	Oral cavity		6% of all cancer	[1]
		Oral Melanoma	30-40%	[132]
		Squamous cell carcinoma	17-25	
		Fibrosarcoma	8-25	
		Acanthomatous epulis	5	
	GI tract	Gastric Adenocarcinoma	1% of all cancers	[1]
		Hepatobiliary Carcinoma	1.5% of all tumors	
		Intestinal Lymphoma	6% of all tumors	
		Intestinal Adenocarcinoma	6% of all tumors	
		Intestinal Leiomyosarcoma	5% of all tumors	
		Perianal Adenoma	58-96	
		Apocrine Gland Adenocarcinoma	17	
	Respiratory tract	Lung Cancer	<1% of all tumor	

	Skeletal system	Osteosarcoma	1% of all tumor	[66]
	Mammary gland	Mammary Tumor	2- >20% lifetime risk	[1]
			50-70% of all tumors in female dogs. 258/100,000	[130].
			23.5% of all tumors	[66]
			476/100'000	[135]
	Urinary system	Transitional Cell Carcinoma	2% of all tumors	[1]
	CNS	Brain tumor	14.5/100'000	[1]
	Hematopoietic system	Lymphoma	13-24/100'000 7-24% of all tumors	[1]
		Plasma Cell Tumor	<1% of all tumor 8% of hematopoietic tumors	[1]
		Hemangiosarcoma	12-21% of mesenchymal tumors, 50% of splenic tumors 2.8% of all tumors	[1] [66]
	Histiocytic Disease	Histiocytic Sarcoma	25% of death in Bernese Mountain dogs 50% of malignant tumors in Flat Coated Retriever 25% of tumors in Bernese Mountain Dogs	[136] [137]

Horse				
	Skin	Equine Sarcoid (ES)	24 - 51.4	[8]
		Squamous Cell Carcinoma	18.3-19	[9]
		Melanoma (grey horses)	6 4-6	[10]
		Gonadal Stromal Tumors	6	
		Mast Cell Tumor	3 – 4 %	
	Hematopoietic system	Lymphoma	14	[8] [9]

Table 2: Scoring system for two- and three-armed *in vivo* and *in vitro* experiments with Viscum album extract (VAE); scoring was conducted per each parameter measured within each experiment.

Result	Score
Two-armed experiments	
VAE was significantly inferior to untreated or negative controls	- 1
VAE was equally efficient compared to untreated or negative controls or significantly inferior to chemotherapy	0
VAE was significantly superior to untreated or negative controls	1
VAE was significantly superior or equal to chemotherapy	1
Three-armed experiments	
VAE was significantly inferior to untreated or negative controls	- 1
VAE was significantly inferior to chemotherapy or both equal to untreated or negative controls	0
VAE was equally efficient to chemotherapy when chemotherapy was significantly superior to untreated or negative controls	1
VAE was significantly superior to chemotherapy, and chemotherapy was significantly superior to untreated controls	2

Table 3: Effect levels of Viscum album extract (VAE) treatment

Effect level	**Relation of score points out of the maximally attainable score (sc_{max})**
Low effects	< 25% of sc_{max}
Moderate effects	25 - 50 % of sc_{max}
Good effects	50 - 75% of sc_{max}
High effects	> 75% of sc_{max}

Table 4: Effect of Viscum album extract (VAE) in cancer bearing animals: assessment of cancer-related outcomes

