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Prospective study of successful autologous dendritic cell therapy in dogs with splenic stage II hemangiosarcoma

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ABSTRACT

Hemangiosarcoma is an aggressive tumour that most frequently occurs in larger, middle-aged dogs of certain breeds. The spleen is the most commonly affected organ. The aim of this prospective therapy study was to evaluate the clinical effect of autologous, monocyte-derived dendritic cell (DC) therapy in canine hemangiosarcoma stage II after splenectomy. Dogs (n=452) diagnosed with splenic hemangiosarcoma that underwent splenectomy were enrolled. Of these, 42 dogs with stage II entered the DC therapy study. The median survival time for the total group of 42 dogs was 203 days. The median survival for the group (n=34) that received the full DC therapy (≥3 vaccines) was 256 days, with a 29 % one-year survival rate and a hazard ratio of 0.30, adjusted to age and bodyweight (P=0.010). We further observed a significant increase in DC yield after each application and demonstrated that DC yield at the beginning of treatment is significantly related to patient survival. While further evidence is needed, we conclude that autologous, monocyte-derived DC therapy is a viable alternative to standard treatment methods of canine splenic stage II hemangiosarcoma.

Introduction

Hemangiosarcoma (malignant hemangioendothelioma) is an aggressive tumour of mesenchymal origin, frequently occurring in larger, middle-aged dogs (Hammer et al., 1991; Sorenmo et al., 2000). The spleen is the most common organ primarily affected by hemangiosarcoma (Kessler, 2022). In addition to rapid primary tumour growth, they often show widespread metastatic disease (Clifford et al., 2000; Schultheiss, 2004; Szivek et al., 2012; Ward et al., 1994) due to free fluid containing tumour cells that circulate throughout the body after frequent rupture of the spleen. The median survival time for splenic hemangiosarcomas is between 19 and 87 days (with one-year survival rates of 6–13 %) when treated by splenectomy alone (Bray et al., 2018; Wendelburg et al., 2015; Wood et al., 1998). Surgical intervention is considered to be palliative and adjuvant treatments like chemotherapy are necessary (Kahn et al., 2013).

Despite recent developments, studies have made limited progress in demonstrating increased survival time after diagnosis of hemangiosarcoma. One prognostic factor that has consistently been correlated with survival is clinical stage (Hammer et al., 1991; Vail et al., 1995; Wood et al., 1998). The most common adjuvant treatments are doxorubicin-based chemotherapy protocols. These may improve median survival to about 5–6 months (150–180 days), across stages I-III (Hammer et al., 1991; Kahn et al., 2013; Ogilvie et al., 1996; Sorenmo et al., 2000). The one-year survival rate is reported at 16 % (Moore et al., 2017). Additions to conventional doxorubicin-based protocols, such as dacarbazine, metronomic or targeted therapies (Finotello et al., 2017a, b; Vail et al., 1995) or off-label use of additional substances such as thalidomide (Bray et al., 2018) have also been investigated, yet show limited improvements in prognosis.

Alternative approaches to conventional chemotherapy regimens used as stand-alone, including metronomic therapies, tyrosine kinase inhibitors, autologous vaccines, and immune-based and anti-angiogenic therapies have not yet been able to demonstrate a significantly improved prognosis for dogs with hemangiosarcoma (Clifford et al., 2000; Dervisis et al., 2011; Gardner et al., 2015; Kahn et al., 2013; Lana et al., 2007; Sahora et al., 2012; U'Ren et al., 2007).

Seeking a new treatment approach, immunotherapies have recently gained attention. Some showed comparable results regarding overall survival (OS) as standard chemotherapy treatment. For an allogeneic lysate-vaccine, U'Ren et al. (2007) reported a median OS of 182 days. Among immunotherapies, dendritic cell (DC) therapy is one of the

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current treatment options in translational veterinary medicine. DCs present (tumour) antigens to T-cells, enabling the immune system to establish a response toward these (Benteyn et al., 2015; Bordon, 2016). Due to these characteristics, cancer immunotherapies with DCs have been used in multiple clinical trials (Santos and Butterfield, 2018; Yu et al., 2022b).

The majority of studies discussed primed DCs and focused on the maturation of DCs through addition of tumour antigens during cultivation (Castiello et al., 2019). These studies, however, do not consider the migratory capacity of DCs (Wculek et al., 2020). It is well-accepted that tumour lysates from patient tumours provide the most effective source of antigen for DC maturation, as a broad range of epitopes occurring on these antigens may elicit a multiple immune response. Therefore, in situ or in vivo priming should be taken into consideration. An approach like this is based on the ability of DCs to internalise and process antigens released by cancer cells into the tumour microenvironment (Castiello et al., 2019). Delay or, at best, inhibition of tumour progression is induced by establishing an anti-tumour effect via prevention of metastasis and cytotoxic reaction of the immune system.

In clinical veterinary medicine, autologous (primed) DC therapy was first described for horses with equine sarcoids (Bischoff, 2009). Here, the adjuvant use of DCs resulted in a high partial or complete remission of the tumour. In subsequent years, case studies investigating various types of tumours and localizations have been published, demonstrating the effect of autologous unprimed DCs (Arnold et al., 2018; Pantke et al., 2021). Further, DC therapy has been applied in dogs in combination with the cytokine IFN-γ to investigate the effect on clinical outcome (Mito et al., 2010). Treatment of splenic hemangiosarcoma with DC therapy in dogs has been investigated in a pilot study (n=10), showing a median survival time of 611 days (Grammel, 2016). Building on this pilot research, this broader prospective study was initiated in order to obtain significant findings from a larger sample size.

The aim of this prospective therapy study was to evaluate the clinical impact of autologous monocyte-derived DC therapy in canine hemangiosarcoma after splenectomy.

Materials and methods

Study design, patient and tumour characteristics

For this prospective study, the ideal patient met the inclusion criteria of undergoing splenectomy upon confirmed diagnosis of splenic hemangiosarcoma, and owner consent to DC-based immunotherapy and data usage for research purposes (January 1, 2016 to December 31, 2021). Of the total number of candidate patients (n=452), the critical selection procedure based on various exclusion criteria revealed 42 patients considered fit for study purpose. In detail: exclusion criteria referred to the presence of other tumours (benign or malignant), blood transfusion during the primary hemangiosarcoma treatment or concurrent chemotherapy or radiation therapy. Patients who died before a treatment decision or before the treatment was administered, as well as owners who withdrew their consent before the start of the therapy were omitted (n=353). For the remaining dogs, all available records were inspected to confirm the presence of disease. The hemangiosarcoma patients meeting inclusion criteria as described above, were then reviewed to determine whether sufficient information (clinical and surgical reports, pathohistological examination) was available to perform disease staging. As per these criteria, 99 dogs were considered to obtain DC therapy. During the course of therapy, 49 dogs had to be excluded due to loss of contact to the owner. Lastly, we decided to focus only on stage II patients as these are most frequently diagnosed and would present a homogeneous group. 42 patients with splenic hemangiosarcoma stage II entered the DC therapy study (Fig. 1).

