View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Edinburgh Research Explorer

THE UNIVERSITY of EDINBURGH

Edinburgh Research Explorer

Feline injection site sarcoma

Citation for published version:
Davidson, C & Lawrence, J 2016, 'Feline injection site sarcoma: current paradigms and future directions', *UK-VET Companion animal*, vol. 21, no. 5, pp. 286-292. https://doi.org/10.12968/coan.2016.21.5.286

Digital Object Identifier (DOI):
10.12968/coan.2016.21.5.286

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
UK-VET Companion animal

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 23. Jul. 2021

Feline Injection Site Sarcoma: Current Paradigms and Future Directions

ABSTRACT

Feline injection site sarcoma (FISS) is an uncommon but important tumour in cats due to its locally aggressive biologic behaviour, poor prognosis and that it is linked to routinely administered vaccinations, which raises ethical questions regarding vaccine practices. Post vaccinal granulomas are a common occurrence in cats therefore client education is vital and careful monitoring should be adopted. Incisional biopsy is recommended to confirm diagnosis of FISS and full staging is recommended prior to determination of a treatment strategy. Tumour palpation has been shown to vastly underestimate the extent of tumour infiltration and therefore prospective treatment planning with advanced imaging is highly recommended. Although further studies are required to determine the most effective combination of treatment modalities for definitive treatment, a multi-modal approach is often required, based on expeditious and aggressive surgery in combination with radiotherapy +/- chemotherapy. Importantly early detection by careful post vaccination monitoring as well as a shift in vaccination practices is key to improving the outcome of FISS.

Key Words: Neoplasms, Sarcoma, Cat Diseases: Pathology, Vaccinations, Tumour, Radiation Therapy, Surgery

INTRODUCTION

In 1991, a letter to the editor of the Journal of the American Veterinary Medical Association, written by Dr Hendrick and Dr Goldschmidt (Hendrick & Goldschmidt 1991), highlighted an increasing concern relating to a link between cat vaccination and the development of soft tissue sarcomas. Further studies supporting these claims were quick to follow; the debate within the veterinary community about this issue continues today and it remains a viable concern for both the veterinary industry and pet owners alike. While it is now suspected that feline injection site sarcoma (FISS), originally known as vaccine site-associated sarcomas, may be linked to other iatrogenic inflammatory insults such as insulin, antibiotics and steroid injections, (Kass et al 2003), feline leukaemia and rabies vaccinations have the strongest epidemiological link (Kass et al. 1993; Hendrick & Goldschmidt 1991; Coyne et al. 1997).

The true incidence and prevalence of this worldwide issue is likely an under-estimate due to under-reporting, however research has demonstrated that the incidence of sarcoma development as a result of vaccination in the United States is between 0.63-10 in every 10,000 vaccinated cats, depending on the study consulted and most commonly occurs after administration of rabies virus and FeLV (feline leukaemia virus) vaccines (Gobar & Kass 2002; Hendrick et al. 1994; Coyne et al. 1997; Lester et al.). Interestingly, in the United Kingdom, where the frequency of rabies vaccination is lower than in North America, the incidence is similar and is estimated to be between 1 in 16,000 and 1 in 50,000 cats (Dean et al. 2013).

Veterinary surgeons have an ethical duty to protect our patients from disease. Our vaccine protocols have been developed to prevent easily acquirable, life limiting conditions, and as such, in the UK, the annual booster is often accepted as gold

standard practice. In human medicine, some might consider vaccine products that have cancer causing potential at this level of risk unacceptable. In response to this concern, the Vaccine-Associated Feline Sarcoma Task Force (VAFSTF) was created, and various clinical recommendations were developed. Many of these recommendations have been adopted in North America; yet, while guidance has been published, these recommendations have not generally been adopted as standard practice in the UK.

In this article, the pathogenesis, case management and prognosis of FISS, including novel treatments and future directions, will be highlighted. Special emphasis on clinical work up and surgical planning is imperative in improving treatment outcome. Due to the considerable morbidity and expense involved in treatment coupled with the challenges associated with achieving long-term tumour control, prevention should focus on thoughtful vaccination protocols and adherence to VAFSTF recommendations.

PATHOGENESIS

In some species such as canines and humans, inflammatory associated sarcomas occur at very low rates; indeed the reported incidence of this disease in cats was also low prior to the 1980's, at which time killed adjuvanted feline vaccines were marketed. Shortly after this time the pathology department at the School of Veterinary Medicine at Pennsylvania University identified an increase in inflammatory injection site reactions in feline biopsy specimens (Hendrick & Dunagan 1991). FISS manifests most commonly in cats as fibrosarcoma histologically, although, chrondrosarcoma, malignant fibrous histiocytoma and rhabdomyosarcoma have also been reported (Hendrick & Brooks 1994). Tumours appear to originate from proliferating fibroblasts

and myofibroblasts (Hendricks and Brooks, 1994). This may be a reflection of the differing feline fibroblastic response to injury relative to other species (Eggers Carroll et al. 2002). Localised vaccine reactions are common in cats vaccinated with rabies and feline leukemia virus vaccines, with one report suggesting that up to 80-100% of cats will have a reaction to rabies vaccination (Macy & Hendrick 1996; McEntee & Page 2001; Gobar & Kass 2002; Wilcock et al. 2012). In one study, it was found that 2.7% of these post vaccinal nodules will result in malignant transformation over several months to years (Gobar & Kass 2002). It is not clear why some nodules become malignant while many others resolve, but it has been suggested that some cats may be genetically predisposed to neoplastic change (Hartmann et al. 2015). Ferrets have been reported to develop injection site sarcomas spontaneously while rodents can be experimentally induced to develop injection site sarcomas, suggesting this phenomenon is not unique to cats (Munday et al. 2003; Huggins & Grand 1963; Hendrick 2011).

