CHEMOTHERAPEUTIC TREATMENT OF ANAL SAC ADENOCARCINOMA IN DOGS

by

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Steam review

as part of the Master’s Dissertation

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PREFACE

The summer before I started to study Veterinary Medicine, my beloved dog Kajla died due to the recurrence of anal sac adenocarcinoma. After fighting the disease for over a year we reached a point where there was nothing else left to do than letting her go. Ever since I am wondering whether treating her with chemotherapy was the right thing to do, or was it a selfish act of mine, because I was not ready to lose her; and whether the treatment she received was indeed the best option for her. This is the reason why I chose to write my master’s dissertation about this topic.

I would like to thank my promoter Professor dr. Mathias Devreese for first of all being my promoter and thereby allowing me to write about this topic. I also would like to thank him for his flexibility, support and very useful input when writing this master’s dissertation and for understanding my special situation of being a mom.

I also would like to thank my co-promoter Professor dr. Patrick De Backer for being my co-promoter and for his useful input for this master’s dissertation.

I would like to thank my mom and my husband for supporting me during my studies which includes working on this master’s dissertation, and my two children for letting me work on it.

This master’s dissertation is dedicated to Kajla.

Kajla

ABSTRACT
Apocrine gland adenocarcinoma of the anal sac is an infiltrative tumor rising from the apocrine glands of the anal sac occurring uncommonly in dogs. There is no gender predilection, while certain breeds are overrepresented. The various clinical symptoms include rectal mass and tenesmus, the tumor however can be an accidental finding. Metastasis occurs early in the course of the disease and hypercalcemia of malignancy is often present. The diagnosis can be confirmed using cytological or histological examination, blood and urine analysis, and using medical imaging. The treatment options include surgery, radiotherapy, chemotherapy and any combination of those. A multimodal approach is most commonly applied. Although it is difficult to compare the different kind of treatment approaches - considering most of the studies are of retrospective nature - an attempt has been made to do so. The prognosis of anal sac adenocarcinoma is poor to fair. The best available treatment based on available literature is surgery followed by radiotherapy and mitoxantrone chemotherapy. Alternatives are surgery followed by radiotherapy, radiotherapy alone, surgery followed by mitoxantrone or melphalan chemotherapy, and surgery alone. In case of far progressed disease palliative care should be provided. Treatment with electrochemotherapy combined with cisplatin and treatment with nitrosylcobalamin should be further investigated. Especially nitrosylcobalamin seems promising, should its effects be confirmed by a larger scale study than the prognosis of this type of tumor could even become good. A large scale standardized study including the currently best and most promising treatment options would provide more clarity; the relatively infrequent nature of the tumor and the limitations to standardize the treatment protocols due to owner willingness and the financial aspects however make it difficult to conduct such a study.

KEY WORDS
Anal sac adenocarcinoma, dog, chemotherapy

SAMENVATTING
Adenocarcinoma van de anaalzak is een agressieve vorm van tumor dat niet vaak voorkomt bij honden (17% van de tumoren in de perianale regio, en 2% van alle huidtumoren). De gemiddelde leeftijd van de aangetaste honden is 10-11 jaar, de jongste aangetaste hond in de literatuur was 3,5 jaar oud. In tegenstelling van wat in het verleden gerapporteerd werd, blijkt er geen geslachts predispositie te zijn, de tumor komt zowel bij reuen en teven evenveel voor. Wat betreft ras zijn labradors, Engelse cocker spaniels en Duitse herdershonden gepredisponeerd. De klinische symptomen zijn uiteenlopend, onder andere massa bij de rectum, tenesmus. Hypercalcemie is vaak ook aanwezig. De tumor - die vaak en vroeg in het verloop van de ziekte metastaseert - wordt vaak bij toeval ontdekt tijdens routinematig klinisch onderzoek. De diagnose kan gesteld worden op basis van cytologie of histologie, bloed- en urineonderzoek en medische beeldvorming. Er zijn verschillende mogelijkheden voor de behandeling: chirurgie, radiotherapie, chemotherapie, en de combinatie van deze. Meestal wordt er een combinatie van de verschillende behandelingstechnieken toegepast. Het is niet gemakkelijk om de verschillende behandelingstherapien te vergelijken met elkaar, aangezien de methoden niet gestandaardiseerd zijn, en er vaak geen controlegroepen ingesloten
werden. Toch wordt het in deze masterthesis geprobeerd om na te gaan welke opties momenteel de beste uitkomst bieden in het behandelen van adenocarcinoma van de analezak, gebaseerd op wetenschappelijke literatuur.

De prognose van adenocarcinoma van de analezak is slecht tot matig. De beste behandelingsmogelijkheid in het geval van geen metastase, of metastase alleen ter hoogte van de regionale lymfeknopen is chirurgie, gevolgd door radiotherapie en mitoxantrone chemotherapie. Mocht dit niet haalbaar zijn, dan de best mogelijke volgende opties zijn chirurgie gevolgd door radiotherapie, radiotherapie als enige therapie, chirurgie gevolgd door mitoxantrone of melphalan chemotherapie of chirurgie als enige behandeling. Chemotherapie als enige type behandeling zal niet toegepast worden in verband met slechte prognose. In het geval van verdere uitzetting is de beste optie chirurgie, gevolgd door chemotherapie (mitoxantrone of melphalan per os). Indien de ziekte verder vergroeide is, dan is palliatieve verzorging aangewezen in de vorm van het toedienen van NSAID's, eventueel gecombineerd met prednisone, furosemide en intraveneuze vochttoediening.

Er zijn een aantal behandelingsmogelijkheden in de literatuur die nog verder onderzocht moeten worden, maar erg veelbelovend zijn. Een van de mogelijkheden is elektrochemotherapie (gecombineerd met cisplatin) na chirurgie. Een hond behandeld met elektrotherapie (in combinatie met cisplatin) na chirurgisch verwijderen van de tumor was 19 maanden na behandeling nog steeds in remissie.

De meest veelbelovende toekomstige therapie is de behandeling met nitrosylcobalamine. In een studie waarbij een hond met analezak adenocarcinoma behandeld werd met nitrosylcobalamine, overleeft de patiënt gedurende 67 maanden en was nog steeds in remissie. Deze periode is meer dan een jaar langer dan het langste gerapporteerd overleven van een hond, die behandeld was met chirurgie, radiotherapie en mitoxantrone chemotherapie. Mocht nitrosylcobalamin ook tijdens verder onderzoek in een grootschaliger studie effectief blijken bij het behandelen van adenocarcinoma van de analezak, dan wordt de prognose van deze ziekte misschien zelfs nog goed.

SLEUTELWOORDEN
Adenocarcinoma van de analezak, hond, chemotherapie
INTRODUCTION
Over the past decades the life expectancy of canines became longer, while the development of (veterinary) medicine makes more accurate diagnosis and treatment of tumors - more commonly occurring in elderly dogs - possible. Cancer is a disease frequently occurring in canines with 0.31 to 1% of dogs being affected during their total life span. (Tennant, 2015) Owners are also willing to treat their pets with cancer, making veterinary oncology an upcoming specialization. Although chemotherapy is considered an accepted therapy when treating canine tumors, the aspects of such protocols are different than that of humans. In veterinary oncology often metronomic chemotherapy is used, meaning that the administered dose of chemotherapeutic agent is lower, in order to minimize adverse side effects, since owners find the quality of life of their pets crucial. Consequently the goal of chemotherapy is not necessarily remission, often the extension of the life span of the animal with acceptable quality of life (stable disease) is considered as a successful therapy.

Anal sac adenocarcinoma is an infrequent but infiltrative tumor occurring in dogs, and since humans and dogs have significant differences in physiology, anatomy and biochemical processes – the research for specific treatment protocols is limited and more importantly successful protocols from human medicine cannot be adapted into veterinary medicine.