The following patient data were collected: dog breed, sex, age at start of disease, bodyweight, clinical stage of disease, primary tumour site, presence of local or distant metastases, dates of clinical diagnosis and surgery, histopathological and surgery report results, number of dendritic cell vaccines administered, amount of blood collected per vaccination, cell count at beginning and end of each cultivation, share of DCs cultivated (CD-1 positive cells), and overall survival (based on date of death). OS was determined by calculating the time between the date of surgery and the date of death.

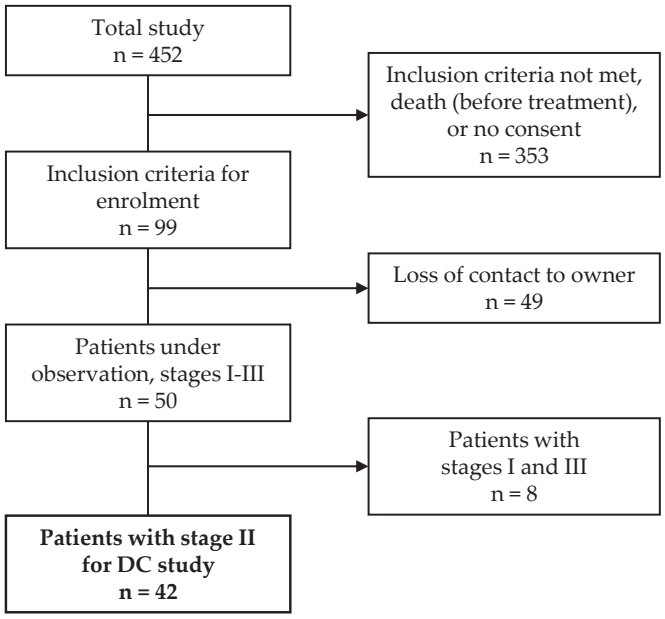

Fig. 1. Consort diagram illustrating critical selection procedure of 42 candidate patients fit for study purpose. Abbreviations: DC: dendritic cell.

Tumour classification

The records of all dogs with diagnosed hemangiosarcoma of the spleen were reviewed. One third to one half of bleeding spleen tumours are benign (Eberle et al., 2012), thus a histopathological diagnosis of the tumour tissue was performed. The clinical stages were determined based on these reports and the tumours were graded according to the appropriate Staging Scheme (Supplement Table 1) (Vail et al., 2020).

Preparation of autologous, monocyte-derived dendritic cells

The monocyte-derived cell suspension was produced under GMP-certified (Good Manufacturing Practice) conditions at PetBioCell GmbH, Germany. To generate these cells, canine patient blood was aseptically drawn into Monovettes™ coated with sodium citrate. Once the blood underwent initial inspection, peripheral blood monocytes (PBMCs) were isolated with conventional gradient centrifugation and through adherence to the culture dish surface. After addition to the gradient solution, the samples were centrifuged and the enriched cell fractions consisting of mononucleated blood cells were washed to remove platelets. At this point, the cells were counted. A minimum number of four million PBMCs was required prior to adherence to ensure

Table 1
Number of patients by number of DC applications.

Number of dendritic cell applications	Number of patients	Percentage
1or 2	8	19 %
≥ 3	34	81 %

DC therapy consists of a minimum of three treatments on different occasions in three-week intervals.
Abbreviations: DC: dendritic cells

a sufficiently high number for the production and quality control (flow cytometry) processes. After centrifugation, adherence medium containing 5 % patient plasma was added. The cells were then transferred onto a tissue culture dish to let them adhere. The lymphocytes were washed out and after the addition of culture medium and the cytokines canine GM-CSF (Inaba et al., 1992; Lazarus et al., 2023; Ushach and Zlotnik, 2016; Witmer-Pack et al., 1987) and canine IL-4 (Lauener et al., 1990; Paul, 1991; te Velde et al., 1988), the cells were incubated for seven days, allowing for differentiation of the monocytes into monocyte-derived DCs (Hopewell and Cox, 2020). On day seven, the cells were harvested and counted.

Of the total cell count, a minimum count of 350.000 cells was technically required for flow cytometry analysis of CD-1b (cluster of differentiation) positive cells. A quantity of at least 10 % CD-1b positive cells was required. The cell viability was set at $\geq$ 90 %.

The surplus cells were suspended into sterile physiological sodium chloride solution and transferred into the syringe used as primary packaging for transportation. Of those, an aliquot was sampled to test for sterility according to European Pharmacopoeia (2023). Details are described in Supplement Fig. 1.

Therapy scheme and administration

Splenectomy was performed at the general veterinarian or at a surgical practice situated at various locations in Germany. For the adjuvant DC therapy, the patients received a minimum of three applications of unprimed, autologous monocyte-derived DCs. For the first DC therapy, the blood was aseptically collected in Monovettes within a period of 14 days after surgery, or at the moment the diagnosis of malignant splenic stage II hemangiosarcoma was confirmed by the histopathological report.

The Monovettes were shipped to PetBioCell GmbH under GMP-compliant conditions (transport validation) by overnight and cooled cargo service. After the ex-vivo cell differentiation period of seven days, the syringes loaded with DC suspension were returned to the attending general veterinarians under GMP conditions as described before. Upon arrival, the total number of cells was immediately administered intracutaneously. Thus, the number of DCs applied – in absolute terms and per kilogram bodyweight – varied between patients and also between vaccines.

For each production of the DC vaccine, the process of blood collection, production, and application was repeated at intervals of three weeks for the first three applications. Thereafter, the cycle increased to every three months. In this study, the dogs received a minimum of one application and a maximum of eight applications of autologous DCs, where a minimum of three applications was defined as a completed treatment. On average, the dogs received 3.9 applications (Table 1). Eight patients did not receive the complete therapy comprised of three DC applications, but one or two applications, because the owners discontinued the therapy. As these patients were excluded from further treatment, they were considered the control group of our data analysis.

Endpoints and statistical analysis

Descriptive statistics were used to assess the primary objective, yield of autologous DCs from patient whole blood. A two sample T-Test was applied to compare means between groups during the first application. Additionally, correlation analysis was used to determine a relationship between average cell yield across all applications of a patient and survival. To find the most meaningful cut-off value for the relationship of DC yield in the first application and overall survival, the maximum-likelihood method was used. By generating the highest chi-square value and the lowest p-value, the cohort was divided into groups with high and low OS. The cut-off was confirmed by receiver operating characteristic (ROC) analysis as per the Youden index method (Perkins and Schisterman, 2005).

In order to evaluate the clinical effect of the autologous DCs, (OS) curves were generated as Kaplan-Meier estimates, with differences described by log-rank test. The Cox regression model was applied, adjusted to age and bodyweight to calculate hazard ratios (HR) with 95 % confidence intervals (95 % CI).

Overall survival included death from hemangiosarcoma, non-hemangiosarcoma and unknown causes. Six-month and one-year survival probabilities with confidence intervals were calculated. All statistical tests were two-sided and a p-value $\leq$ 0.05 was considered significant. Statistical analyses were carried out using SPSS 28 (IBM, Armonk, NY, USA).