One theory in FISS formation is that in a genetically 'tumour-susceptible' cat, the chronic inflammatory stimuli provided by vaccine components leads to neoplastic transformation of these fibroblastic cells (Jelínek 2003; Eggers Carroll et al. 2002). Vaccine adjuvants such as aluminium have frequently been identified within these sarcomas on histological sections and ultra-structural studies and strengthen the suspicion of an injection-related tumour (Madewell et al. 2001; Hendrick et al. 1992). Although non-adjuvanted vaccines have been associated with FISS, adjuvanted vaccines, and particularly those containing aluminium products, are known to enhance localized inflammatory reactions and therefore are frequently highlighted as a risk factor for tumour development (Day et al. 2007). Further supporting the theory that injections or vaccines are involved is the fact that tumours occur most frequently in

the sub-cutis of vaccine injection sites. Historically, tumours were more likely to occur in the interscapular region, the most common site for injection, yet there has been a statistically significant shift in tumour location to regions corresponding to recommended vaccine sites in North America (Hendricks and Brooks, 1999, Shaw et al 2009). To date, rabies and feline leukemia virus vaccines remain the injections most clearly associated with the development of FISS; it is estimated that the number of vaccines given simultaneously at the same location`n greatly increases the risk of FISS development (Kass et al. 1993).

Precise mechanisms underlying tumour formation and progression in FISS are not known although several studies have attempted to elucidate some information regarding the molecular biology of these tumours. To date, researchers have investigated receptor and non-receptor protein tyrosine kinases that are involved in cell signalling, growth, differentiation and/or survival, p53 mutations that disrupt cell death pathways, and chromosomal aberrations that may influence tumour development and progression (Smith et al. 2009; Katayama et al. 2004; Lawrence et al. 2012; Nambiar et al. 2000; Hershey et al. 2005; Banerji & Kanjilal 2006; Banerji et al. 2007; Mayr et al. 1995; Petterino et al. 2006; Thomas et al. 2009). Understanding changes and alterations in tumour cells is important as it provides possible avenues for novel treatment approaches,

PRESENTATION AND DIAGNOSTIC WORK UP

FISS is most commonly identified at the site of previous vaccination and although there is a rapid rate of growth of this aggressive, locally invasive tumour, the lag time from vaccination to tumour formation can be variable. Compared to other non-injection site related sarcomas, cats with FISS tend to be younger. Epidemiological

evidence points to two peak presentations; 6-7 years and 10-11 years (Kass et al. 1993). Recognising that many cats will develop post vaccinal nodules at the site of injection, the VAFSTF established a protocol in 1999 leading to the creation of the **3-2-1 rule** (box 1). These guidelines recommend that any mass persisting for more than **3** months after injection (vaccination), measuring more than **2** cm in diameter and increasing in size **1** month after injection should be biopsied (Morrison & Starr 2001).

For any mass that occurs at an injection site associated with a vaccination, incisional biopsy is recommended for confirmation of diagnosis. As with other soft tissue sarcomas, a wedge, punch or needle biopsy should not extend into healthy tissue in order to avoid compromising future surgical or irradiated margins. Although fine needle aspiration cytology can be performed to rule out other causes of soft tissue masses such as mast cell tumour or abscess, biopsy is recommended where sarcoma is suspected due to the frequency of false negative results with fine needle aspirates (Morrison & Starr 2001; Wilcock et al. 2012).

STAGING

Once a histological diagnosis has been made from biopsy, thorough staging should be performed in order to identify the extent of disease and aid in determining treatment options. Staging should include urinalysis, FIV and FeLV testing, haematology and biochemistry profiles to rule out concurrent diseases that may affect treatment decisions. Three view inflated thoracic radiographs and/or computed tomography (CT) should be performed to identify potential metastatic disease. The pulmonary parenchyma is the most common metastatic site although other sites such as lymph node and abdominal viscera have been reported. The overall rate of metastasis in FISS is reported to be between 10 and 28% (Couto & Macy 1998; Hendrick et al. 1994;

Hershey et al. 2000; Romanelli et al. 2008), which is higher than in other feline soft tissue sarcomas. An early paper investigating positive prognostic factors for FISS indicated that the first surgical attempt was the most effective in terms of achieving long term control (Hershey et al. 2000). While this has not been well established in more recent studies, prospective treatment planning remains vital prior to determining a definitive approach to treatment. Advanced imaging with both pre and post contrast studies (CT or magnetic resonance imaging (MRI)) is strongly recommended prior to determining optimal treatment as it has been shown that palpation vastly underestimates the true extent of the tumour (Ferrari et al. 2015). CT in particular can be used for surgical or radiation therapy planning and can provide a reasonable estimate as to whether or not surgery alone is likely to be successful or if multimodality therapy is more appropriate (Figure 1). It is also important to recognize that microchips may be associated with imaging artefact, particularly on MRI thus knowledge about the presence and location of a microchip is beneficial (SAITO et al. 2010).

TREATMENT AND PROGNOSIS

Prior to commencing treatment, as per the VASFSTF guidelines, it would be advisable to consult a veterinary oncologist for advice on the most up to date treatment recommendations based on the current literature (Box 2). Additionally, it has been demonstrated that there is a significant improvement in disease-free interval (DFI) when a complete first excision is performed at a referral institution compared to excision with a referring veterinary surgeon (274 days versus 66 days) (Hershey et al. 2000).

FISS should be treated both promptly and aggressively for the best long-term

outcome. Wide, en-bloc surgical excision, with a margin of 5cm of macroscopically healthy tissue and two fascial planes deep which therefore may include partial scapulectomy, hemipelvectomy or osteotomy of spinous processes, is the surgical treatment of choice (Phelps et al. 2011). Histopathologic assessment is important to confirm the diagnosis of FISS and to assess the margins; clean surgical margins do not guarantee long-term survival and approximately 20-30% of tumours that are cleanly excised will have recurrence (Romanelli et al. 2008; Cronin et al. 1998; McEntee & Page 2001; Phelps et al. 2011; Kobayashi et al. 2002). However, it is likely that surgical success rate is improved if complete radical surgical excision is achieved at the first surgery whereas recurrence is higher if clean margins are not achieved (Giudice et al. 2010). Notably, following wide en bloc excision, a recurrence rate of 14% was documented in a series of cats (Phelps et al. 2011), although this may reflect a number of temporal changes, such as surgical dose, surgeon experience, initial size of the tumour at diagnosis, and histopathology evaluation. Current vaccination guidelines stress the importance of vaccinating as distally as possible on a limb (below the elbow or stifle) or tail so that if a tumour develops, high amputation, potentially involving scapular or femoral disarticulation may have a higher likelihood of achieving complete margins (Hartmann et al. 2015; Shaw et al. 2009). While radical surgery is currently pursued by many veterinary oncologic surgeons, smaller, more conservative surgeries are frequently performed. The optimal surgical approach will vary with each patient and concise guidelines for all cats are not currently available. Less is known about more conservative approaches, as there has been a strong desire to achieve complete margins on histopathology. Additional knowledge of outcome and surgical dose and approach is still being garnered and may contribute to future recommendations regarding treatment.