In this master’s dissertation – after a brief presentation of the aspects of the disease and its diagnosis – the treatment options of anal sac adenocarcinoma are discussed, especially focusing on chemotherapy, based on available literature. The mode of action of each chemotherapeutical agents used for the treatment of anal sac adenocarcinoma is presented alongside the treatment protocol, and the effects and side effects of the treatment. The prognosis of the disease considering different kind of treatment protocols is discussed in a separate section. Beside the most commonly used agents as cisplatin, carboplatin, mitoxantrone and melphalan; other non-conventional agents considered for the treatment of this tumor are included as well.
LITERATURE REVIEW

1. ETIOLOGY AND PATHOGENESIS

Anal sac problems are common in dogs with approximately 12% being affected throughout their lives. (Van Duijkeren, 1995) Tumors of the perianal region occur frequent as well; most of the tumors however turn out to be benign. Anal sac adenocarcinoma is a malignant, invasive form of cancer with a high prevalence of metastasis; originating from the apocrine glands located in the walls of the anal sac. (Berrocal et al., 1989) 17% of the tumors occurring in the perianal region are diagnosed to be anal sac adenocarcinoma and 2% of all skin tumors in dogs. (Withrow and Vail, 2007) Anal sac adenocarcinoma occurs very rarely in cats, there are only a couple of documented cases. (Elliot and Blackwood, 2011)

According to the study of Emms (2005), 53% of dogs had metastasis at initial presentation to the veterinarian. Metastatic sites include regional (iliac) lymph nodes, lungs, liver and spleen, bone, inguinal lymph node, pancreas, heart and mediastinum (Bennett, 2002; Emms, 2005) There are two documented cases of anal sac adenocarcinoma associated with the development of hypertrophic osteopathy; the hypertrophic osteopathy was developed early in one and late in the other case of the disease. Hypercalcemia was present as well. (Giuliano et al., 2015) Brisson et al. (2004) reported a case presented to the veterinarian with acute hind limb paralysis due to metastasis in the vertebral body and vertebral canal.

Although mainly elderly dogs are affected, with a median age of approximately 10-11 years at presentation; dogs as young as 3.5 years old are reported. (Bennett et al., 2002; Williams et al., 2003) Berrocal et al. (1989) showed a gender predisposition in adenocarcinoma of the anal sac, other studies (Bennett et al., 2002; Emms, 2005; Hobson et al., 2006; London et al., 2015) however did not confirm this finding. Polton et al. (2004) found no gender predilection, but being neutered appeared to be a risk factor, more significantly in male than in female dogs.

According to Polton et al. (2004) and Aguirre-Hernandez et al. (2012) English cocker spaniels are overrepresented when it comes to anal sac adenocarcinoma and so are other spaniel breeds (Aguirre-Hernandez et al., 2012). Aguirre-Hernandez (2010) found an association between the dog leukocyte antigen DQB1 (the most common dog leukocyte antigen in English cocker spaniels) and anal sac carcinoma in English Cocker Spaniels. In another study in 2012 Aguirre-Hernandez et al. mapped a locus on chromosome two significantly to anal sac adenocarcinoma in the English cocker spaniel.

The study of London et al. (2011) suggests a predisposition in labrador retrievers (9 out of the 32 cases of anal sac adenocarcinoma were labrador retrievers, while 2 further dogs were labrador mixed breeds).

In the study of Williams et al. (2003) the most frequently affected breed dogs were German shepherds (20%), followed by English cocker spaniels (10%) and Golden retrievers (8.7%). The overrepresentation of German shepherds is confirmed by Potanas et al. (2002): 23% of the breed dogs affected were German shepherd dogs.
2. SYMPTOMS AND DIAGNOSIS

The clinical symptoms are very variable, including rectal mass (26% of the cases), tenesmus, anorexia, polyuria and polydipsia, lethargy, weight loss, urinary incontinence, constipation and posterior weakness. Clinical signs are often due to metastasis in the regional (iliac and lumbar) lymph nodes (teneasms) or hypercalcemia (polyuria and polydipsia). Hypercalcemia occurs in approximately 50% at the cases at the time of diagnosis. These dogs may also develop renal azotemia. (Bennett et al. 2002) Hypercalcemia of malignancy in dogs with anal sac adenocarcinoma is due to the production of parathormone-related peptide by the tumor. (Withrow and Vail, 2007) Weir et al. (1988) isolated the 16,000 Dalton parathormone-related peptide - a protein with a high potential to activate the parathormone receptor-coupled adenylate cyclase in bone cells - from canine apocrine gland adenocarcinomas. Hypercalcemia might be negatively correlated to the survival (Williams et al., 2003), although the studies of Emms (2005) and Bennett et al. (2002) do not confirm this. The study by Bennett et al. (2002) however had a low power due to the small number of dogs in the study and the great variation in survival time. According to Bennett et al. (2002) in 21 to 34% of the cases the tumor is an accidental finding. Bilateral development of anal sac adenocarcinoma is rare; in the study of Emms (2005) two out of 14 canines were presented with bilateral tumors.

The differential diagnosis of anal sac diseases includes (Schaer, 2010):
- Anal sac impaction
- Anal sacculitis
- Anal sac neoplasia
- Perianal neoplasia
- Trauma
- Perianal fistula or abces

The diagnostic techniques of anal sac diseases include (Côte, 2015):
- Visual examination of the anal sac and rectal palpation
- Cytological examination of contents of the anal sac
- Culture of anal sac contents
- Cytological examination of anal sac masses

*Figure 1: Fine needle aspirate cytology of an anal sac adenocarcinoma (400x) (Withrow and Vail, 2007)*

When diagnosing anal sac adenocarcinoma the cytological differential diagnosis looks as follows (Campbell, 2004):
- Anal sac adenocarcinoma
- Perianal adenoma (mainly occurs in intact male dogs, rarely in ovariohysterectomized females)
- Perianal adenocarcinoma

For the staging of the tumor the following diagnostic techniques can be used (Bowlt et al., 2014):
- Laboratory analysis (urine, hematology, serum biochemistry - including serum calcium)
- Medical imaging to determine the presence and localization of metastasis (thoracic radiographs and abdominal ultrasound or CT / MRI scan)

3. TREATMENT OPTIONS
The possible treatment options of anal sac adenocarcinoma include surgical excision, radiation therapy, chemotherapeutical treatment and any possible combination of these above. (Bennett et al., 2002)

3.1. SURGICAL EXCISION
Surgery is the first choice treatment option in case of anal sac adenocarcinoma without apparent metastasis or metastasis only at the site of the regional lymph nodes at presentation. In case of further metastasis surgery can be palliative - with a higher morbidity and mortality risk. (Bennett et al., 2002) Anal sacculectomy is the surgical treatment of anal sac adenocarcinoma. Complete excision should be aimed for whenever possible, using the closed technique. Postoperative complications include tenesmus, hemorrhage, wound infection and dehiscence on the short-term; long-term complications include fistula formation, fecal incontinence, persistent infection and anal stricture. Fecal incontinence is the most serious complication of surgery, in case of unilateral injury the incontinence is temporarily. (Ragni, 2012) In the 2015 study of Potanas et al. very few cases of surgical complications were reported (three out of 42 dogs) and only one dog developed fecal incontinence, but was not euthanized as a result of it. Hobson et al. (2006) reported a dog with a survival time of 54 months after diagnosis as a result of five consecutive surgeries, including lymphadenectomy. They concluded that the surgical removal of tumors and metastatic lymph nodes can result in a prolonged survival time with minimal post-operative complications. According to Bennett et al. (2002) tumor recurrence occurred in 45% of the cases post-surgery.