Results

Epidemiological characteristics

For the prospective DC therapy study, the following breeds were included: mixed breed (n=9), five German Shepherds (three purebred, two Shepherd mix), four Labrador Retrievers (three purebred, one Labrador mix), three animals of the breeds Australian Shepherd, French Bulldog and Rhodesian Ridgeback, respectively. Two Bernese Mountain dogs and two Bracken were included as well. One dog each of the following breeds completed the study: Appenzeller Mountain Dog, Border Collie, Collie, Dalmatian, English Bulldog, Flat Coated Retriever, German Wirehaired, German Shorthair, Irish Terrier, Magyar Vizsla, and Poodle.

The mean age of the animals was 10.1 years (range: 6.5–14.1 years) with a median age of 10.5 years. 19 dogs were less than 10 years old at diagnosis, 23 dogs were 10 years and older. The sex ratio of the animals was almost balanced, 20 were male, 22 were female. Ten dogs weighed less than 20 kg (range: 10-49 kg).

Dendritic cell yield

In our DC therapy study, we were able to prepare monocyte-derived, autologous dendritic cells from whole blood of the respective patients. No clinical complications after DC application to the dogs were reported.

Overall, there was a wide range in the number of DCs cultivated per millilitre of blood among patients, ranging from 2745 to 235,940 DCs/ml of blood in the first application and 8453 to 241,554 in the third application (Supplement Figs. 2A and 2B). This translated into an average share of 27.5 % DCs (CD-1 positive cells) in the first application to a maximum average share of 38.5 % DCs in the sixth application (Supplement Table 2). The Chi Square Test for inequality showed no significant difference between the treated and control groups with regard to DCs/ml cultivated in the first application (Supplement Table 5), providing evidence that the two groups did not differ systematically in terms of initial DC yield. When further analysing the DCs/ml for every application, we observed an increase in the median DC yield after each application compared to the first application, this effect being most pronounced after the first and fourth applications (Supplement Table 3). Similarly, the administered DCs per kilogram bodyweight increased after each application (compared to the first), with the sharpest increase after the first and fourth applications (Supplement Table 4).

Considering the relationship of DC yield with OS, the average DC yield per ml blood across all administered applications per patient showed a significant correlation with OS (P=0.008), as did the administered dose of DC/kg bodyweight (P=0.003) (Supplement Table 6). No correlation was observed between DCs/ml blood and bodyweight or age (Supplement Table 5). A significant difference in the mean number of DCs/ml of blood in the first application (P=0.045) was found between patients with lower-than-median (203 days) and higher-than-median survival time. Analysing this relationship further and using 18,000 DCs/ml blood in the first application as a cut-off regarding OS as endpoint, the study was dichotomised into groups with a longer and shorter OS. This approach showed that the patients with a DC/ml yield

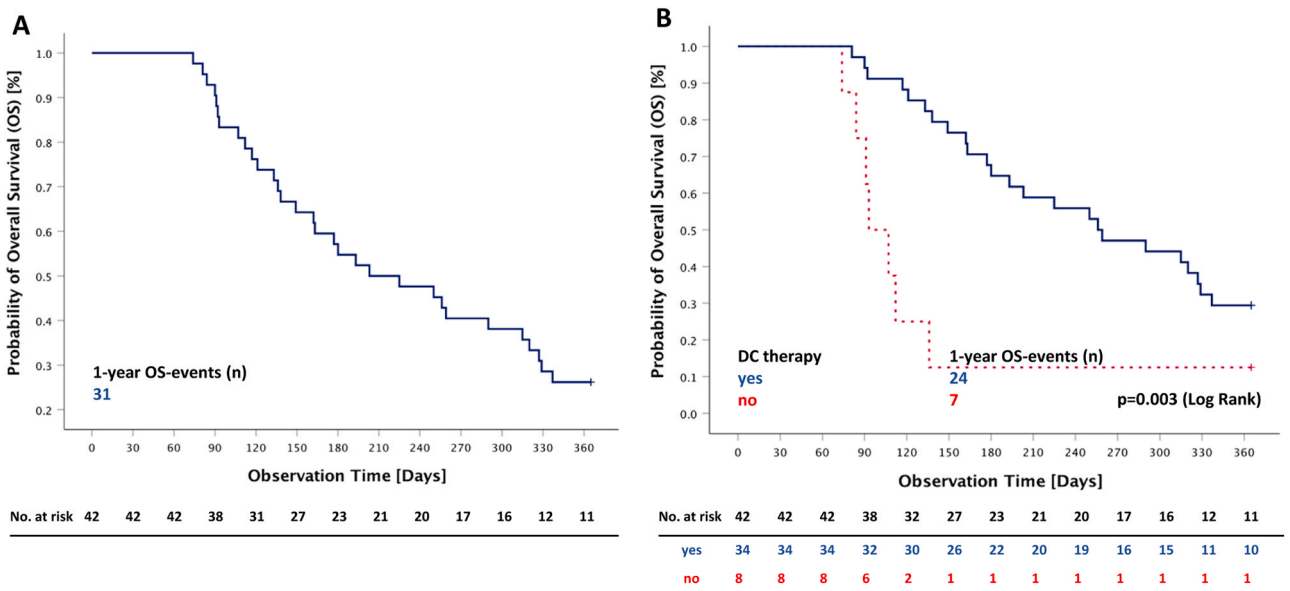

Fig. 2. Survival estimates of dogs diagnosed with stage II hemangiosarcoma of the spleen. Upon splenectomy, dogs were subjected to dendritic cell therapy. A: Overall survival probability of the total DC therapy study. B: Overall survival probability considering dendritic cell therapy. Abbreviations: DC: dendritic cell.

Table 2
Six-month and one-year overall survival probability for the total DC therapy study in selected groups.

Group	Median survival in days	95 % CI	6-month overall survival (%)	95 % CI	1-year overall survival (%)	95 % CI
Total cohort (*n*=42)	203	119.38–286.62	54.8	39.71–69.89	26.2	12.87–39.53
DC therapy						
No	93	70.83–115.18	12.5	-10.43–35.43	12.5	-10.43–35.43
Yes	256	163.14–348.86	64.7	48.63–80.77	29.4	14.11–44.69
Bodyweight						
< 20 kg	327	-	70.0	41.58–98.42	50.0	19.03–80.97
≥ 20 kg	180	124.56–235.44	50.0	32.75–67.25	18.8	5.28–32.32
Age						
< 10 years	320	143.68–496.32	68.4	47.43–89.37	36.8	15.04–58.56
≥ 10 years	149	106.74–191.26	43.5	23.31–63.69	17.4	1.92–32.88
DCs / ml blood						
≤ 18,000	112	90.31–133.69	10.0	-8.62–28.62	10.0	-8.62–28.62
> 18,000	259	174.73–343.27	71.4	54.74–88.06	32.1	14.85–49.35

p-value ≤ 0.05 considered significant; abbreviations: DC: dendritic cell, CI: confidence interval

Table 3
Univariate and multivariate survival analysis of dogs adjusted to bodyweight and age.