Whilst surgery alone for some tumours may provide long term control, the majority of FISS occurring in the interscapular region are difficult to control with surgery alone and many require a combination of radiation therapy and surgery to decrease the incidence and time to recurrence (Kobayashi et al. 2002; Cronin et al. 1998; Eckstein et al. 2009). Prospective treatment planning is vital and typically involves an oncologist, radiation oncologist and surgeon. Radiation therapy can be prescribed preoperatively or postoperatively, depending on the clinical scenario and planned approach. Preoperative radiation therapy attempts to sterilise the margins of bulky disease in order to decrease the likelihood of tumour recurrence. The benefit of preoperative approaches is that a smaller treatment field is typically treated and a lower overall dose can be utilised, thus may be more tolerable for the cat (Figure 2). Alternatively, postoperative radiation therapy attempts to kill residual tumour cells following surgery but typically requires that a larger volume of normal tissue is included in the irradiated field to a higher overall dose. Both preoperative and postoperative radiation therapy are associated with similar outcomes and delay the time to tumour recurrence (Eckstein et al. 2009; Cronin et al. 1998; Romanelli et al. 2008; Bregazzi et al. 2001; Kobayashi et al. 2002). Currently there is insufficient evidence in the literature to assess the role of adjunctive chemotherapy in definitive treatment, however chemotherapy (often anthracycline-based) is currently considered to be potentially useful when postoperative radiation therapy is not available (Poirier et al. 2002; Bray & Polton 2014). More advanced radiation therapy equipment and planning software that allow for intensity modulated radiation therapy (IMRT) or stereotactic radiation therapy (SRT) may improve the ability of radiation to target tumour volumes while sparing normal tissue, thus placing more importance on the role of radiation in cats with tumours not amenable to surgery alone (Nolan et al.

2013).

A number of palliative options exist for cats with advanced injection site sarcomas where definitive treatment is not possible. Chemotherapy or palliative radiation therapy can be used as single modalities or as part of a multimodality approach. Chemotherapy drugs (Barber et al. 2014; Poirier et al. 2002; Saba et al. 2012; Kobayashi et al. 2002) such as doxorubicin alone or in combination with cyclophosphamide and CCNU have shown responses in the gross disease setting although other drugs may be tried as well given the undefined role of chemotherapy. Palliative intent radiation therapy, in which large doses of radiation are used infrequently, may improve quality of life and offer temporary tumour control in many cases (Nolan et al. 2013; Eckstein et al. 2009). Medical management with analgesia is also important as tumours can cause significant pain as they grow and invade normal structures.

NOVEL THERAPIES

As many injection site sarcomas recur in cats despite multimodality approaches, it is clear that novel treatment options are needed. FISS has been shown to express a number of dysregulated growth factor receptors, including the tyrosine kinase receptor platelet derived growth factor receptor (PDGFR), making this an attractive target. Initial in-vivo preclinical work investigating imatinib and masitinib demonstrate inhibition of PDGFR signalling, have shown some beneficial effects but require additional investigation to validate their clinical benefit and to determine their role in definitive management (Katayama et al. 2004; Lawrence et al. 2012; Turek et al. 2014; Holtermann et al. 2016).

There is interest in the use of immunotherapy following approval of a

recombinant feline IL-2 vaccine in Europe (conditional licensure in the USA) for the treatment of FISS in conjunction with surgery and radiation therapy (Jas et al. 2015; Jahnke et al. 2007; Quintin-Colonna et al. 1996). IL-2 is a cytokine known to stimulate an anti-tumour immune response and it is thought that it may upregulate cytotoxic T lymphocyte and natural killer (NK) cell activity. Preliminary work with the vaccine suggested that cats treated with recombinant feline IL-2 in conjunction with surgery and brachytherapy had longer median time to recurrence compared to cats treated with local therapy alone, however additional work should evaluate its utility when conventional external beam radiation therapy is utilised (Jas et al. 2015). As the recombinant feline IL-2 vaccine is administered intra-tumourally or via subcutaneous injection at the surgery site, there is theoretical concern regarding the use of an injection that induces inflammation in an 'FISS disposed' cat; further studies will hopefully alleviate these concerns.

PREVENTION

While novel treatments are needed to improve long-term control of FISS, the most important factor to decrease the impact FISS has on cats is prevention. In response to the concern over the link between vaccination and FISS, several strong recommendations have been published regarding frequency and site of vaccination in cats (box 3). The World Small Animal Veterinary Association (WSAVA) and the American Association of Feline Practitioners (AAFP) recommend vaccination of feline herpes virus (FHV), feline calicivirus (FCV) and feline panleukopenia virus (FPV) at an interval of 3 years and vaccination against feline leukaemia virus (FeLV) at a yearly interval only if that particular cat is at risk on contracting FeLV (Day et al. 2010; Richards et al. 2006). Therefore an indoor cat with a very low chance of

exposure to FeLV does not require or benefit from this vaccine. The European Advisory Board on Cat Diseases (ABCD) advises FeLV vaccination every 2-3 years in cats over 3-4 years old. Guidelines published by the ABCD and the AAFP recommend rabies vaccination in the right hindlimb and FeLV in the left hindlimb while all other vaccinations should be given in the right foreleg; vaccinations should be administered distal to the elbows and stifles (Hartmann et al. 2015; Richards et al. 2006) (Figure 3). Of particular importance, all guidelines recommend avoidance of the interscapular region of any injections. To date, vaccinations are still recommended while considering the risk of disease in each cat although one may muse that discontinuing vaccinations may be the most effective means of prevention.