3.2. RADIATION THERAPY
Radiation therapy can be used pre- or post-surgical or as palliative care. Prior to surgery radiation therapy can be used to enhance the chance of the surgical removal with clear margins. An example of pre-operative radiation treatment includes 2.5 Gy fractions for 22 days (cumulative dose of 60 Gy). Surgery takes place two to four weeks after the last treatment: after the radiated tissues healed. (Burgess et al., 2009)
Post-operative radiotherapy can be applied in case the tumor margins have not been clear, or to irradiate the sublumbar lymph node when metastasis is present, but lymphadenectomy was not (fully) possible. The doses are higher, 2.5 Gy for 24 days (cumulative dose of 55 Gy). Adverse side effects include colitis, tenesmus, discomfort and skin desquamation; these side effects can occur during therapy, or weeks after. (Burgess et al., 2009)
Palliative care includes higher doses (6-8 Gy) of radiation given weekly in order to minimize adverse side effects and improving the quality and length of life. (Burgess et al., 2009)

In the study of Turek et al. (2003) 15 dogs affected by anal sac adenocarcinoma were treated with radiotherapy and mitoxantrone chemotherapy post-surgery. The administered doses of the irradiation were 3.2 Gy, given five consecutive days three weeks in a row, with a cumulative dose of 48 Gy. Four out of the 15 dogs had tumor recurrence in the irradiated field (27%) One or more adverse side effects occurred in all dogs, including skin desquamation, colitis/proctitis, tenesmus, rectal stricture and change in defecation.

3.3. PALLIATIVE CARE

Palliative care can be a choice of the owner of the dog or it can be the best option in case of far progressed disease when surgery is not recommended or possible. (Withrow and Vail, 2007). Surgery as well as chemotherapy however can also be used as palliative treatment. Several studies showed that medical treatment with non-steroid anti-inflammatory drugs beneficial is for various kinds of cancer patients. It might cause an anti-tumor (via COX-2 inhibition) or an anti-angiogenic effect. (Bennett et al., 2002) Knudsen et al. (2013) showed that COX-2 is expressed in the glandular epithelial cells in anal sac adenocarcinomas and in the glandular cells of lymph nodes, as well as in the ductal epithelial cells in non-neoplastic anal sacs. COX-2 inhibitors therefore can be an effective way of treating anal sac adenocarcinoma.

Piroxicam can be used after surgery or chemotherapy or as palliative care alone. It can be combined with furosemide, prednisone and fluid therapy in case of hypercalcemia. (Bennett et al., 2002) Gregório et al. (2012) found that administration of a selective COX-2 inhibitor (for example firocoxib 5 mg/kg per os dose one time) as only treatment in dogs with different kinds and stage cancer significantly improved the overall quality of life as well as specific aspects (happiness, mental status, pain, appetite and mobility) of it.

Radiation therapy can also be used as palliative care. (Burgess et al., 2009)

3.4. CHEMOTHERAPY

Cytotoxic cancer chemotherapy agents are routinely used in veterinary medicine. The doses are determined to cause minimal hospitalization and mortality due to toxicity. Adverse side effects however do occur. Gastrointestinal toxicity occurs as lack of appetite, nausea, vomiting and diarrhea; due to stimulation of the chemoreceptor trigger zone, or secondary due to damaged epithelial cells of the intestines. Myelosuppression occurs due to the damage of bone marrow stem cells, resulting in mainly neutrophilia en low blood platelet count. Alopecia occurs rare, although it does occur in dogs with continuously growing hair, cats lose their whiskers. (Withrow and Vail, 2007)

Exposure to cytotoxic drugs can be hazardous to the veterinarian as well as to the owner and the family of the pet. Therefore specific safety measures should be applied, including specific storage of the drugs, wearing special gloves, gown and protective eyewear during administration of the agents, and separate waste disposal. Measures have to be taken when it comes to patient care as well. As a
general rule specific gloves should be worn when cleaning up urine and feces 48 hours past administration of the drugs and all waste should be double bagged. (Withrow and Vail, 2007) Even though the general guideline should be applied for 48 hours and mentions urine and feces only, Janssens et al. (2012) found that carboplatin is excreted in saliva, sebum and cerumen as well and the excretion was still measurable three weeks after administration (happened mainly in the first four days post administration though).

The administered dose of the chemotherapeutic agents in traditional chemotherapy is the maximal tolerated dose in intervals as short as possible, while in metronomic chemotherapy a low dose of the chemotherapeutic drug is administered daily. The working mechanism is different as well, while traditional chemotherapy targets tumor cells in an effort to induce tumor regression, metronomic chemotherapy is anti-angiogenic by targeting growing tumor vascular endothelial cells. The administered dose is traditionally determined based on the body surface area, where $\text{BSA} = W^{1/3}$. When it comes to cats and dogs that weigh less than 15 kg, the recommendation is however to use the body weight instead of body surface area to determine the dose, in order to avoid increased toxicity with the administration of certain agents, including doxorubicin, cisplatin, carboplatin en melphalan. (Withrow and Vail, 2007)

According to the study of Bennett et al. (2002) 75% of the 20 dogs receiving chemotherapeutical treatment were treated at some point with platinum containing agents: cisplatin or carboplatin, indicating that platinum agents are considered the standard therapy when it comes to chemotherapeutical treatment of anal sac adenocarcinoma in dogs. Two other agents were used in studies involving a larger amount of canines: in the study of Turek et al. (2003) 15 dogs has been treated with radiotherapy combined with mitoxantrone and in the study by Emms (2005) 14 dogs with anal sac adenocarcinoma were treated with melphalan. Most of the other agents described below are usually administered after failure of treatment of the agents mentioned previously, and are considered non-conventional agents for the treatment of this tumor. (Bennett et al., 2002)

3.4.1. Platinum based anti-neoplastic agents
The platinum based anti-neoplastic agents cisplatin and carboplatin are generally used to treat osteosarcoma, carcinoma and sarcoma. The platinum based anti-neoplastic agents are not strictly alkylating agents; even though these agents also inhibit protein synthesis due to binding to DNA. The effect of these agents is independent from the cell cycle phase. (Withrow and Vail, 2007)

3.4.1.1. Cisplatin
Cisplatin - a platinum (II) compound - is one of the most potent anti-cancer agent. Although cisplatin has been used as an anti-tumor agent for almost 30 years it is still not completely understand how the effects are caused. Cisplatin enters cells mainly via active transport through a specific copper ion transporter, but also via passive diffusion. In the cell cisplatin loses the chloride ions (leaving groups) creating the possibility to covalently bind to the N7 site of purine bases (particularly the N7 sites of
guanine) in DNA forming intra- or interstrand cross-links. Cisplatin sequesters the DNA damage recognition proteins that bind to the DNA adducts thereby prevents their participation in transcription which may triggers DNA damage signals transducing. The DNA damage - due to the binding of cisplatin - activate several pathways, including a pathway that activates cell-cycle check points, resulting in a temporary S-phase followed by a G2/M-phase cell cycle arrest. The activation of the p53 tumor suppressor protein is induced by DNA damage caused by cisplatin, and plays a role in inducing apoptosis. The MAPK pathway is also activated and it plays a role in inducing apoptosis as well. The elimination half-life of cisplatin is 1.5-3.6 hours. (Siddik, 2003; Palmer et al. 2012; Dasari and Tchounwou, 2014)

Figure 1: Chemical structure of cisplatin (Weiss and Christian, 1993)

The adverse side effects of cisplatin include nephrotoxicity, therefore cisplatin is administered alongside 0.9% saline diuresis. Another potential side effect is severe nausea and vomiting. The administered dose of cisplatin is 70 mg/m² in dogs intravenously in three weeks cycles. (Withrow and Vail, 2007) According to the retrospective study of Bennett et al. (2002) the platinum agents were effective: out of 13 dogs treated with cisplatin four had partial response to the treatment, and in one dog was the disease stable. Azotemia, an adverse side effect due to the nephrotoxicity of cisplatin has been developed in five out of the 13 dogs.