Parameters	Sample size	Univariate analysis			Multivariate analysis		
	n=42	HR	95 % CI	p-value	HR	95 % CI	p-value
DC therapy							
No	8	1			1		
Yes	34	**0.29**	0.120–0.684	**0.005**	**0.30**	0.123–0.753	**0.010**
Bodyweight							
< 20 kg	10	1			1		
≥ 20 kg	32	2.19	0.838–5.745	0.110	**2.85**	1.024–7.915	**0.045**
Age							
< 10 years	19	1			1		
≥ 10 years	23	1.89	0.909–3.921	0.088	**2.75**	1.241–6.095	**0.013**
Sex							
Female	22						
Male	20		0.903–3.733	0.093			

Abbreviations: Confidence Interval (CI), Dendritic Cell (DC), HR Hazard ratio (HR),
bold: p-value (Pearson $\chi 2$ test) < 0.05

18,000 cells or below in the first application had a 10 % probability of six-month and one-year survival, while patients with a DC/ml yield of more than 18,000 cells in the first application had significantly longer six-month and one-year survival rates (71 % and 32 %, respectively) (Supplement Figure 3 C).

Overall survival analysis

Considering the second objective of this prospective DC therapy study, we observed a median overall survival of 203 days (6.8 months) post surgery. The survival probability after six months was 55 % and after one year 26 % (Table 2 and Fig. 2A). Of the 42 patients, 31 deaths (74 %) were documented. Of these, 29 were due to disease progression and two patients died of kidney failure.

A therapy cycle of at least three DC applications was associated with a significantly better clinical outcome compared to the control patients (log rank P=0.003, Fig. 2B). After six months, patients with DC therapy showed a survival probability of 65 % and after one year 29 %. Only one patient of the control group was still under observation after six months, with a one-year survival probability of 12.5 %. Also underlined by the Cox regression analysis: Patients with DC therapy showed a significant, threefold lower risk of death, with a hazard ratio (HR) of 0.29, compared to patients of the control group (Table 3). In a multivariate analysis of OS adjusted to age and weight, the effect stayed significant, with a hazard ratio of 0.30 (Table 3).

Clinical parameters used in the multivariate analysis of the DC therapy showed the following results: Considering bodyweight, patients weighing 20 kg or more demonstrated a 2.9 times higher risk of death. Accordingly, the six-month survival probability for the group with less than 20 kg was 70 % and the one-year survival rate was 50 %, compared to a six-month survival probability of 50 % and a one-year survival probability of 19 % for the patients with bodyweight of 20 kg or higher (Table 2).

For differences in age, a significant impact with a HR of 2.75 (Table 3) was calculated. The six-month survival probability for patients younger than 10 years was 68 % and the one-year survival probability was 37 %, compared to a six-month survival probability of 44 % and a one-year survival probability of 17 % for the patients 10 years and older (Table 2). Survival estimates for the clinical parameters age and bodyweight can be seen in Supplement Figures 3A-B.

Discussion

In our prospective DC therapy study we evaluated the clinical effect of autologous, monocyte-derived dendritic cell therapy in canine hemangiosarcoma stage II after splenectomy. To our knowledge, this is the first time that the feasibility of preparing individualised vaccines from canine patient whole blood followed by a significant impact of DC therapy on overall survival for this disease has been demonstrated.

The DCs were administered by intradermal injection, as application directly into the tumour or tumour site may lead to the development of dysfunctional DCs through tumour immune escape mechanisms (DeVito et al., 2019). Intradermal injection has been practiced in clinical veterinary medicine for two decades (Bischoff, 2009) and has gained acceptance on the assumption that local DCs of the skin can be involved in immune response initiation (Scholz, 2005). As tumours spread shedded cell material, e.g. exosomes, via body fluids such as blood, DCs may take up antigens away from the tumour origin (Yu et al., 2022a). Despite the fact that tumour-released exosomes are able to inhibit DC function, they are also able to serve as antigen source for in situ priming. DCs interact with different immune cell subsets like T cells and B cells to initiate and shape the adaptive immune response targeted against the tumour.

Dendritic cell yield

With respect to our first objective, vaccines of autologous, monocyte-derived, unprimed DCs were prepared. The yield of cultivated DCs differed strongly between animals. Using the median survival time in order to dichotomise the total DC therapy group, patients with a higher amount of DCs/ml blood in the first application showed longer overall survival. We also showed an increase in DC yield after each application and a correlation of average DC yield with survival. We therefore infer that a higher number of DCs (18,000 DCs or more per ml blood) at the beginning of treatment may positively influence treatment efficacy and therefore clinical outcome. The initiation of a T-cell response as important step in eliciting an anti-tumour immune response is dependent on the interaction of naive T-cells with antigen-presenting cells like DCs. There is growing evidence of a minimum threshold of necessary cell-to-cell interactions between T-cells and DCs for this immune reaction to take place (Celli et al., 2012; Hawlina et al., 2022), providing further support to our findings.

Clinical staging has consistently been associated with longer survival in canine splenic hemangiosarcoma (Hammer et al., 1991; Vail et al., 1995; Wood et al., 1998), yet differential diagnosis including clinical characterisation has not changed substantially over the last decades. Recently, however, tumour biology including molecular pathogenesis, cellular ontogenesis, tumour microenvironment and heterogeneity specifically of hemangiosarcomas have received more attention in veterinary medicine (Gardner et al., 2015; Kim et al., 2015; Wong et al., 2022). For example, the examination of histone lysine demethylase 2B seems to provide indications for the prognosis in canine hemangiosarcoma (Gulay et al., 2022). These advances may in future allow for a broader assessment of tumour grade and malignancy of the disease, giving further insight into the mechanisms that drive DC therapy efficacy in canine splenic hemangiosarcoma.

Overall survival

Regarding our second research objective, we found a significant clinical impact of autologous dendritic cell therapy following splenectomy on overall survival in dogs with stage II hemangiosarcoma. Dogs that received the complete DC therapy comprising at least three treatments, showed a threefold lower risk of death than dogs without the therapy. Of the 42 patients in the study, 26 % were still alive after one year. Age and weight of the patients seemed to reduce the impact of the DC therapy, while it still remained significant. In addition, we focused on the survival data of other studies examining the treatment of canine hemangiosarcoma stage II.

In their larger study on splenic hemangiosarcoma (stages I-III) in dogs, Wendelburg and colleagues looked at the survival of 208 animals treated with splenectomy alone compared to patients with chemotherapy after splenectomy (doxorubicin or cyclophosphamide-based metronomic or combination of both) (Wendelburg et al., 2015). They found a significant difference in survival time of the combined treatments for all chemotherapy protocols (median overall survival 102 days, HR=0.6 for any chemotherapy and HR=0.4 for combined conventional and metronomic protocol), but only during the first four months observation time. After this time, the effect was not significant. Similar results regarding studies of adjuvant chemotherapy in hemangiosarcoma of the spleen were generated by Teske and colleagues who studied different forms of doxorubicin protocols only in patients with stage II hemangiosarcoma (Teske et al., 2011). The authors found a median survival of 166 days, and a survival probability of 41 % after six months and 23 % after one year observation time. Moore et al. (2017) reported a median survival time of 158 days and a one-year survival probability of 16 % for dogs with stage II hemangiosarcoma treated with splenectomy and chemotherapy (combination of anthracyclines and lomustine). In evaluating adjuvant doxorubicin-based chemotherapy combined with daily dacarbazine, Finotello et al. (2017b) found a median survival of > 550 days and a 69 % one-year survival rate for their cohort of nine stage II and III patients. Another study evaluated the effect of a bispecific angiotoxin (eBAT) followed by adjuvant doxorubicin-based chemotherapy, finding a six-month survival rate of 70 % and median survival of 258 days (8.1 months) (Borgatti et al., 2017). Further studies of the last decades demonstrated a range of 125–273 days overall median survival across all stages after splenectomy followed by different chemotherapy protocols (Dervisis et al.,

2011; Finotello et al., 2017a; Kim et al., 2007; Sorenmo et al., 2000; Vail et al., 1995).