Tumor classification	Organ	Number of references/ experiments/ VAE groups	Outcome of cancer related parameters: sum of score[a]/masc[b], in brackets number of experiments where the respective parameter was analysed							Sum of score for all parameters[a] / masc[b]
			Tumor				Immuno-modulation	Survial time	Other effects	
			Number/ animal	Morphology	Volume or size	Weight				
Sarcoma	Bone	1/3/7	-	-	8/11 (7)	-	-	-	-	8/11
	Lung	2/4/16	6/12 (12)	-	3/4 (4)	3/4 (4)	12/16 (12)	-	-	24/32
	Skin	4/10/23	6/7 (7)	-	-		3/8 (8)[†]	8/16 (13)	1/1 (1)	18/32
	Others[c]	3/4/5*	-	-	2/9 (6)*	-	-	1/2 (2)	3/10 (8)*	6/21
All sarcoma		**10/19/51**	**12/19 (19)**	**-**	**13/24 (17)**	**3/4 (4)**	**15/20 (20)**	**9/18 (15)**	**4/11 (9)***	**56/96**
Carcinoma	Colon	2/2/9	-	-	2/13 (7)	-	0/1 (1)	-	3/5 (1)	5/19
	Kidney	1/1/8	-	-	3/11 (7)	-	-	-	-	3/11
	Lung	1/1/4	-	-	0/3 (3)	-	-	-	-	0/3
	Mammary gland	10/16/33*	8/8 (8) -	10/10 (8) -	14/27 (23)	16/16 (17)*	-	1/ 20 (11)	22/31 (19)	71/112
	Pancreas	1/2/4	-	-	0/5 (4)	-	-	-	-	0/5
	Testicles	1/1/8	-	-	2/6 (6)	-	-	-	-	2/6
	Urinary tract	1/2/3*	0/2 (2)	0/3 (3)	-	-	-	1/2 (2)	1/2 (2)	2/9
All carcinoma		**14/23/68****	**8/10 (10)**	**10/13 (11)**	**21/65 (49)**	**16/16 (17)***	**0/1 (1)**	**2/22 (13)**	**26/38 (22)**	**83/165**
Carcino-sarcoma	Mammary gland	**1/1/3**	**-**	**0/3 (3)**	**-**	**0/3 (3)**	**-**	**-**	**-**	**0/6**
HLM[d]	Leucemia	5/5/17*	-	-	-	4/8 (4)*	0/10 (5)	5/26 (13)	1/24 (12)	10/68
	Lymphoma	3/5/16	8/12 (12)	-	3/3 (3)	-	17/19 (12)	1/1 (1)	-	29/35
All HLM		**8/10/33***	**8/12 (12)**	**-**	**3/3 (3)**	**4/8 (4)***	**17/29 (17)**	**6/27 (14)**	**1/24 (12)**	**39/103**
Melanoma	Skin	9/12/29	8/11 (11)	1/1 (1)	5/15 (15)	1/2 (2)	6/7 (5)	10/13 (13)	19/19 (19)	50/68
	Others	1/1/1	-	1/1 (1)	0/1 (1)	-	-	1/1 (1)	-	2/3
All melanoma		**10/13/29**	**8/11 (11)**	**2/2(2)**	**5/16 (16)**	**1/2 (2)**	**6/7 (5)**	**11/14 (14)**	**19/19 (19)**	**52/71**
Others	Equine sarcoid	**1/1/1**	**1/1 (1)**	**1/1 (1)**	**1/1 (1)**	**-**	**-**	**-**	**-**	**3/3**
All clinical experiments		**4/4/8***	**1/1 (1)**	**1/1 (1)**	**1/1 (2)**	**-**	**-**	**1/2 (2)**	**2/2 (2)**	**6/7**

Organ Tumor classification	Number of references/ experiments/ VAE groups	Outcome of cancer related parameters: sum of score[a]/masc[b], in brackets number of experiments where the respective parameter was analysed							Sum of score for all parameters[a] / masc[b]
		Tumor				Immuno-modulation	Survial time	Other effects	
		Number/ animal	Morphology	Volume or size	Weight				
All VAE x chemotherapy vs chemotherapy	**4/4/4***	**-**	**-**	**2/5 (3)**	**4/6 (3)**	**-**	**-**	**4/7 (5)***	**10/18**
All	**36/67/188**	**37/53(53)**	**13/19 (17)**	**43/109 (86)**	**24/33 (30)**	**38/57 (43)**	**28/81 (56)**	**45/92 (62)**	**228/444**

Table 5: Effects of viscum album extract (VAE) in cancer cell lines *in vitro* and *ex vivo*