Figure 2: Cisplatin induced cellular effects (Dasari and Tchounwou, 2014)
3.4.1.2. Carboplatin

Carboplatin is a similar platinum (II) compound to cisplatin: the chloride ions are replaced by 1,1-cyclobutanecarboxylato ligands (leaving groups). The leaving groups of carboplatin are more stable, hence less reactive than those of cisplatin. (Dasari and Tchounwou, 2014)

According to Knox et al. (1986) cisplatin and carboplatin differs only in the kinetics of their aquation and subsequent reactions with DNA, but regarding toxicity carboplatin is superior to cisplatin: ten times more carboplatin can be administered before the dose limiting toxicity is reached. Due to toxicity cisplatin cannot be administered to cats; the agent induces fatal pulmonary edema. (Withrow and Vail, 2007) Although in vitro the same reaction products are formed, carboplatin is less potent than cisplatin: the required dose of carboplatin is 4:1 compared to the dose of cisplatin. The half-life of carboplatin is 30 hours. (Dasari and Tchounwou, 2014)

The adverse side effects of carboplatin include myelosuppression, and possibly severe gastrointestinal problems. (Withrow and Vail, 2007) The dose of carboplatin is 300 mg/m² intravenously. (Janssens et al. 2012) According to Bennett et al. (2002) out of three dogs treated with carboplatin one had partial response to the treatment and in two dogs was the disease stable. Azotemia, an adverse side effect has been developed in one out of the three dogs. Janssens et al. (2012) found that although the excretion (in mainly urine, but also in saliva, sebum and cerumen) of the platinum agent happens mainly in the first four days after administration, excretion is still measurable even three weeks after treatment.

Carboplatin chemotherapy is also documented to be used for the treatment of anal sac adenocarcinoma in cats. (Wright et al. 2010; Elliot and Blackwood, 2011)

3.4.1.3. Satraplatin

Satraplatin is a platinum (IV) compound cytotoxic agent with a similar mode of action as cisplatin. The advantage of satraplatin is due to its lipophilicity, which makes administration per os possible. Satraplatin has two chloride atoms as leaving groups (like cisplatin), and two asymmetrical stable ligands: a single amine and cyclohexylamine group, which change the DNA adduct profile of satraplatin, making the inhibition of DNA synthesis more efficient, as well as reducing the possibility of being recognized by DNA mismatch-repair, or high-mobility group proteins, causing less resistance than cisplatin. (Bhargava and Vaishampayan, 2009)

Satraplatin has been administered to 23 dogs in the study of Selting et al. (2011). One of the dogs was treated for anal sac adenocarcinoma. Based on this study satraplatin is recommended to be administered in doses of 30-35 mg/m² daily for five days in three weeks cycles. The authors gave four
doses in total. Administering these doses resulted in myelotoxicity, alongside of minimal gastrointestinal problems as adverse side effects. Neuro- and nephrotoxicity; adverse side effects of the other cytotoxic platinum agents cisplatin and carboplatin; did not occur. There was measurable response to the treatment; the study however does not mention the response specifically in the dog treated for anal sac adenocarcinoma.

Figure 4: Chemical structure of satraplatin (Bhargava and Vaishampayan, 2009)

3.4.2. Alkylating anti-neoplastic agents

The working of alkylating anti-neoplastic agents are cell cycle independent by changing the structure of DNA by covalently binding to it, and inserting an alkyl group making DNA, RNA and protein synthesis impossible for the cell. (Withrow and Vail, 2007)

3.4.2.1. Melphalan

Melphalan is a nitrogen mustard bi-functional alkylating anti-neoplastic agent, including an amino-ethyl bridge between the aromatic ring and the carboxyl group and having two chloride leaving groups. Melphalan enters the cells via chlorine or certain amino acid transporters (which are often being overexpressed in cancer cells), and – like other alkylating anti-neoplastic agents – binds to the nucleophilic sites on purine bases in DNA. The most reactive nucleophilic site of DNA is the N7 site of guanine, followed by the N3 site of adenine and the N3 site of cytosine. (Brown et al, 2009; Palmer et al., 2009)

Figure 5: Chemical structure of melphalan (Palmer et al., 2012)

Due to the alkylation of the N7 site the guanine adopts the tautomeric iminol form, which resembles that of adenine, making guanine bind to thymine rather than to cytosine. This mechanism plays an important role in the mutagenic effects of alkylating agents. (Palmer et al., 2012)

The main indications for the treatment with melphalan include multiple myeloma and anal sac adenocarcinoma. Melphalan can be administered per os, based on two different schemes: an initial dose of 0.1 mg/kg daily for 10 days followed by a reduced dose of 0.05 mg/kg per day; or pulse dose administration of 7 mg/m² for five days in three weeks cycles. Adverse side effects include myelosuppression and cumulative thrombocytopenia. Melphalan is excreted via urine and feces. (Withrow and Vail, 2007) In the retrospective study of 14 dogs with anal sac adenocarcinoma by Emms (2005), the dogs have been treated with melphalan per os after surgery. The canines have been divided into two groups: one group with patients with metastasis in the sublumbar lymph nodes present at the time of diagnosis, the other group included the dogs without metastasis found at initial presentation (each group contained seven dogs). Bilateral anal sac adenocarcinoma occurred in one
dog in each group. The dogs with metastasis also underwent lymph node extirpation during surgery. The administered dose of melphalan was 7 mg/m² with a 16 days pause after administering the drug for five days. The treatment did not cause any apparent toxicity according to the dog owners, the doses did not have to be modified based on hematological monitoring (that took place accidentally). The treatment started two weeks post-surgery and lasted a lifelong. The median survival in the group with metastasis was 20 months (range: 5-28 months), while in the group without metastasis the median survival time was 29 months (range: 12-42 months). Two of the fourteen dogs were still alive at the last follow-up: one dog with a survival of 10 and another with 37 months. From the other twelve dogs only two died due to not cancer related problems; the death of the other 10 was tumor-related.

3.4.2.2. Cyclophosphamide
Cyclophosphamide is a DNA alkylating agent including a dichloroethylamine moiety and two chloride leaving groups. Cyclophosphamide is extensively metabolized in the liver, initiated by a cytochrome P450 enzyme and includes several enzymatic and non-enzymatic steps. One of the products of the metabolisation of cyclophosphamide is acrolein – also present in smoke of cigarettes and in frying oil – that binds to the N7 site of guanine forming tricyclic adducts, thereby inhibiting DNA synthesis and repair. (Palmer et al., 2012)

Figure 6: Chemical structure of cyclophosphamide (Palmer et al., 2012)

Cyclophosphamide is usually administered (per os or intravenously) to treat lymphoma, carcinoma, and sarcoma. The total dose can be given in one administration (200-300 mg/m²), or divided over four days and administered daily. Adverse side effects include bone marrow suppression and sterile hemorrhagic cystitis, the latter does not occur frequently in dogs. Cyclophosphamide is excreted via the urine. (Withrow and Vail, 2007)

The one dog treated with cyclophosphamide for anal sac adenocarcinoma did suffer from sterile hemorrhagic cystitis as a side effect. (Williams et al. 2003)

3.4.3. Anti-tubulin agents: vinca alkaloids

Figure 7: Equilibrium of tubuline-dimers and microtubule (Pratt et al., 1994)
Vinca-alkaloids bind to the free tubulin-dimers, inhibiting the formation of microtubule – more specifically the forming of the mitotic spindles - and thereby mitosis during the metaphase. The working of vinca alkaloids is cell cycle phase dependent. At high concentration after binding with free tubuline-dimers, the tubuline-dimers cannot form microtubule; hence there are less free tubuline-dimers available for microtubule assembly, which shifts the equilibrium of microtubule-tubulin-dimers towards disassembly and shrinkage of microtubule. Vinca alkaloids cause the formation of paracrystalline aggregates which shifts the equilibrium more towards disassembly. (Pratt et al., 1994)

At lower concentration vinca-alkaloids inhibit the dynamics of microtubule, without changing the microtubule organization. While first generation vinca-alkaloids - such as vincristine - suppress the rate and extent of microtubule shortening, second generation vinca-alkaloids as vinorelbine suppress the rate and extent of microtubule growing events and therefore inhibits the dynamics of microtubule differently. (Ngan et al., 2001)

3.4.3.1. Vincristine

Vincristine is primarily used for the treatment of lymphoma and mast cell tumor administered at a dose of 0.5-0.7 mg/m$^2$ weekly intravenously. Adverse side effects include myelosuppression, perivascular vesicant and peripheral neuropathy (mainly in humans). Excretion through feces and since the metabolization mainly happens in the liver, liver disease is a contraindication for use (Withrow and Vail, 2007).