Studies on alternative treatments were based on much smaller sample sizes and heterogeneity in staging, yet still warrant comparisons. Investigating the effect of low-dose oral chemotherapy (etoposide and cyclophosphamide) in nine dogs, Lana and colleagues reported a median survival of 178 days (Lana et al., 2007). A median survival of 182 days was reported for a novel vaccine produced with lysates of allogeneic canine hemangiosarcoma cell lines in combination with chemotherapy for patients of stage II (U'Ren et al., 2007). Other approaches included studies with various medications in addition to chemotherapy, such as thalidomide (median OS of 303 days, stage II) (Bray et al., 2018) or toceranib (172 days median OS, stages I and II) (Gardner et al., 2015).

One study with DCs reported primed monocytic DCs that were administered alternately with doxorubicin (n=5). In addition, the animals received five million units of veterinary type I interferon omega injected subcutaneously with each application of DCs. They showed a median survival time of 109 days and stated no significant improvement of OS compared to chemotherapy (Konduri et al., 2019).

Regarding the overall survival comparisons across published studies, our prospective study showed that the effect of DC therapy is comparable to standard chemotherapy and alternative treatment protocols. The treatment of splenic hemangiosarcoma in dogs with DC therapy can therefore be seen as a viable alternative to the currently common treatment approaches.

Examining the survival statistics for bodyweight, we showed that patients weighing less than 20 kg had a two times lower risk of death than patients weighing 20 kg and more, with an increasing effect and significance after adjustment to age and DC therapy. Comparable findings are presented in the study of Story et al. (2020) who found a trend in the difference of survival times of small (< 20 kg) and large breed dogs with hemangiosarcoma of the spleen, treated with splenectomy and chemotherapy. Further research is needed to investigate the reported multivariate effect in the present study for significance beyond the general finding that small breed dogs have longer lifetimes than large breed dogs (Kraus et al., 2013). Additionally, the dosage related to bodyweight should be further examined to infer a potential relationship.

Adverse effects and quality of life

Various studies on chemotherapy treatment (doxorubicin or combination protocols) report a number of severe adverse effects for the animal, among which are anorexia, vomiting, diarrhoea, fever, extravasation, gastroenteritis, lethargy, heart failure, allergic reactions, anaemia, liver dysfunction, palmar-plantar erythrodysesthesia, thrombocytopenia, and neutropenia, and in some cases led to termination of study participation (Chavalle et al., 2022; Dervisis et al., 2011; Kim et al., 2007; Teske et al., 2011; Treggiari et al., 2022; U'Ren et al., 2007). In comparison, studies on immunologic treatment with DCs reported few and mild adverse effects. In a meta-analysis of 29 clinical trial studies of human dendritic cell therapies the authors identified only mild side effects and toxicities (Draube et al., 2011), such as reactions around the injection site, fever and flu-like symptoms. Similarly, no reduction in quality of life during DC therapy of 55 human patients with metastatic renal cell carcinoma was found, concluding that DC therapy was an attractive and non-toxic treatment option (Leonhartsberger et al., 2012). The studies report no dropouts due to adverse effects.

The presented prospective study aimed to capture quality of life data from the owners as an indicator for side effects of the treatment, however, due to a low response rate, the results were not presented here. Yet, the quality of life of dogs treated with DCs was examined previously in a study with 373 dogs (656 completed questionnaires) of various tumour types (Grammel et al., 2019). The authors were able to show a statistically significant improvement in perceived general health and quality of life from one week before to one week post injection. A strong case for DC therapy can thus be made not only regarding clinical outcome but also regarding adverse effects.

Strengths and limitations of this study

On the one hand, the present study has the core strength of being one of the first to observe the sole effect of DC therapy on canine splenic stage II hemangiosarcoma. It also benefits from the large sample size rarely reported elsewhere. Furthermore, the GMP-certified production of the monocyte-derived DC suspensions ensures a highly standardized process at the lowest possible contamination risk for the patients.

On the other hand, the small number of the control group (n=8) and its status as not being entirely untreated may limit the generalizability of its results. However, for several reasons the group with less than three DC applications was drawn upon as a comparison to measure the impact of DC treatment. In a clinical context, animal owners are rarely identified to accept their dogs being excluded from any treatment (in this case including splenectomy only), while viable and standard options are readily available. Most often, owners opt for no treatment or splenectomy only when the animal is already in a palliative state. This approach would introduce significant bias into the survival data. Moreover, our data already show a significant effect of the DC treatment when patients receive more than two DC applications. This constitutes a study setting where achieving significance is even biased toward underestimating the treatment efficacy, compared to a group without any DC application.

Future research

Future research should focus on comparing DC treatment with different combination treatment protocols. We suggest to expand on studying a doxorubicin protocol in combination with DC therapy as Konduri and colleagues had shown in a smaller study (Konduri et al., 2019). Another recent publication (Korpela et al., 2021) presented cell death inducing effects of propranolol on canine hemangiosarcoma by inhibiting the uptake of lipid building blocks needed for cell proliferation of tumour cells, thereby reducing their capacity to emit exosomes. Both these treatment combinations have the potential to enhance the effectiveness of in situ priming of the DCs, as with increased cell death through the medication, more tumour antigens would be available for maturation. Evidence for the propranolol approach is growing, yet points toward an alternative treatment method to chemotherapeutics due to fewer side effects (Ammons et al., 2023). Studies should additionally consider allowing for recovery time for the immune system or boosting monocyte production after medication, in order to obtain sufficient quantity and quality of monocytes for DC cultivation. The existing genetic alterations of hemangiosarcomas should also be included in future investigations (Estabrooks et al., 2023).

Lastly, we suggest standardizing the DC dose administered to patients by parameters such as weight, as lack of this practice currently makes it difficult to compare results across studies. This approach should be combined with different protocols of DC administration, for example by varying the number of applications given and the time between applications, providing further evidence for clinical implementation.

Conclusions

Our prospective DC therapy study is the first published study that prospectively evaluated the clinical impact of autologous, unprimed dendritic cell therapy in dogs with stage II splenic hemangiosarcoma. We showed that patients DC-treated on three or more occasions survived significantly longer than those without complete DC therapy. With a median overall survival time of 256 days, our DC therapy option showed among the highest overall survival compared to published data using chemotherapy or alternative treatments. Moreover, DC yield observed at the beginning of treatment significantly favoured period of patient survival. We therefore conclude that DC therapy is as a feasible and viable therapeutic option with limited adverse effects in treating canine

splenic hemangiosarcoma. Further studies to validate these findings are required.