Classification of tumor	Affected organ (tissue)	References / number of cell lines / experiments	Outcome of cancer related parameters: sum of score[a]/ SC_{max}[b]; in brackets: number of experiments, where the respective parameter was analysed										Sum of score for all parameters[a] / SC_{max}[b]	
			Tumor cell											
			Proliferation		Cytotoxicity		Apoptosis		Cell viability		Other cancer related parameters			
			MET [c]	LET [c]	MET [c]	LET [c]	MET[c]	LET [c]	MET [c]	LET [c]	MET [c]	LET [c]	MET [c]	LET [c]
Sarcoma	Bones	4[1.]/ 6 /8	5[1.] /8 (6)	1/8 (6)	0/4 (2)	0/4 (2)	6/9 (7)	1/9 (7)	-	-	4/6 (4)	1/6 (4)	15/27 [x]	3/27 [x]
	Muscle	3[2.]/ 7 /7	4/6 (6)	0/6 (6)	-	-	5/5 (5)	0/5 (5)	-	-	2/2 (2)	0/2 (2)	11/13	0/13
	Others [d]	3[3.]/ 4 /4	1/3 (3)	0/3 (3)	1/1 (1)	1/1 (1)	-	-	-	-	-	-	2/4	1/4
All	Sarcoma	**8/ 16 /19**	**10/17 (15)**	**1/17 (15)**	**1/5 (3)**	**1/5 (3)**	**11/ 14 (12)**	**1/ 14 (12)**	**-**	**-**	**6/8 (6)**	**1/8 (6)**	**28/44** [x]	**4/44** [x]
Carcinoma	Colon	3[4.]/10/13	3/13 (13)	0/13 (13)	0/2 (2)	0/2 (2)	0/4 (4)	0/4 (4)	-	-	-	-	3/19	0/19
	Kidney	2[5.]/ 7 /8	5/7 (7)	0/7 (7)	0/2 (2)	0/2 (2)	-	-	-	-	-	-	5/9	0/9
	Lung	6[6.]/ 24 /27	8/18 (18)	4/20 (20)[†]	7/12 (12)	3/12 (12)	9/ 12 (12)	6/ 12 (12)	1/2 (2)	-	0/8 (8)	1/7 (7)	25/52	15/ 57
	Mamma	10[7.]/ 15 /20	7/16 (16)	2/16 (16)	4/6 (6)	0/6 (6)	2/6 (6)	1/6 (6)	1/1 (1)	0/1 (1)	1/1 (1)	1/1 (1)	15/30	4/30
	Cervix	2[8.]/2/2	-	-	1/1 (1)	0/1 (1)	-	-	-1/1 (1)[†]	-1/1 (1)[†]	-1/1 (1)[†]	-1/1 (1)[†]	-1/3	-2/3
	Prostate	2[9.]/4/4	1/4 (4)	0/4 (4)	0/2 (2)	0/2 (2)	-	-	-	-	-	-	1/6	0/6
	Stomach	2[10.]/3/3	1/3 (3)	0/3 (3)	-	-	-	-	-	-	-	-	1/3	0/3
	Skin	2[11.]/2/2	0/1 (1)	0/1 (1)	-	-	1/1 (1)	0/1 (1)	-	-	-	-	1/2	0/2
	Others [e]	6[12]/16/14	6/11 (11)	0/9 (9)	1/5 (5)	1/5 (5)	2/2 (2)	1/2 (2)	-	0/2 (2)	1/1 (1)	0/2 (2)	5/19	1/14
All	**Carcinoma**	**16/78/93**	**31/73 (73)**	**3 (73)**	**13/30 (30)**	**4/30 (30)**	**14/ 25 (25)**	**8/25 (25)**	**1/4 (4)[†]**	**-1/4 (4)[†]**	**1/11 (11)[†]**	**1/11 (11)[†]**	**60/143**	**15/143[†]**
HLM [f]	Leukemia	13[13.]/17/27	7/17 (16)	0/17 (16)	4/5 (4)	2/5 (4)	8/9 (8)	1/9 (8)	8/11 (11)	0/11 (11)[†]	2/2 (2)	0/2 (2)	29/44 [x]	3/44 [x]
	Lymphoma	9[14.]/16/27	2/8 (8)	0/8 (8)	3/3 (3)	0/3 (3)	0/3 (3)	0/3 (3)	7/14 (14)	- 2/14(14)[††]	2/4 (4)	0/4 (4)	14/32	-2/32 [††]