*Figure 8: Chemical structure of vincristine (Pratt et al., 1994)*

In the study of Bennett et al. (2002) one dog treated with chemotherapy alone has been treated with only non-conventional agent vincristine in combination with cyclophosphamide. There has been no response to the therapy.

3.4.3.2. Vinorelbine

Vinorelbine- a second generation semi-synthetic vinca alkaloid developed as a less neurotoxic alternative to vincristine - has primary long tumor as its main indication and is administered intravenously in a dose of 15-18 mg/m$^2$ weekly or every second week. Adverse side effects include myelosuppression and perivascular vesicant. (Withrow and Vail, 2007)

*Figure 9: Chemical structure of vinorelbine (Ngan et al., 2001)*
Vinorelbine is expensive compared to vincristine. Even though biotransformation occurs in the liver, vinorelbine can be used in dogs with liver disease; the dose should be lowered though. (Withrow and Vail, 2007)

Wouda et al. (2015) treated 58 dogs with vinorelbine, including a nine years old German shepherd look-a-like cross breed female with metastatic anal sac adenocarcinoma. The dog had previously been treated with surgery and chemotherapy (mitoxantrone, paclitaxel and carboplatin) prior to the administration of 12 doses vinorelbine (at a dose of 15 mg/m²). The canine responded to the therapy, reached a state of stable disease and the hypercalcemia resolved as well (after 5 months of treatment). The most common adverse side effect in the group of 58 dogs was neutropenia (affecting 40% of the dogs) - the treatment of two dogs had to be stopped due to grade four neutropenia and fever - followed by thrombocytopenia (affecting 7% of the dogs).

3.4.4. Anti-neoplastic antimicrobial drugs

3.4.4.1. Doxorubicin

Doxorubicin, a very frequently used chemotherapeutic agent (for the treatment of lymphoma, carcinoma and sarcoma) in canine cancer, can only be administered a limited amount of times to a patient due to cumulative cardiotoxicity (limit: 180 mg/m²). (Withrow and Vail, 2007; Serres et al., 2012) Doxorubicin is a cell cycle phase independent anti-neoplastic agent, an original product of the *Streptomyces peucetius var. caesius* yeast; the antitumor activity is due to inhibiting DNA and protein synthesis, free radical formation and also the activity of the topoisomerase enzymes. (Withrow and Vail, 2007) Doxorubicin has a planar ring structure and with its polycyclic moiety it intercalates two base pairs of the DNA double strand. Iron (Fe³⁺) - that is chelated by the ring and is being reduced by glutathione – reduces the drug molecule leading to the production of reactive oxygen species - causing oxidative damage to DNA, oxidative stress, lipid peroxidation, membrane damage; activating the apoptotic pathways in the cell -; and formaldehyde, binding the amino-sugar moiety of doxorubicin to the N7 site of a guanine (or adenine) base, inhibiting DNA replication. (Thorn et al., 2011; Palmer et al., 2012) According to Tewei et al. (1984) another mechanism - doxorubicin entering the nucleus and targeting topoisomerase IIα and IIβ both - also results in DNA damage and cell death. Doxorubicin
stimulates the DNA cleavage by DNA topoisomerase II. (Wassermann et al., 1990) The oxidative damage to mitochondrial lipids has been linked to the cardiotoxicity of doxorubicin (Palmer et al., 2012), although recent studies suggest that cardiotoxicity is due to damage to topoisomerase IIβ enzymes present in cardiomyocytes poisoned by the agent. (Evison et al., 2015)

Adverse side effects beside cumulative cardiotoxicity include myelosuppression, gastro-intestinal problems, hypersensitivity during administration (mainly gastro-intestinal and skin related), perivascular damage with extravasation and alopecia. (Withrow and Vail, 2007) Doxorubicin can be used as chemotherapeutical treatment for anal sac adenocarcinoma. The required doses are 30 mg/m² or 1 mg/kg (for dogs under 10 kg bodyweight) intravenously every three weeks. Excretion of the drug happens mainly via feces. (Withrow and Vail, 2007) According to the retrospective study of Bennett et al. (2002) out of four dogs treated with doxorubicin in two dogs was the disease becoming stable (50%). In the study of Williams et al. (2003) one dog suffered from cardiac toxicity as a side effect of the administration of doxorubicin (out of 27 dogs that had been treated with doxorubicin-mitoxantrone). Serres et al. (2012) studied cardiac arrhythmia in dogs treated with doxorubicin by using 24 hours Holter-monitoring in 20 dogs. They found that premature ventricular complexes occurred in 30% of the dogs receiving doxorubicin treatment, the arrhythmia however did not occur during administration, nor was it related to the cumulative doses.

Doxorubicin can be administered as doxorubicin HCl liposome injection - which is much pricier than doxorubicin - due to the liposome encapsulation however the cardiotoxicity is reduced to a minimum. (Withrow and Vail, 2007)

Figure 11: Chemical structure of doxorubicin (left) and epirubicin (right) (Evison et al, 2015)

3.4.4.2. Epirubicin

Epirubicin also known as 4′-epidoxorubicin is an isomer of the anthracycline antibiotic doxorubicin. Despite the fact that the alteration of the chemical structure is minimal, there are some differences regarding the biological activity: most importantly the treatment with epirubicin results in reduced cardio- and overall-toxicity. (Goldin et al., 1985)

In the study of Bennett et al. (2002) the disease of the one dog that has been treated with epirubicin remained progressive.
3.4.4.3. Actinomycin D

Actinomycin D, an original product of the *Streptomyces* yeast, is a cell cycle phase independent antineoplastic agent active by inhibiting RNA and protein synthesis (Withrow and Vail, 2007). Actinomycin is a cyclic polypeptide that intercalates to DNA between guanine-cytosine base pairs, while the lactone rings position in the minor groove of the DNA helix spanning the base pairs in opposite directions (Chen et al., 1996; Koba and Konopa, 2005). Actinomycin D stimulates the DNA cleavage by both DNA topoisomerase I and II (Wassermann et al., 1990). Actinomycin D is mainly used for the treatment of lymphoma (usually after failure of treatment with other agents), nephroblastoma and as a non-cardiotoxic alternative for doxorubicin once the cumulative cardiotoxic limit have been reached. The required dose is 0.75-0.8 mg/m\(^2\) intravenously in three weeks cycles. Adverse side effects include myelosuppression, gastro-intestinal problems (nausea, vomiting, and diarrhea) and perivascular damage with extravasation. Actinomycin D is eliminated via urine and feces (Withrow and Vail, 2007). Actinomycin D has been administered to four dogs in the study of Bennett et al. (2002), the disease remained progressive in all four dogs despite the treatment.

![Figure 12: Chemical structure of actinomycin D (Lo et al., 2013)](image)

3.4.4.4. Mithramycin

Mithramycin is the product of *Streptomyces argillaceus*. Mithramycin - a DNA groove binder - binds to DNA preferably at guanine-cytosine sites - for this interaction the presence of a bivalent metal ion is required – and inhibits the transcription of genes with guanine-cytosine rich promoter sequences.
while it can also bind to histones - which interaction does not require the presence of a bivalent metal ion. Mithramycin increases apoptosis mediated by tumor necrosis factor α and Fas death receptor. (Duverger et al., 2004; Sleiman et al., 2011; Banerjee et al., 2014)

In the study of Bennett et al. (2002) the dog treated with non-conventional agents alone also received mithramycin with no response to the treatment.