To assess the effectiveness of this guidance, a study within the USA comparing vaccine location and incidence of FISS, before and after the VAFSTF guidance was published in 1996. Results were encouraging despite the fact that the highest incidence of FISS remained in the interscapular region, as there was a significant decrease in the incidence of interscapular FISS from 53% to 40% and a significant increase in the incidence of FISS in the right thoracic limb from 1% to 10% (Shaw et al. 2009), indicating a shift in vaccine practices. A study by Dean et al (2012) evaluated vaccine protocols used by UK veterinarians and found that despite published guidelines, most UK practices vaccinate for FHV, FPV, FCV and FeLV annually, most commonly in the interscapular region. Although distal limb can be a difficult site to vaccinate, a recent study suggested that cats not only tolerate vaccination in both locations, but also that the tail may represent an alternative site to the distal limb (Hendricks et al. 2014).

In addition to adherence to vaccine locations, efforts should be made to

facilitate early detection and reduce inflammation at the injection site. All clients should be educated at the time of vaccination on post vaccinal nodules and how to monitor the injection site. Intramuscular vaccination should be avoided if possible, as tumours that develop within muscle are more difficult to detect. Administration of a cold vaccine has been associated with higher risk of FISS (Kass et al. 2003), therefore, while vaccines need to be kept refrigerated to maintain efficacy, consideration should be given to taking feline vaccines out of the fridge 15 minutes before administration. (Hartmann et al. 2015). Finally, although any vaccine can induce inflammation that may led to malignant transformation, non-adjuvanted vaccines, modified-live or recombinant vaccines, and/or those vaccines that provide prolonged immunity should be preferred over adjuvanted, killed, or short-acting vaccines. In an effort to reduce the number of vaccinations each cat receives in its lifetime, there is an argument to consider antibody titre measurement in order to assess the immune status of each individual cat prior to vaccination but this is not currently considered standard practice.

CONCLUSIONS

It is now known that vaccination of cats, once thought to be associated with little or no risk, is not without significant risk. Development of invasive injection-site sarcomas is arguably the most serious of the reported adverse reactions in cats, despite its relatively low incidence. While we as veterinarians and veterinary nurses must continue to protect cats against life limiting, preventable conditions with vaccination, the vaccination protocol for each individual patient should be carefully thought through from a holistic viewpoint, rather than a routine procedure. Administration of any irritant injection such as a vaccination or other injectable substance should be performed only if deemed necessary and as infrequently as possible, seeking oral

alternatives where available. When vaccines or other injections are administered, the interscapular area should be avoided. It appears that there may be resistance to published vaccine guidelines in the UK but it is unclear if this observation is uniform across the UK, if it is due to lack of awareness of these guidelines or if the guidelines are viewed as impractical.

There is an array of vaccination recommendations available to practitioners, not limited to the originally published VAFSTS guidelines. These include those by the World Small Animal Veterinary Association (WSAVA), the American Association of Feline Practitioners (AAFP), the European Advisory Board on Cat Diseases (ABCD), in addition to the vaccine manufacturer's datasheets published in the National Office of Animal Health compendium (NOAH, 2016). These guidelines all differ slightly from each other and as yet there is no cohesive conclusions or standardized approach regarding frequency, type and location of vaccination. For example, the AAFP recommend that FeLV vaccination should be repeated at intervals depending on an individual cat's risk (Richards et al. 2006). The WSAVA recommend this vaccination should be repeated not more than every three years but only if there is a sustained risk of exposure (Day et al. 2010), whilst the ABCD recommends that cats over the age 3-4 years, should be re-vaccinated at a 2-3 year interval (Dean et al. 2012; Hartmann et al. 2015). On the other hand, data sheet recommendations produced by vaccination manufacturers for the majority of FeLV vaccinations available in the UK (NOAH, 2016) advise yearly re-vaccination. A survey analysing vaccine practices by UK veterinarians (Dean et al. 2012) showed that 84% of practices routinely gave FeLV vaccination annually, 1% every 2 years and no practices reported that their standard policy was to repeat FeLV vaccination every three years. The remainder of the practices surveyed decided on vaccination

frequency on an individual cat basis. Arguably, practitioners may be more inclined to follow manufacturer data sheet recommendations rather than other guidelines, particularly as these guidelines do not have unified conclusions and therefore perhaps come across as confusing. Gentle reminders to carry out thoughtful vaccination practices that include routine monitoring of post vaccinal nodules, as well as to intervene and consider referral early may help reduce the impact that injection site sarcomas have on cats in the UK.

KEY POINTS

1. Vaccine practices with specific attention to the location and frequency of vaccines should be carefully considered and protocols determined based on the cat's individual risk.
2. Careful monitoring of vaccination sites and client education should be routinely performed in practice.
3. Early intervention and adequate local control of the disease is important to treatment outcome.

REFERENCES

ABCD. The european Advisory Board on Cat Diseases. Guidelines. http://abcdvets.org/guidelines/index.asp.2012

Banerji, N. & Kanjilal, S., 2006. Somatic alterations of the p53 tumor suppressor gene in vaccine-associated feline sarcoma. *American journal of veterinary research*, 67(10), pp.1766–72. Available at: http://avmajournals.avma.org.ezproxy.is.ed.ac.uk/doi/abs/10.2460/ajvr.67.10.1766 [Accessed August 17, 2015].

Banerji, N., Kapur, V. & Kanjilal, S., 2007. Association of germ-line polymorphisms in the feline p53 gene with genetic predisposition to vaccine-associated feline sarcoma. *The Journal of heredity*, 98(5), pp.421–7. Available at: http://jhered.oxfordjournals.org.ezproxy.is.ed.ac.uk/content/98/5/421.full [Accessed March 2, 2016].