Classification of tumor	Affected organ (tissue)	References / number of cell lines / experiments	Outcome of cancer related parameters: sum of score[a]/ sc_{max}[b]; in brackets: number of experiments, where the respective parameter was analysed										Sum of score for all parameters[a] / sc_{max}[b]	
			Tumor cell											
			Proliferation		Cytotoxicity		Apoptosis		Cell viability		Other cancer related parameters			
			MET[c]	LET[c]	MET[c]	LET[c]	MET[c]	LET[c]	MET[c]	LET[c]	MET[c]	LET[c]	MET[c]	LET[c]
	Myeloma	4[15.]/7/9	6/7 (7)	2/7 (7)	2/13 (7)	2/13 (7)	3/12 (6)	3/ 12 (6)	1/2 (1)	0/2 (1)	5/12 (6)	0/12 (6)	17/46[x]	7/46[x]
All	HLM[f]	**20/39/63**	**15/32 (31)**	**2/32 (31)**	**9/21 (14)**	**4/21 (14)**	**11/ 24 (17)**	**4/ 24 (17)**	**16/27 (26)**	**-2/27 (26)†††**	**9/18 (12)**	**0/18 (12)**	**60/122**[x]	**8/122**[x]
Melanoma	Skin	8[16.]/12/14	6/13 (13)	2/13 (13)	2/4 (4)	0/4 (4)	1/1 (1)	0/1 (1)	2/2 (2)	1/1 (1)	1/1 (1)	0/1 (1)	12/21	3/19
	Others[g]	2[17.]/2/2	0/2 (2)	0/2 (2)	-	-	-	-	-	-	1/1 (1)	0/1 (1)	1/ 3	0/3
All	Melanoma	**8/14/16**	**6/15 (15)**	**2/15 (15)**	**2/4 (4)**	**0/4 (4)**	**1/1 (1)**	**0/1 (1)**	**2/2 (2)**	**1/1 (1)**	**2/2 (2)**	**0/2 (2)**	**13/24**	**3/24**
Other	Brain, uterus	**1[18.]/2/2**	**1/2 (2)**	**0/2 (2)**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**1/4**	**0/4**
All	**All**	**35/147/193**	**63/139 (136)**	**8/139 (136)**	**25/60 (51)**	**9/60 (51)**	**37/ 64 (55)**	**13/64 (55)**	**19/33 (32)**	**-2/33 (32)**	**18/39 (31)**	**2/39 (31)**	**162/335**	**30/335**

[a] Sum of scoring points (compare table 2); [b] Sum of maximally attainable score (sc_{max}) per parameter and experiment, based on experimental design: one in case of experiments with placebo or untreated control; two in case of experiments with chemotherapytherpeutic control (compare table 2); [c] MET: most efficient treatment protocol, LET: least efficient treatment protocol; [d]sarcoma others: acetabulum, transformed embryonal fibroblasts, uterus-muscle layer of blood; vessel; [e]carcinoma others: head and neck (tongue), liver, ovar, pancreas, testicles, urinary bladder; [f]HLM: hematopoetic and lymphatic malignancies; [g] others melanoma: eye, lung; [x]including three armed experiments, [†]Number of negative score († = -1, †† = -2 etc.); References: 1. [95, 96, 138, 164] ; 2.[144, 165, 166]; 3. [96, 165, 167]; 4. [166, 168, 169]; 5. [165, 166]; 6. [96, 165, 166, 168-170]; 7. [109, 148, 153, 165, 166, 168, 169, 171-173]; 8. [140, 174]; 9. [165, 166]; 10. [165, 166]; 11. [96, 169]; 12. [89, 166, 167]; 13. [23, 110, 115, 116, 157, 165-167, 175-179]; 14. [23, 109, 153, 166, 169, 171, 173, 176, 180]; 15. [165, 166, 179, 181]; 16. [24, 153, 165, 172, 178, 182-184]; 17. [165, 182]; 18. [16)