3.4.4.5. Mitoxantrone

Mitoxantrone is a cell cycle phase independent synthetic antitumor antibiotic, its anti-tumor effect is due to the inhibition of the topoisomerase II enzyme. (Withrow and Vail, 2010) Mitoxantrone has been developed in an effort to synthesize a novel doxorubicin analog with reduced cardiotoxicity. Mitoxantrone enters the cells via passive diffusion and intercalates on the planar chromophore between pyrimidine and purine steps of DNA, preferably guanine and cytosine. The relatively flexible side chain prefers the major groove for intercalative binding while an alternative mode of binding is the possibility of a weak electrostatic interaction between the cationic side-chains of mitoxantrone and the anionic phosphate residues of DNA. The DNA damage is achieved by four different mechanisms: mitoxantrone stabilizes the topoisomerase II(α) cleavage complex, by intercalating in the duplex; the formation of reactive oxygen species by reducing the quinone moiety of the agent; mitoxantrone can be activated by formaldehyde or myeloperoxidase to form DNA adducts bound covalently; and mitoxantrone can also condense nucleic acids. (Evison et al., 2015)

![Chemical structure of mitoxantrone](Evison et al., 2015)

Mitoxantrone is mainly used to treat lymphoma and transitional cell carcinoma. The required dose is 5-5.5 mg/m² intravenously every three weeks. Adverse side effects include myelosuppression, gastrointestinal problems (vomiting and diarrhea), perivascular damage with extravasation, in some patients the urine can be blue-green colored and the sclera can become blueish as well. Another disadvantage of mitoxantrone is its high price. Excretion of mitoxantrone happens via urine and feces. (Withrow and Vail, 2007)

15 dogs with anal sac adenocarcinoma were treated with radiotherapy and mitoxantrone chemotherapy after surgery. The dose of mitoxantrone was 5 mg/m² intravenously administered in three weeks cycles with a total of five times (three dogs however did not received all five doses due to detection of treatment failure, and one more due to poor owner compliance). Adverse side effects as of mild gastrointestinal problems, neutropenia (two dogs needed to be hospitalized as a result of severe neutropenia and fever) and thrombocytopenia occurred. 11 of the 15 dogs had disease recurrence; seven of them were treated with one or more other chemotherapeutic agents and one dog received additional radiotherapy. The median disease free interval was 9.4 months (range: 2.6-34.9 months) and the median overall survival time was 31 months (range: 5-46.7 months). At the time of
analysis three dogs were still alive, out of the 12 dogs that had died; the death of eight was tumor-related, 4 dogs had died because of other reasons (the survival time of these dogs has not been taken into account for the overall survival time analysis). The overall survival time has been calculated based on the overall survival time of the eight dogs whom death was tumor related. (Turek et al., 2003)

3.4.5. Other agents
3.4.5.1. Nitrosylcobalamin

Nitrosylcobalamin is a nitric oxide carrier based on cobalamin (vitamin B12). The cobalamin uptake by cells is regulated by the ubiquitous plasma membrane transcobalamin II receptor that might be overexpressed in tumor cells, considering tumor cells often use more cobalamin than regular cells. Nitrosylcobalamin induces mRNAs for among others tumor necrosis factor-related apoptosis-inducing ligand; initiator and effector caspases. The anti-tumor effect is due to the induction of apoptosis by activating the extrinsic apoptotic pathway. (Bauer et al. 2002) In the 2009 study of Bauer et al., four dogs with different kinds of tumors were treated with nitrosylcobalamin, among them a ten years old dog with anal sac adenocarcinoma and enlarged regional lymph nodes. The Bichon Frise castrated male underwent surgery after the initial diagnosis of the disease and was also treated with mitoxantrone, radiation therapy and adryamicin prior to the administration of nitrosylcobalamin. The dog remained slightly hypercalcemic and had elevated liver enzymes even after surgery and the administration of the drugs and radiation.

Figure 15: Chemical structure of nitrosylcobalamin (Hannibal et al., 2007)

The treatment with nitrosylcobalamin started 28 weeks post diagnosis and was administered for 46 weeks. The initial dose of 28 mg/kg has been raised to 42 mg/kg after 12 weeks of treatment and to 56 mg/kg after eight more weeks. After 46 weeks of treatment with nitrosylcobalamin alone a combination of nitrosylcobalamin and carboplatin was used for six cycles (until week 56 post diagnosis). Carboplatin administration was stopped while the administration of nitrosylcobalamin continued. After 61 weeks of treatment the size of the anal sac tumor was reduced by 43% while the iliac lymph node was reduced in size by 90%.
61 months after starting the nitrosylcobalamin therapy the dog is still alive (18 months off study since the treatment has been stopped, 41 months after the disease was found stable, since the treatment with nitrosylcobalamin started 6.5 months post diagnosis the total survival time of the dog until analysis is 67.5 months). In the last three months of the study the blood urea nitrogen level had been increased and the dog had developed mild renal azotemia. The other three patients did not suffer from azotemia; this indicates that the cause of azotemia might be secondary dehydration.

The nitrosylcobalamin treatment has also been effective in the other three dogs participating in the study, suffering from inoperable thyroid carcinoma, malignant peripheral nerve sheath tumor and spinal meningioma. The daily administration of nitrosylcobalamin did not result in toxicity.

Based on this preliminary study nitrosylcobalamin seems to be a promising cytostatic with a longer survival rate than the longest survival reported in literature for anal sac adenocarcinoma. More research is needed considering only one dog has been treated for anal sac adenocarcinoma.

3.4.5.2. Toceranib phosphate

![Chemical structure of toceranib phosphate](image)

*Figure 16: Chemical structure of toceranib phosphate (Anonymus, 2012)*

Toceranib phosphate is a tyrosine kinase receptor inhibiting agent - a competitive inhibitor of ATP resulting in preventing receptor phosphorylation and the following downstream signal transduction - used for the treatment of solid tumors in dogs. (London et al., 2012; Bernabe et al., 2013) Toceranib was developed as an anti-angiogenic drug, therefore is active against various type of tumors. (London et al., 2012) The approved doses of toceranib phosphate is 3.25 mg/kg per os every second day (Bernabe et al., 2013). London et al. (2012) indicates that a dose lower than the approved dose still results in sufficient clinical response with less adverse side effects, making a long term treatment possible. Bernabe et al. (2013) confirmed this, showing that lower doses of 2.5-2.75 mg/kg per os every second day causes less adverse side effects while the plasma concentration is still high enough for biological activity (inhibition).

London et al. (2012) analyzed in a retrospective study 85 cases of dogs with various tumors treated with toceranib (some dogs were treated with NSAID’s and cyclophosphamide in combination with toceranib), including 32 cases of anal sac adenocarcinoma. 25 of the 32 dogs were treated with surgery, chemotherapy, radiation therapy (or a combination of these) before the administration of toceranib. 28 dogs had metastasis in lymph nodes, lungs, liver and other sites. The median dosage used for the treatment was 2.81 mg/kg per os every other day, two or three times a week for a median treatment period of 25 weeks. 8 dogs had partial response for the therapy (medial duration of partial
response was 22 weeks) and 20 out of the 32 dogs had stable disease (for a median interval of 30.5 weeks) as a result of the treatment.

Adverse side effects occurred in 77.6% of all dogs participating in this retrospective study including diarrhea, weight loss, vomiting, musculo-skeletal pain and/or weakness, anorexia, hemo-occult positive feces, lethargy, neutropenia and skin disorders.

Toceranib phosphate was also administered to dogs to treat (metastatic) osteosarcoma, thyroid carcinoma, head and neck carcinoma and nasal carcinoma according to this study.