Barber, L.G. et al., 2014. Combined doxorubicin and cyclophosphamide chemotherapy for nonresectable feline fibrosarcoma. *Journal of the American Animal Hospital Association*, 36(5), pp.416–21. Available at: http://jaaha.org.ezproxy.is.ed.ac.uk/doi/abs/10.5326/15473317-36-5-416 [Accessed March 20, 2016].

Bray, J. & Polton, G., 2014. Neoadjuvant and adjuvant chemotherapy combined with anatomical resection of feline injection-site sarcoma: results in 21 cats. *Veterinary and comparative oncology*. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24502401 [Accessed September 29, 2015].

Bregazzi, V.S. et al., 2001. RESPONSE OF FELINE ORAL SQUAMOUS CELL CARCINOMA TO PALLIATIVE RADIATION THERAPY. *Veterinary Radiology <html_ent glyph="@amp;" ascii="&"/> Ultrasound*, 42(1), pp.77–79. Available at: http://doi.wiley.com/10.1111/j.1740-8261.2001.tb00907.x [Accessed September 29, 2015].

Couto, C.G. & Macy, D.W., 1998. Review of treatment options for vaccine-associated feline sarcoma. *Journal of the American Veterinary Medical Association*, 213(10), pp.1426–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9828936 [Accessed August 17, 2015].

Coyne, M.J., Reeves, N.C. & Rosen, D.K., 1997. Estimated prevalence of injection-site sarcomas in cats during 1992. *Journal of the American Veterinary Medical Association*, 210(2), pp.249–51. Available at: http://europepmc.org/abstract/med/9018362 [Accessed August 14, 2015].

Cronin, K. et al., 1998. RADIATION THERAPY AND SURGERY FOR FIBROSARCOMA IN 33 CATS. *Veterinary Radiology <html_ent glyph="@amp;" ascii="&"/> Ultrasound*, 39(1), pp.51–56. Available at: http://doi.wiley.com/10.1111/j.1740-8261.1998.tb00325.x [Accessed August 23, 2015].

Day, M.J. et al., 2007. A kinetic study of histopathological changes in the subcutis of cats injected with non-adjuvanted and adjuvanted multi-component vaccines. *Vaccine*, 25(20), pp.4073–84. Available at: http://www.sciencedirect.com/science/article/pii/S0264410X0700196X [Accessed August 16, 2015].

Day, M.J., Horzinek, M.C. & Schultz, R.D., 2010. WSAVA Guidelines for the

Vaccination of Dogs and Cats. *Journal of Small Animal Practice*, 51(6), pp.1–32. Available at: http://doi.wiley.com/10.1111/j.1748-5827.2010.00959a.x [Accessed September 6, 2015].

Dean, R.S., Pfeiffer, D.U. & Adams, V.J., 2012. Feline vaccination practices and protocols used by veterinarians in the United Kingdom. *Veterinary journal (London, England : 1997)*, 194(1), pp.113–7. Available at: http://www.sciencedirect.com/science/article/pii/S1090023312000949 [Accessed August 23, 2015].

Dean, R.S., Pfeiffer, D.U. & Adams, V.J., 2013. The incidence of feline injection site sarcomas in the United Kingdom. *BMC veterinary research*, 9(1), p.17. Available at: http://www.biomedcentral.com/1746-6148/9/17 [Accessed August 14, 2015].

Eckstein, C. et al., 2009. A retrospective analysis of radiation therapy for the treatment of feline vaccine-associated sarcoma. *Veterinary and comparative oncology*, 7(1), pp.54–68. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19222831 [Accessed August 23, 2015].

Eggers Carroll, E., Dubielzig, R.R. & Schultz, R.D., 2002. Cats Differ from Mink and Ferrets in Their Response to Commercial Vaccines: A Histologic Comparison of Early Vaccine Reactions. *Veterinary Pathology*, 39(2), pp.216–227. Available at: http://vet.sagepub.com.ezproxy.is.ed.ac.uk/content/39/2/216 [Accessed August 17, 2015].

Ferrari, R. et al., 2015. Clinical and computed tomography tumour dimension assessments for planning wide excision of injection site sarcomas in cats: how strong is the agreement? *Veterinary and comparative oncology*. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26364691 [Accessed March 2, 2016].

Giudice, C. et al., 2010. Feline injection-site sarcoma: recurrence, tumour grading and surgical margin status evaluated using the three-dimensional histological technique. *Veterinary journal (London, England : 1997)*, 186(1), pp.84–8. Available at: http://www.sciencedirect.com/science/article/pii/S1090023309002925 [Accessed February 15, 2016].

Gobar, G.M. & Kass, P.H., 2002. World Wide Web-based survey of vaccination practices, postvaccinal reactions, and vaccine site-associated sarcomas in cats. *Journal of the American Veterinary Medical Association*, 220(10), pp.1477–1482. Available at: http://avmajournals.avma.org.ezproxy.is.ed.ac.uk/doi/abs/10.2460/javma.2002.220.1477 [Accessed August 17, 2015].

Hartmann, K. et al., 2015. Feline injection-site sarcoma: ABCD guidelines on prevention and management. *Journal of Feline Medicine and Surgery*, 17(7), pp.606–613. Available at: http://jfm.sagepub.com/content/17/7/606.short [Accessed June 27, 2015].

Hendrick, M.J., 2011. Musings on feline injection site sarcomas. *Veterinary journal (London, England : 1997)*, 188(2), pp.130–1. Available at: http://www.sciencedirect.com/science/article/pii/S1090023310002376 [Accessed March 20, 2016].