Declaration of Competing Interest

T.G. owns stocks of PetBioCell. C.D.-R., V.S. are employees of PetBioCell. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data that could inappropriately influence or bias the content of the paper. All other authors have no relevant financial or non-financial interests to disclose.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tvjl.2024.106196.

References

Ammons, D.T., MacDonald, C.R., Chow, L., Repasky, E.A., Dow, S., 2023. Chronic adrenergic stress and generation of myeloid-derived suppressor cells: Implications for cancer immunotherapy in dogs. Vet. Comp. Oncol. 21, 159–165. https://doi.org/10.1111/vco.12891.

Arnold, C., Dreher, I., Grammel, T., Schusser, G.F., 2018. Immunotherapy of a squamous cell carcinoma in the perianal region using autologous dendritic cells in a horse. Equine Vet. Educ. 30, 576–582. https://doi.org/10.1111/eve.12741.

Benteyn, D., Heirman, C., Bonehill, A., Thielemans, K., Breckpot, K., 2015. mRNA-based dendritic cell vaccines. Expert Rev. Vaccines 14, 161–176. https://doi.org/10.1586/14760584.2014.957684.

Bischoff, S., 2009. Therapie equiner Sarkoide unter Verwendung dendritischer Zellen. Freie Universität Berlin, Berlin. https://refubium.fu-berlin.de/handle/fub188/4869?show=full.

Bordon, Y., 2016. Sorting, sorted!, 657–657 Nat. Rev. Immunol. 16. https://doi.org/10.1038/nri.2016.115.

Borgatti, A., Koopmeiners, J.S., Sarver, A.L., Winter, A.L., Stuebner, K., Todhunter, D., Rizzardi, A.E., Henriksen, J.C., Schmechel, S., Forster, C.L., Kim, J.-H., Froelich, J., Walz, J., Henson, M.S., Breen, M., Lindblad-Toh, K., Oh, F., Pilbeam, K., Modiano, J. F., Vallera, D.A., 2017. Safe and Effective Sarcoma Therapy through Bispecific Targeting of EGFR and uPAR. Mol. Cancer Ther. 16, 956–965. https://doi.org/10.1158/1535-7163.MCT-16-0637.

Bray, J.P., Orbell, G., Cave, N., Munday, J.S., 2018. Does thalidomide prolong survival in dogs with splenic haemangiosarcoma? J. Small Anim. Pract. 59, 85–91. https://doi.org/10.1111/jsap.12796.

Castiello, L., Aricò, E., D'Agostino, G., Santodonato, L., Belardelli, F., 2019. In situ Vaccination by Direct Dendritic Cell Inoculation: The Coming of Age of an Old Idea? Front. Immunol. 10 https://doi.org/10.3389/fimmu.2019.02303.

Celli, S., Day, M., Müller, A.J., Molina-Paris, C., Lythe, G., Bousso, P., 2012. How many dendritic cells are required to initiate a T-cell response? Blood 120, 3945–3948. https://doi.org/10.1182/blood-2012-01-408260.

Chavalle, T., Chamel, G., Denoeux, P., Lajoinie, M., Sayag, D., Berny, P., Ponce, F., 2022. Are severe adverse events commonly observed in dogs during cancer chemotherapy? A retrospective study on 155 dogs. Vet. Comp. Oncol. 20, 393–403. https://doi.org/10.1111/vco.12782.

Clifford, C.A., Mackin, A.J., Henry, C.J., 2000. Treatment of canine hemangiosarcoma: 2000 and beyond. J. Vet. Intern Med. 14, 479–485. https://doi.org/10.1111/j.1939-1676.2000.tb02262.x.

Dervisis, N.G., Dominguez, P.A., Newman, R.G., Cadile, C.D., Kitchell, B.E., 2011. Treatment with DAV for Advanced-Stage Hemangiosarcoma in Dogs. J. Am. Anim. Hosp. Assoc. 47, 170–178. https://doi.org/10.5326/JAAHA-MS-5525.

DeVito, N.C., Plebanek, M.P., Theivanthiran, B., Hanks, B.A., 2019. Role of Tumor-Mediated Dendritic Cell Tolerization in Immune Evasion. Front. Immunol. 10 https://doi.org/10.3389/fimmu.2019.02876.

Draube, A., Klein-González, N., Mattheus, S., Brillant, C., Hellmich, M., Engert, A., von Bergwelt-Baildon, M., 2011. Dendritic cell based tumor vaccination in prostate and renal cell cancer: a systematic review and meta-analysis. PLoS One 6, e18801. https://doi.org/10.1371/journal.pone.0018801.

Eberle, N., von Babo, V., Nolte, I., Baumgärtner, W., Betz, D., 2012. Splenic masses in dogs. Part 1: Epidemiologic, clinical characteristics as well as histopathologic diagnosis in 249 cases (2000-2011). Tierarztlich- Praxis Ausgabe K Kleintiere Heimtiere 40, 250–260.

Estabrooks, T., Gurinovich, A., Pietruska, J., Lewis, B., Harvey, G., Post, G., Lambert, L., Miller, A., Rodrigues, L., White, M.E., Lopes, C., London, C.A., Megquier, K., 2023. Identification of genomic alterations with clinical impact in canine splenic hemangiosarcoma. Vet. Comp. Oncol. 21, 623–633. https://doi.org/10.1111/vco.12925.

European Pharmacopoeia, 11.0. ed, 2023.

Finotello, R., Henriques, J., Sabattini, S., Stefanello, D., Felisberto, R., Pizzoni, S., Ferrari, R., Marconato, L., 2017a. A retrospective analysis of chemotherapy switch suggests improved outcome in surgically removed, biologically aggressive canine haemangiosarcoma. Vet. Comp. Oncol. 15, 493–503. https://doi.org/10.1111/vco.12193.

Finotello, R., Stefanello, D., Zini, E., Marconato, L., 2017b. Comparison of doxorubicin-cyclophosphamide with doxorubicin-dacarbazine for the adjuvant treatment of canine hemangiosarcoma. Vet. Comp. Oncol. 15, 25–35. https://doi.org/10.1111/vco.12139.

Gardner, H.L., London, C.A., Portela, R.A., Nguyen, S., Rosenberg, M.P., Klein, M.K., Clifford, C., Thamm, D.H., Vail, D.M., Bergman, P., Crawford-Jakubiak, M., Henry, C., Locke, J., Garrett, L.D., 2015. Maintenance therapy with toceranib following doxorubicin-based chemotherapy for canine splenic hemangiosarcoma. BMC Vet. Res. 11, 131. https://doi.org/10.1186/s12917-015-0446-1.

Grammel, T., 2016. A pilot uncontrolled study of postsurgical treatment with autologous dendritic cell-based immunologic therapy in 10 dogs with splenic hemangiosarcoma, in: 3rd World Veterinary Cancer Congress, Foz Do Iguassu/Brazil May 25-29, 2016.

Grammel, T., Hawacker, T., Hettling, H., Grammel, S., 2019. Quality of life of dogs during immunologic treatment with monocyte-derived dendritic cells, in: 25th FECAVA EuroCongress, St. Petersburg, Russia, September 2019.