Urie et al. (2012) reported an objective response rate of 25% and the occurrence of stable disease in 50-60% of dogs with anal sac adenocarcinoma treated with toceranib phosphate.

3.5. ELECTROCHEMOTHERAPY

Electrochemotherapy uses electric pulses to deliver the chemotherapeutic agent into the (tumor) tissues. Since with the use of electrochemotherapy local treatment is possible, no systemic adverse side effects appear. Electrochemotherapy combined with cisplatin has been used in the case of a 14 year old giant schnauzer dog with anal sac carcinoma after surgery (with incomplete surgical excision of the tumor). Electrochemotherapy was administered under anesthesia two weeks post-surgery. The tumor bed has been prepared using lidocaine and hyaluronidase prior to the infiltration with cisplatin (at a concentration of 0.5 mg/ml, administering a total dose of 8 mg) after which eight biphasic electrical pulses were delivered. After a second treatment two weeks following the first one, the dog was scheduled for monitoring. The dog is in complete remission 18 months after the second treatment (19 months post diagnosis) and still being monitored by the authors at the time of publishing. (Spugnini et al., 2008a) The authors also successfully treated a dog with metastatic apocrine gland carcinoma of the submandibular site: the dog is in remission six months after the last treatment and still being monitored, indicating that electrochemotherapy is a safe and efficient treatment option for metastatic carcinoma in dogs. (Spugnini et al., 2008b)

4. PROGNOSIS

Table 1: Survival times according to the different kinds of treatment per study

<table>
<thead>
<tr>
<th>Number of dogs</th>
<th>Treatment</th>
<th>Median survival time (months)</th>
<th>Range of survival time (months)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Surgery followed by nitrosylcobalamin</td>
<td>67.5 + (dog still in remission at time of publishing)</td>
<td>Not applicable</td>
<td>Bauer et al. (2009)</td>
</tr>
<tr>
<td>8 (15)</td>
<td>Surgery followed by radiotherapy and mitoxantrone chemotherapy</td>
<td>31</td>
<td>5-46.7</td>
<td>Turek et al. (2003)</td>
</tr>
<tr>
<td>15</td>
<td>Surgery followed by</td>
<td>24.4</td>
<td>&lt;6-&gt;36</td>
<td>Williams et al.</td>
</tr>
</tbody>
</table>
Anal sac adenocarcinoma has a reported recurrence rate of 45% (Bennett et al., 2001).

There are several factors that can influence the prognosis:

- **Tumor size**
  Williams et al. (2003) found that tumor size $\geq 10 \text{ cm}^2$ is associated with a shorter overall survival time than tumors smaller than $10 \text{ cm}^2$, with a median survival time of 9.6 months in the group of dogs with tumor larger than $10 \text{ cm}^2$ and 19.2 months in the group of dogs with

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Median Survival</th>
<th>Range</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiotherapy alone</td>
<td>21.6</td>
<td>&lt;6-&lt;36</td>
<td>Williams et al. (2003)</td>
</tr>
<tr>
<td>Surgery followed by melphalan chemotherapy</td>
<td>20</td>
<td>5-42</td>
<td>Emms (2005)</td>
</tr>
<tr>
<td>Surgery followed by electrochemotherapy combined with cisplatin</td>
<td>19+ (dog still in remission at time of publishing)</td>
<td>Notapplicable</td>
<td>Spugnini et al. (2008a)</td>
</tr>
<tr>
<td>Surgery alone (including lymphadenectomy)</td>
<td>19</td>
<td>4-54</td>
<td>Hobson et al. (2006)</td>
</tr>
<tr>
<td>Surgery followed by chemotherapy</td>
<td>17.8</td>
<td>&lt;6-&gt;36</td>
<td>Williams et al. (2003)</td>
</tr>
<tr>
<td>Surgery alone</td>
<td>16.4</td>
<td>&lt;6-&gt;36</td>
<td>Williams et al. (2003)</td>
</tr>
<tr>
<td>Surgery followed by chemotherapy</td>
<td>14.4</td>
<td>9-22</td>
<td>Bennett et al. (2002)</td>
</tr>
<tr>
<td>Surgery followed by chemotherapy in 50% of the cases</td>
<td>13</td>
<td>55</td>
<td>Potanas et al. (2015)</td>
</tr>
<tr>
<td>Chemotherapy alone (11 dogs received cisplatin or carboplatin at some point)</td>
<td>8.7</td>
<td>1-14</td>
<td>Bennett et al. (2002)</td>
</tr>
<tr>
<td>Palliative care</td>
<td>8.7</td>
<td>0-9</td>
<td>Bennett et al. (2002)</td>
</tr>
<tr>
<td>Surgery alone</td>
<td>7.9</td>
<td>0-41</td>
<td>Bennett et al. (2002)</td>
</tr>
<tr>
<td>Toceranib phosphate</td>
<td>7 (stable disease)</td>
<td>2.5-11 (stable disease)</td>
<td>London et al. (2011)</td>
</tr>
<tr>
<td>Chemotherapy alone</td>
<td>7</td>
<td>&lt;6-&lt;12</td>
<td>Williams et al. (2003)</td>
</tr>
</tbody>
</table>
smaller tumors. Potanas et al. (2015) however did not found a significant difference in disease-free interval or survival time in dogs with tumor length smaller than 4.2 cm or equal or larger than 4.2 cm.

- Hypercalcemia of malignancy

Bennett et al. (2002) found that there was no significant decrease in survival time in dogs with hypercalcemia. Hypercalcemia was not associated with poor response to therapy in the study by Emms (2005) either. Williams et al. (2003) however found hypercalcemia a significant predictor of survival time; dogs with hypercalcemia had a median survival time of 8.4 month while the median survival time of normocalcemic dogs was 19.2 months. Hypercalcemia can be a predictor of relapse, since it occurs often at time of relapse. (Emms, 2005)

- Metastasis

Williams et al. (2002) found that the third significant predictor of survival time was the presence or absence of pulmonary metastasis: dogs with the evidence of pulmonary metastasis had a median survival time of 7.2 months, while the median survival time of dogs without apparent metastasis was 18 months. Although Turek et al. (2003) did not find significant difference between the event free survival and the tumor staging; dogs without metastasis at presentation had longer median event free survival than dogs with metastasis at presentation. Statistical significance was not expected due to the small number of dogs (15) in the study, the difference in the median event free survival is however noticeable. Similarly, dogs with lymph node metastasis that underwent lymph node removal during surgery had a longer overall survival time, that dogs that did not. The difference was once again not statistically significant. According to Potanas et al. (2015) dogs without sublumbar lymphadenopathy had a longer disease-free interval and median survival time than dogs with the condition present prior to surgery. Williams et al. (2003) and Emms (2005) found no significant difference in survival in dogs with or without sublumbar metastasis.

- Surgical excision of the sublumbar lymph-node

Hobson et al. (2006) reported a dog with a survival time of 54 months after presentation treated with surgeries alone (including lymphadenectomy). Dogs that underwent surgical excision of the sublumbar lymph node (because of lymphadenopathy present) had a shorter disease-free interval and median survival time than dogs that did not need to undergo lymphadenectomy. (Potanas et al., 2015)

Bennett et al. (2002) reported that dogs receiving only surgical treatment had the shortest-, while dogs treated with surgery and chemotherapy the longest median survival time. There was, however, no significant difference found in the median survival time between the groups of dogs treated with surgery alone, palliative care, and surgery and chemotherapy both. The study however had a low power because of the small number of canines in the study.
According to Potanas et al. (2015) the two factors that have significant influence on the survival time: the presence (or absence) of sublumbar lymphadenopathy and the surgical removal of the lymph node are also associated with the disease free interval and so is the treatment with platinum chemotherapy. Dogs that received platinum-containing chemotherapeutic treatment had a shorter median disease-free interval than dogs that did not. This might be because of the owners of dogs with further progressed disease are more likely to choose for additional chemotherapeutical treatment after surgery. (Potanas et al., 2015)

DISCUSSION
Apocrine gland adenocarcinoma of the anal sac does not occur common in dogs, due to the infiltrative character; however, with a metastatic rate of approximately 50% at the time of diagnosis it is considered an important malignancy in canines. Recent studies do not confirm gender predilection, while certain breeds such as English cocker spaniel, Labrador and German shepherd are overrepresented. The diagnosis can be made by palpation, fine needle aspiration, blood- and urine analysis, and medical imaging. Hypercalcemia is especially important, considering the tumor is often an accidental finding. Since hypercalcemia is most often caused by malignancies in dogs, canines – especially elderly ones – presented with hypercalcemia should be examined via palpation of the anus in order to localize a potential anal sac neoplasia.