Hendrick, M.J. et al., 1994. Postvaccinal sarcomas in cats. *Journal of the National Cancer Institute*, 86(5), pp.341–3. Available at:

http://www.ncbi.nlm.nih.gov/pubmed/8308925 [Accessed September 29, 2015].
Hendrick, M.J. et al., 1992. Postvaccinal Sarcomas in the Cat: Epidemiology and Electron Probe Microanalytical Identification of Aluminum. *Cancer Res.*, 52(19), pp.5391–5394. Available at: http://cancerres.aacrjournals.org.ezproxy.is.ed.ac.uk/content/52/19/5391 [Accessed August 17, 2015].
Hendrick, M.J. & Brooks, J.J., 1994. Postvaccinal Sarcomas in the Cat: Histology and Immunohistochemistry. *Veterinary Pathology*, 31(1), pp.126–129. Available at: http://vet.sagepub.com/lookup/doi/10.1177/030098589403100121.
Hendrick, M.J. & Dunagan, C.A., 1991. Focal necrotizing granulomatous panniculitis associated with subcutaneous injection of rabies vaccine in cats and dogs: 10 cases (1988-1989). *Journal of the American Veterinary Medical Association*, 198(2), pp.304–5. Available at: http://europepmc.org/abstract/med/2004997 [Accessed March 2, 2016].
Hendrick, M.J. & Goldschmidt, M.H., 1991. Do injection site reactions induce fibrosarcomas in cats? *Journal of the American Veterinary Medical Association*, 199(8), p.968. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1748617 [Accessed August 14, 2015].
Hendricks, C.G. et al., 2014. Tail vaccination in cats: a pilot study. *Journal of feline medicine and surgery*, 16(4), pp.275–80. Available at: http://jfm.sagepub.com.ezproxy.is.ed.ac.uk/content/16/4/275.full [Accessed September 24, 2015].
Hershey, A.E. et al., 2005. Aberrant p53 expression in feline vaccine-associated sarcomas and correlation with prognosis. *Veterinary pathology*, 42(6), pp.805–11. Available at: http://vet.sagepub.com.ezproxy.is.ed.ac.uk/content/42/6/805.full [Accessed September 29, 2015].
Hershey, A.E. et al., 2000. Prognosis for presumed feline vaccine-associated sarcoma after excision: 61 cases (1986-1996). *Journal of the American Veterinary Medical Association*, 216(1), pp.58–61. Available at: http://avmajournals.avma.org.ezproxy.is.ed.ac.uk/doi/abs/10.2460/javma.2000.216.58 [Accessed August 17, 2015].
Holtermann, N., Kiupel, M. & Hirschberger, J., 2016. The tyrosine kinase inhibitor toceranib in feline injection site sarcoma: efficacy and side effects. *Veterinary and comparative oncology*. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26762970 [Accessed February 16, 2016].
Huggins, C. & Grand, L.C., 1963. Sarcoma Induced Remotely in Rats Fed 3-Methylcholanthrene. *Cancer Research*, 23(3), pp.477–480. Available at: http://cancerres.aacrjournals.org/content/23/3/477.abstract [Accessed March 20, 2016].
Hüttinger, C. et al., 2008. Neoadjuvant gene delivery of feline granulocyte-macrophage colony-stimulating factor using magnetofection for the treatment of feline fibrosarcomas: a phase I trial. *The journal of gene medicine*, 10(6), pp.655–67. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18338834 [Accessed August 23,

2015].
Jahnke, A. et al., 2007. Intra-tumoral gene delivery of feIL-2, feIFN-gamma and feGM-CSF using magnetofection as a neoadjuvant treatment option for feline fibrosarcomas: a phase-I study. *Journal of veterinary medicine. A, Physiology, pathology, clinical medicine*, 54(10), pp.599–606.
Jas, D. et al., 2015. Adjuvant immunotherapy of feline injection-site sarcomas with the recombinant canarypox virus expressing feline interleukine-2 evaluated in a controlled monocentric clinical trial when used in association with surgery and brachytherapy. *Trials in Vaccinology*, 4, pp.1–8.
Jelínek, F., 2003. Postinflammatory sarcoma in cats. *Experimental and toxicologic pathology : official journal of the Gesellschaft für Toxikologische Pathologie*, 55(2-3), pp.167–72.
Kass, P.H. et al., 1993. Epidemiologic evidence for a causal relation between vaccination and fibrosarcoma tumorigenesis in cats. *Journal of the American Veterinary Medical Association*, 203(3), pp.396–405.
Kass, P.H. et al., 2003. Multicenter case-control study of risk factors associated with development of vaccine-associated sarcomas in cats. *Journal of the American Veterinary Medical Association*, 223(9), pp.1283–1292.
Katayama, R. et al., 2004. Imatinib mesylate inhibits platelet-derived growth factor activity and increases chemosensitivity in feline vaccine-associated sarcoma. *Cancer chemotherapy and pharmacology*, 54(1), pp.25–33.
Kobayashi, T. et al., 2002. PREOPERATIVE RADIOTHERAPY FOR VACCINE ASSOCIATED SARCOMA IN 92 CATS. *Veterinary Radiology <html_ent glyph="@amp;" ascii="&"/> Ultrasound*, 43(5), pp.473–479.
Lawrence, J. et al., 2012. Masitinib demonstrates anti-proliferative and pro-apoptotic activity in primary and metastatic feline injection-site sarcoma cells. *Veterinary and comparative oncology*, 10(2), pp.143–54.
Lester, S., Clemett, T. & Burt, A., Vaccine site-associated sarcomas in cats: clinical experience and a laboratory review (1982-1993). *Journal of the American Animal Hospital Association*, 32(2), pp.91–5.
Macy, D.W. & Hendrick, M.J., 1996. The Potential Role of Inflammation in the Development of Postvaccinal Sarcomas in Cats. *Veterinary Clinics of North America: Small Animal Practice*, 26(1), pp.103–109.
Madewell, B.R. et al., 2001. Feline Vaccine-associated Fibrosarcoma: An Ultrastructural Study of 20 Tumors (1996-1999). *Veterinary Pathology*, 38(2), pp.196–202.
Mayr, B. et al., 1995. Mutations in tumour suppressor gene p53 in two feline fibrosarcomas. *British Veterinary Journal*, 151(6), pp.707–713.
McEntee, M.C. & Page, R.L., 2001. Feline Vaccine-Associated Sarcomas. *Journal of Veterinary Internal Medicine*, 15(3), pp.176–182.
Morrison, W.B. & Starr, R.M., 2001. Vaccine-associated feline sarcomas. *Journal of the American Veterinary Medical Association*, 218(5), pp.697–702.
Munday, J.S., Stedman, N.L. & Richey, L.J., 2003. Histology and immunohistochemistry of seven ferret vaccination-site fibrosarcomas. *Veterinary pathology*, 40(3), pp.288–93.
Nambiar, P.R. et al., 2000. Mutational analysis of tumor suppressor gene p53 in feline vaccine site-associated sarcomas. *American Journal of Veterinary Research*, 61(10), pp.1277–1281.
Nolan, M.W. et al., 2013. Stereotactic body radiation therapy for treatment of