Gulay, K.C.M., Aoshima, K., Kim, S., Kitaguchi, R., Kobayashi, A., Kimura, T., 2022. The expression of histone lysine demethylase 2B in canine hemangiosarcoma is associated with disease progression. Vet. Comp. Oncol. 20, 529–534. https://doi.org/10.1111/vco.12796.

Hammer, Ai.S., Couto, C.G., Filppi, J., Getzy, D., Shank, K., 1991. Efficacy and Toxicity of VAC Chemotherapy (Vincristine, Doxorubicin, and Cyclophosphamide) in Dogs with Hemangiosarcoma. J. Vet. Intern. Med. 5, 160–166. https://doi.org/10.1111/j.1939-1676.1991.tb00943.x.

Hawlina, S., Chowdhury, H.H., Smrkolj, T., Zorec, R., 2022. Dendritic cell-based vaccine prolongs survival and time to next therapy independently of the vaccine cell number. Biol. Direct 17, 5. https://doi.org/10.1186/s13062-022-00318-w.

Hopewell, E.L., Cox, C., 2020. Manufacturing dendritic cells for immunotherapy: monocyte enrichment. Mol. Ther. Methods Clin. Dev. 16, 155–160. https://doi.org/10.1016/j.omtm.2019.12.017.

Inaba, K., Inaba, M., Romani, N., Aya, H., Deguchi, M., Ikehara, S., Muramatsu, S., Steinman, R.M., 1992. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. J. Exp. Med. 176, 1693–1702. https://doi.org/10.1084/jem.176.6.1693.

Kahn, S.A., Mullin, C.M., de Lorimier, L.-P., Burgess, K.E., Risbon, R.E., Fred, R.M., Drobatz, K., Clifford, C.A., 2013. Doxorubicin and deracoxib adjuvant therapy for canine splenic hemangiosarcoma: a pilot study. Can. Vet. J. 54, 237–242.

Kessler, M., 2022. Kleintieronkologie, 4th ed. Georg Thieme Verlag KG, Stuttgart. https://doi.org/10.1055/b-006-163261.

Kim, J.-H., Graef, A., Dickerson, E., Modiano, J., 2015. Pathobiology of Hemangiosarcoma in Dogs: Research Advances and Future Perspectives. Vet. Sci. 2, 388–405. https://doi.org/10.3390/vetsci2040388.

Kim, S.E., Liptak, J.M., Gall, T.T., Monteith, G.J., Woods, J.P., 2007. Epirubicin in the adjuvant treatment of splenic hemangiosarcoma in dogs: 59 cases (1997–2004). J. Am. Vet. Med. Assoc. 231, 1550–1557. https://doi.org/10.2460/javma.231.10.1550.

Konduri, V., Halpert, M.M., Baig, Y.C., Coronado, R., Rodgers, J.R., Levitt, J.M., Cerroni, B., Piscoya, S., Wilson, N., DiBernardi, L., Omarbekov, Z., Seelhoff, L., Ravi, V., Douglass, L., Decker, W.K., 2019. Dendritic cell vaccination plus low-dose doxorubicin for the treatment of spontaneous canine hemangiosarcoma. Cancer Gene Ther. 26, 282–291. https://doi.org/10.1038/s41417-019-0080-3.

Korpela, D., Saha, J., Khammanivong, A., Brown, D., Dickerson, E., 2021. Veterinary Cancer Society, Annual Conference 2021: PROPRANOLOL REDUCES HEMANGIOSARCOMA CELL VIABILITYBY DISRUPTING LIPID HOMEOSTASIS AND INDUCING ER STRESS, in: Abstracts from the 2021 Veterinary Cancer Society (VCS) Annual Conference, 4-6 November 2021.

Kraus, C., Pavard, S., Promislow, D.E.L., 2013. The size–life span trade-off decomposed: why large dogs die young. Am. Nat. 181, 492–505. https://doi.org/10.1086/669665.

Lana, S., U'ren, L., Plaza, S., Elmslie, R., Gustafson, D., Morley, P., Dow, S., 2007. Continuous low-dose oral chemotherapy for adjuvant therapy of splenic hemangiosarcoma in dogs. J. Vet. Intern. Med. 21, 764–769. https://doi.org/10.1111/j.1939-1676.2007.tb03019.x.

Lauener, R.P., Goyert, S.M., Geha, R.S., Vercelli, D., 1990. Interleukin 4 down-regulates the expression of CD14 in normal human monocytes. Eur. J. Immunol. 20, 2375–2381. https://doi.org/10.1002/eji.1830201103.

Lazarus, H.M., Pitts, K., Wang, T., Lee, E., Buchbinder, E., Dougan, M., Armstrong, D.G., Paine, R., Ragsdale, C.E., Boyd, T., Rock, E.P., Gale, R.P., 2023. Recombinant GM-CSF for diseases of GM-CSF insufficiency: Correcting dysfunctional mononuclear phagocyte disorders. Front. Immunol. 13 https://doi.org/10.3389/fimmu.2022.1069444.

Leonhartsberger, N., Ramoner, R., Falkensammer, C., Rahm, A., Gander, H., Höltl, L., Thurnher, M., 2012. Quality of life during dendritic cell vaccination against metastatic renal cell carcinoma. Cancer Immunol. Immunother. 61, 1407–1413. https://doi.org/10.1007/s00262-012-1207-7.

Mito, K., Sugiura, K., Ueda, K., Hori, T., Akazawa, T., Yamate, J., Nakagawa, H., Hatoya, S., Inaba, M., Inoue, N., Ikehara, S., Inaba, T., 2010. IFNγ markedly cooperates with intratumoral dendritic cell vaccine in dog tumor models. Cancer Res. 70, 7093–7101. https://doi.org/10.1158/0008-5472.CAN-10-0600.

Moore, A.S., Rassnick, K.M., Frimberger, A.E., 2017. Evaluation of clinical and histologic factors associated with survival time in dogs with stage II splenic hemangiosarcoma treated by splenectomy and adjuvant chemotherapy: 30 cases (2011–2014). J. Am. Vet. Med. Assoc. 251, 559–565. https://doi.org/10.2460/javma.251.5.559.

Ogilvie, G.K., Powers, B.E., Mallinckrodt, C.H., Withrow, S.J., 1996. Surgery and doxorubicin in dogs with hemangiosarcoma. J. Vet. Intern. Med. 10, 379–384. https://doi.org/10.1111/j.1939-1676.1996.tb02085.x.

Pantke, P., Söhl, K.O., Schwittlick, U., Grammel, Z.-M., 2021. Chirurgische Resektion und adjuvante dendritische Zelltherapie eines nasopharyngealen Mastzelltumors bei einem Schäferhund. Kleintier-Praxis 66, 456–465.

Paul, W., 1991. Interleukin-4: a prototypic immunoregulatory lymphokine. Blood 77, 1859–1870. https://doi.org/10.1182/blood.V77.9.1859.1859.

Perkins, N.J., Schisterman, E.F., 2005. The Youden Index and the Optimal Cut-Point Corrected for Measurement Error. Biom. J. 47, 428–441. https://doi.org/10.1002/bimj.200410133.