The prognosis without treatment is very poor. There are several treatment protocols described in the literature, the most common protocol is a multimodal approach of surgical excision followed by some kind of adjuvant chemotherapy. This is logical, considering the highly infiltrative nature of the tumor and the limitations of medical imaging detecting metastasis. It is also not surprising, that the second and third highest median survival time is achieved by treating with surgery followed by radiotherapy and chemotherapy: combining several therapies is more likely to result in prolonged survival; the question is whether it is necessary to combine it all, considering the combination of surgery, radiotherapy and chemotherapy is very intense and stressful for the dogs, and time consuming and expensive for the owners, not all dog owners have the possibility for it, there should be considerable alternatives for them. Furthermore, considering animal welfare it is not always the best thing for the animal to undergo such an intensive therapy, especially given the bad prognosis and short life expectancy after diagnosis.

It is difficult to compare the treatment protocols, considering there is no standardization of the protocols, follow up, neither are there control groups. Since humans and dogs have significant differences in physiology, anatomy and biochemical processes, it is also not possible to adapt treatment protocols from human medicine into veterinary medicine when it comes to the treatment of this tumor. Furthermore there is often no specific information regarding the outcome per chemotherapeutic agent in the available literature, the result of the treatment by the different agents is often summarized under chemotherapy.
Based on table 1, it is clear that a multimodal approach should be applied when it comes to the treatment of anal sac adenocarcinoma (considering the drawbacks provided above). The single treatment methods are all at the bottom of the table with the shortest medial survival times and ranges of survival times.

In case there is no willingness or possibility to apply a multimodal approach, the best single therapy is radiotherapy in case there is no distant metastasis. Considering radiotherapy is only available in special veterinary cancer centers, surgery alone is an alternative. Although Bennett et al. (2002) reports no statistical significant difference between the survival times of the different protocols (it might be, however, due to the low power of the study), the difference in medium survival time, as well as in the range of survival time is noticeable. The median survival time of treatment with palliative care alone and surgery alone seems already poor prognostically, but considering that the range of survival starts with zero months - meaning that the lives of some dogs did not get extended by even a months, makes these treatment options not appealing, especially when it comes to surgery. Williams et al. (2003) and Hobson et al. (2006) reports more appealing median survival times for treatment with surgery alone though. A possible explanation why the overall survival times differs so much when treating with surgery alone is incomplete tumor excision, the possibility of present metastasis in the regional lymph nodes without lymph node extirpation, and the use of surgery as palliative care. The prognosis of chemotherapy alone is poor as well (Bennett et al., 2002, Williams et al., 2003).

It can be concluded that as single treatment option, radiotherapy is the best option, followed by surgery (including lymphadenectomy in case metastasis present in the local lymph nodes), in case of radiotherapy is not available or wished. Chemotherapy alone should not even be considered due to the poor prognosis, side effects and costs of it. Palliative care alone (COX-2 inhibitors), however, could be used as a treatment option if the other options are not possible due to progressed disease or lack of owner willingness. Surgery should not be used as palliative care due to the poor prognosis and the elevated mortality rate during and after palliative surgeries.

A fair prognosis can be achieved by applying the multimodal approach. Since radiotherapy alone has fair prognosis, it seems logical that the number two choice of multimodal approach (after surgery followed by chemotherapy and radiation therapy) should be surgery followed by radiotherapy, with surgery followed by chemotherapy being a good alternative, in case an effective anti-neoplastic agent is chosen.

Four groups of anti-neoplastic agents are reported being used as chemotherapy in the treatment of anal sac adenocarcinoma.

The platinum-based anti-neoplastic agents are considered as standard protocol in the treatment of this tumor; hence it is surprising that there is no study reporting the treatment outcome of treatment with this group of drugs alone. Bennett et al. (2002) do report response to the treatment. Based on the studies where platinum-agents were used it is questionable why this agents are considered to be the standard treatment since even the highest survival times in the range of survival times are not
standing out. The use of electrochemotherapy combined with cisplatin should be further investigated considering the promising result of Spugnini et al. (2008a) - especially since at the time of publishing the dog was still in remission 19 months post diagnosis.

It would be interesting to learn more about the response to the treatment of this tumor with satraplatin. Selting et al. (2011) report measurable response to the treatment of various kinds of tumors, which seems promising. The oral administration route would make administration of the drug much less stressful and invasive for the dogs, and easier and less time-consuming for the owners.

From the alkylating anti-neoplastic agents melphalan can also be administered per os, making it an appealing treatment option. With a median survival time of 20 months (28 months in the group without metastasis at presentation) it seems like a reasonable option. There was no response to the treatment with cyclophosphamide combined with vincristine – a vinca alkaloid - and since there are other drugs with better prognosis available these agents should not be further investigated.

As of vinblastine – another vinca alkaloid – the one dog treated with it reached a state of stable disease after five months of treatment. There is no data available over the event free or the overall survival time. (Wouda et al., 2015) It could be interesting to follow up on the patient in order to decide whether a larger scale study should be conducted or not. Since there are other agents available with proven better prognosis, this drug should not be used as standard therapy, it could, however, be used after failure of treatment with the conventional agents used for the treatment of anal sac adenocarcinoma.

Using the anti-tumor antibiotics, there was no response to the treatment with epirubicin, actinomycin D, mithramycin, these agents should not be used in the treatment of this tumor. In case of doxorubicin the response rate was 50%. Instead of doxorubicin mitoxantrone should be used, the prognosis is promising; however since the treatment also included radiotherapy there is no data on the effect of mitoxantrone alone, this should be further investigated in a study. Still seems mitoxantrone the best option based on the current available literature.

![Figure 17: Treatment options of anal sac adenocarcinoma with the best prognosis based on available literature](image-url)
The prognosis for the treatment with toceranib phosphate does not seem very appealing, but considering that 87% of the dogs had metastasis prior to the treatment and still in 62% of the dogs the disease became stable for a period of time, the prognosis suddenly seems somewhat better. There is no data regarding the overall survival time, which underestimates the effect of this agent. Follow up of the patients should provide more clarity about the therapeutic possibilities of this agent.

The treatment outcome of the dog treated with nitrosylcobalamin is very promising. The survival time of this dog exceeds the longest reported survival time of dogs with anal sac adenocarcinoma with over a year, while the dog was still in remission at the time of publishing; in fact he might still be alive (as one of the oldest dog in the world since the study has been published in 2009). It is unfortunate, that only one dog with anal sac adenocarcinoma was included in the study – although the treatment with nitrosylcobalamin started approximately a half year post diagnosis after failure of other treatment options, which implies that it was not the mildest case. The three other dogs participating in the study with different kinds of tumors also responded well to the treatment. There is definitely a necessity of a larger scale study regarding the effects of the treatment with nitrosylcobalamin, at this moment it seems the most promising treatment option for the future, but the treatment is not available yet. Should the effect of nitrosylcobalamin be confirmed by a larger scale study than the prognosis of anal sac adenocarcinoma might even becomes good in the future. However, further research is needed.
REFERENCES