injection-site sarcomas in cats: 11 cases (2008-2012). *Journal of the American Veterinary Medical Association*, 243(4), pp.526–31.
Petterino, C. et al., 2006. Immunohistochemical study of STAT3 expression in feline injection-site fibrosarcomas. *Journal of comparative pathology*, 134(1), pp.91–100.
Phelps, H.A. et al., 2011. Radical excision with five-centimeter margins for treatment of feline injection-site sarcomas: 91 cases (1998–2002).
Poirier, V.J. et al., 2002. Liposome-Encapsulated Doxorubicin (Doxil) and Doxorubicin in the Treatment of Vaccine-Associated Sarcoma in Cats. *Journal of Veterinary Internal Medicine*, 16(6), pp.726–731.
Quintin-Colonna, F. et al., 1996. Gene therapy of spontaneous canine melanoma and feline fibrosarcoma by intratumoral administration of histoincompatible cells expressing human interleukin-2. *Gene therapy*, 3(12), pp.1104–12.
Richards, J.R. et al., 2006. The 2006 American Association of Feline Practitioners Feline Vaccine Advisory Panel report. *Journal of the American Veterinary Medical Association*, 229(9), pp.1405–41.
Romanelli, G. et al., 2008. Analysis of prognostic factors associated with injection-site sarcomas in cats: 57 cases (2001-2007). *Journal of the American Veterinary Medical Association*, 232(8), pp.1193–9.
Saba, C.F., Vail, D.M. & Thamm, D.H., 2012. Phase II clinical evaluation of lomustine chemotherapy for feline vaccine-associated sarcoma. *Veterinary and comparative oncology*, 10(4), pp.283–91.
SAITO, M. et al., 2010. Evaluation of the Susceptibility Artifacts and Tissue Injury Caused by Implanted Microchips in Dogs on 1.5 T Magnetic Resonance Imaging. *Journal of Veterinary Medical Science*, 72(5), pp.575–581.
Shaw, S.C. et al., 2009. Temporal changes in characteristics of injection-site sarcomas in cats: 392 cases (1990-2006). *Journal of the American Veterinary Medical Association*, 234(3), pp.376–80.
Smith, A.J., Njaa, B.L. & Lamm, C.G., 2009. Immunohistochemical expression of c-KIT protein in feline soft tissue fibrosarcomas. *Veterinary pathology*, 46(5), pp.934–9.
Thomas, R. et al., 2009. Microarray-based cytogenetic profiling reveals recurrent and subtype-associated genomic copy number aberrations in feline sarcomas. *Chromosome research : an international journal on the molecular, supramolecular and evolutionary aspects of chromosome biology*, 17(8), pp.987–1000.
Turek, M. et al., 2014. Masitinib mesylate does not enhance sensitivity to radiation in three feline injection-site sarcoma cell lines under normal growth conditions. *Research in veterinary science*, 96(2), pp.304–7.
Wilcock, B., Wilcock, A. & Bottoms, K., 2012. Feline postvaccinal sarcoma: 20 years later. *The Canadian veterinary journal. La revue vétérinaire canadienne*, 53(4), pp.430–4.

FIGURE LEGENDS

Figure 1: CT images illustrating the infiltrative nature of injection site sarcoma. (A) represents a rapidly growing, deeply invasive sarcoma that developed in the interscapular region. Note the proximity to critical structures such as the heart and lung. (B) represents a tumour located on the proximal hindlimb but due to its size and proximal location, amputation without hemipelvectomy would not achieve good control.

Figure 2: Sagittal (A) and axial (B) CT images used for radiation treatment planning with dose colour wash displayed. Radiation dose is represented by various colours with red indicating the prescribed dose of radiation, with a transition to yellow, green and blue as radiation dose diminishes. Note the excessively large size of the infiltrative tumour and the normal structures (spinal cord, lungs) that are irradiated due to their proximity to the tumour volume. Cats with extensive disease such as this may benefit from preoperative radiation therapy in order to reduce the overall radiation dose to normal tissues.

Figure 3: Recommended vaccination locations for common feline vaccines based on AAFP and ABCD guidelines.