Sahora, A.I., Rusk, A.W., Henkin, J., McKeegan, E.M., Shi, Y., Khanna, C., 2012. Prospective study of thrombospondin-1 mimetic peptides, ABT-510 and ABT-898, in dogs with soft tissue sarcoma. J. Vet. Intern. Med. 26, 1169–1176. https://doi.org/10.1111/j.1939-1676.2012.00966.x.

Santos, P.M., Butterfield, L.H., 2018. Dendritic Cell–Based Cancer Vaccines. J. Immunol. 200, 443–449. https://doi.org/10.4049/jimmunol.1701024.

Scholz, C., 2005. Dendritische Zellen in der Immuntherapie des Pankreaskarzinoms: Einfluss der Antigenaufbereitung aus vitalen Tumorzellen auf die Immunantwort in vitro. Ludwig-Maximilians-Universität Munich. https://edoc.ub.uni-muenchen.de/4536/1/Scholz_Christoph.pdf.

Schultheiss, P.C., 2004. A Retrospective Study of Visceral and Nonvisceral Hemangiosarcoma and Hemangiomas in Domestic Animals. J. Vet. Diagn. Investig. 16, 522–526. https://doi.org/10.1177/104063870401600606.

Sorenmo, K., Duda, L., Barber, L., Cronin, K., Sammarco, C., Usborne, A., Goldschmidt, M., Shofer, F., 2000. Canine hemangiosarcoma treated with standard chemotherapy and minocycline. J. Vet. Intern. Med. 14, 395. https://doi.org/10.1892/0891-6640(2000)014<0395:CHTWSC>2.3.CO;2.

Story, A.L., Wavreille, V., Abrams, B., Egan, A., Cray, M., Selmic, L.E., 2020. Outcomes of 43 small breed dogs treated for splenic hemangiosarcoma. Vet. Surg. 49, 1154–1163. https://doi.org/10.1111/vsu.13470.

Szivek, A., Burns, R.E., Gericota, B., Affolter, V.K., Kent, M.S., Rodriguez, C.O., Skorupski, K.A., 2012. Clinical outcome in 94 cases of dermal haemangiosarcoma in dogs treated with surgical excision: 1993-2007*. Vet. Comp. Oncol. 10, 65–73. https://doi.org/10.1111/j.1476-5829.2011.00282.x.

te Velde, A.A., Klomp, J.P., Yard, B.A., de Vries, J.E., Figdor, C.G., 1988. Modulation of phenotypic and functional properties of human peripheral blood monocytes by IL-4. J. Immunol. 140, 1548–1554.

Teske, E., Rutteman, G.R., Kirpenstein, J., Hirschberger, J., 2011. A randomized controlled study into the efficacy and toxicity of pegylated liposome encapsulated doxorubicin as an adjuvant therapy in dogs with splenic haemangiosarcoma. Vet. Comp. Oncol. 9, 283–289. https://doi.org/10.1111/j.1476-5829.2011.00266.x.

Treggiari, E., Cossu, G., Valenti, P., Taylor, A., 2022. Risk factors associated with the onset of lomustine-induced neutropenia in tumour-bearing dogs. Vet. Comp. Oncol. 20, 577–586. https://doi.org/10.1111/vco.12809.

U'Ren, L.W., Biller, B.J., Elmslie, R.E., Thamm, D.H., Dow, S.W., 2007. Evaluation of a novel tumor vaccine in dogs with hemangiosarcoma. J. Vet. Intern. Med. 21 https://doi.org/10.1892/0891-6640(2007)21[113:EOANTV]2.0.CO;2.

Ushach, I., Zlotnik, A., 2016. Biological role of granulocyte macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF) on cells of the myeloid lineage. J. Leukocyte Biol. 100, 481–489. https://doi.org/10.1189/jlb.3RU0316-144R.

Vail, D.M., MacEwen, E.G., Kurzman, I.D., Dubielzig, R.R., Helfand, S.C., Kisseberth, W. C., London, C.A., Obradovich, J.E., Madewell, B.R., Rodriguez, C.O., 1995. Liposome-encapsulated muramyl tripeptide phosphatidylethanolamine adjuvant immunotherapy for splenic hemangiosarcoma in the dog: a randomized multi-institutional clinical trial. Clin. Cancer Res. 1, 1165–1170.

Vail, D.M., Thamm, D.H., Liptak, J.M., 2020. Miscellaneous Tumors. in: Withrow and MacEwen's Small Animal Clinical Oncology. Elsevier, pp. 773–810. https://doi.org/10.1016/B978-0-323-59496-7.00034-7.

Ward, H., Fox, L.E., Calderwood-Mays, M.B., Hammer, A.S., Couto, C.G., 1994. Cutaneous hemangiosarcoma in 25 dogs: a retrospective study. J. Vet. Intern. Med. 8, 345–348. https://doi.org/10.1111/j.1939-1676.1994.tb03248.x.

Wculek, S.K., Cueto, F.J., Mujal, A.M., Melero, I., Krummel, M.F., Sancho, D., 2020. Dendritic cells in cancer immunology and immunotherapy. Nat. Rev. Immunol. 20, 7–24. https://doi.org/10.1038/s41577-019-0210-z.

Wendelburg, K.M., Price, L.L., Burgess, K.E., Lyons, J.A., Lew, F.H., Berg, J., 2015. Survival time of dogs with splenic hemangiosarcoma treated by splenectomy with or without adjuvant chemotherapy: 208 cases (2001–2012). J. Am. Vet. Med. Assoc. 247, 393–403. https://doi.org/10.2460/javma.247.4.393.

Witmer-Pack, M.D., Olivier, W., Valinsky, J., Schuler, G., Steinman, R.M., 1987. Granulocyte/macrophage colony-stimulating factor is essential for the viability and function of cultured murine epidermal Langerhans cells. J. Exp. Med. 166, 1484–1498. https://doi.org/10.1084/jem.166.5.1484.

Wong, S., Ehrhart, E.J., Stewart, S., Zismann, V., Cawley, J., Halperin, R., Briones, N., Richter, K., Sivaprakasam, K., Perdigones, N., Contente-Cuomo, T., Facista, S., Trent, J.M., Murtaza, M., Khanna, C., Hendricks, W.P.D., 2022. Genomic landscapes of canine splenic angiosarcoma (hemangiosarcoma) contain extensive heterogeneity within and between patients. PLoS One 17, e0264986. https://doi.org/10.1371/journal.pone.0264986.

Wood, C., Moore, A., Gliatto, J., Ablin, L., Berg, R., Rand, W., 1998. Prognosis for dogs with stage I or II splenic hemangiosarcoma treated by splenectomy alone: 32 cases (1991-1993). J. Am. Anim. Hosp. Assoc. 34, 417–421. https://doi.org/10.5326/15473317-34-5-417.

Yu, D., Li, Y., Wang, M., Gu, J., Xu, W., Cai, H., Fang, X., Zhang, X., 2022a. Exosomes as a new frontier of cancer liquid biopsy. Mol. Cancer 21, 56. https://doi.org/10.1186/s12943-022-01509-9.

Yu, J., Sun, H., Cao, W., Song, Y., Jiang, Z., 2022b. Research progress on dendritic cell vaccines in cancer immunotherapy. Exp. Hematol. Oncol. 11, 3. https://doi.org/10.1186/s40164-022-00257-2.