Figure 1 A

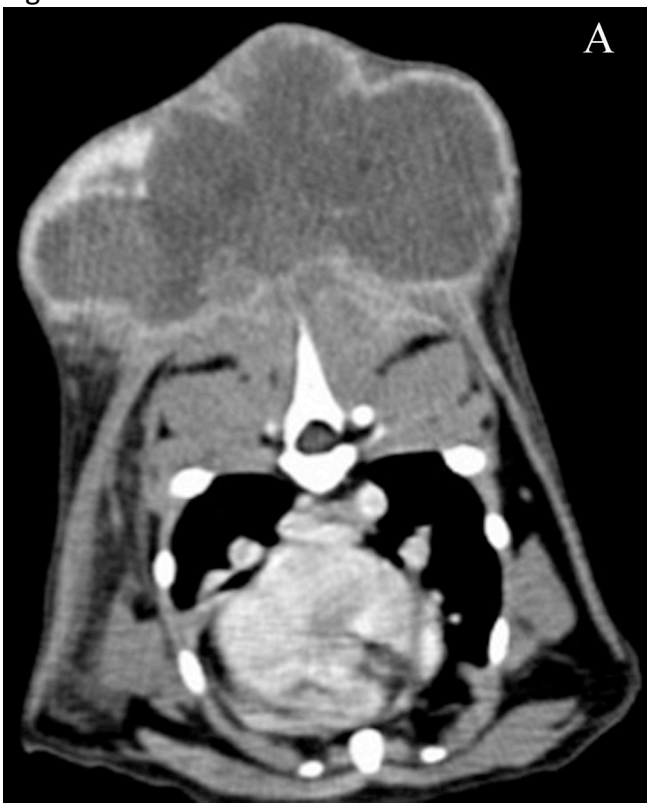


Figure 1 B

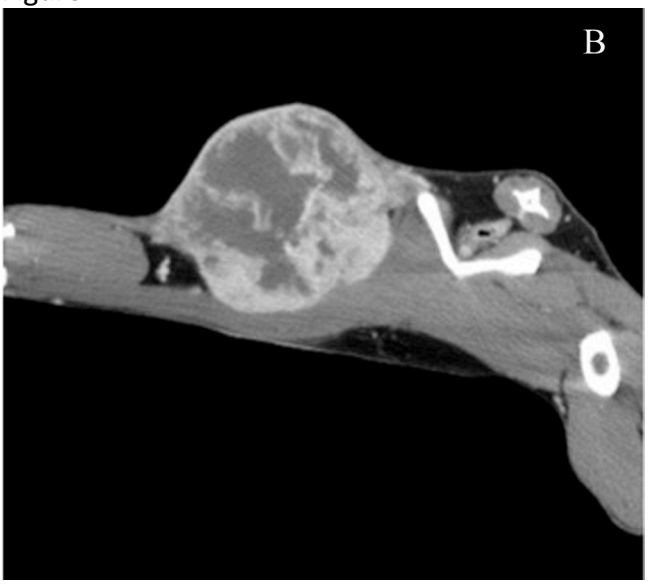


Figure 2 A

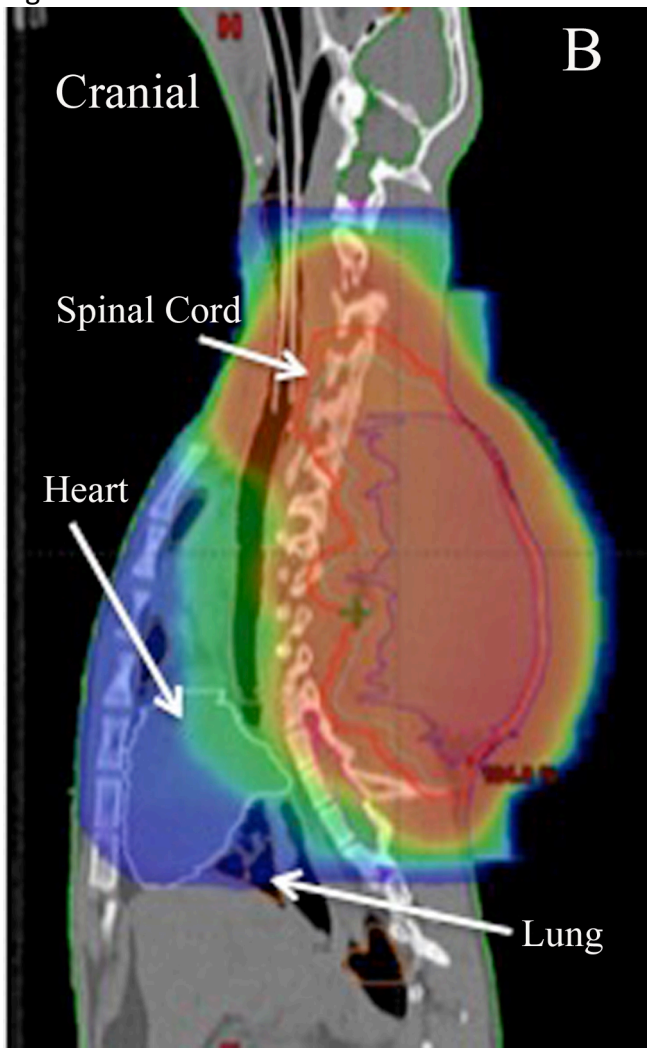


Figure 2 B

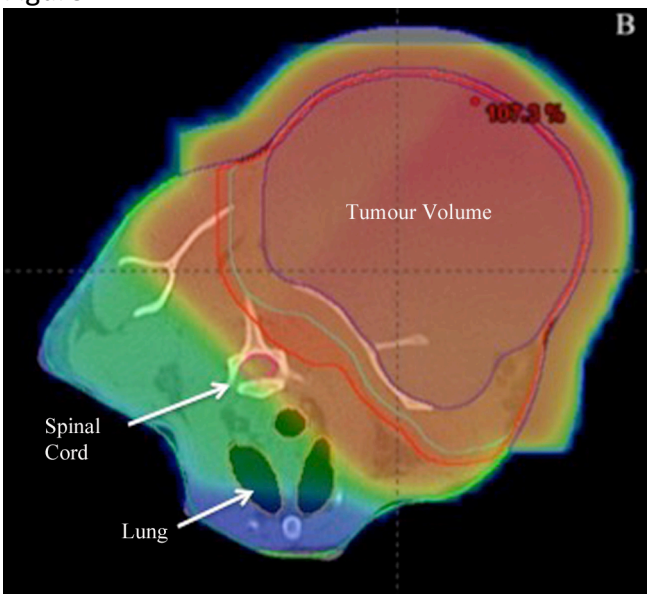
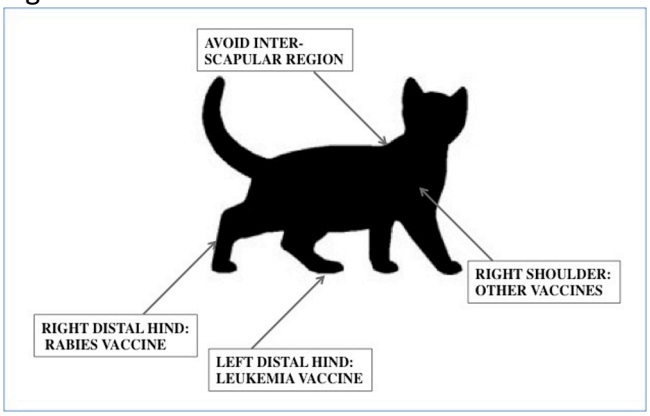


Figure 3


AVOID INTER-
SCAPULAR REGION
RIGHT SHOULDER:
OTHER VACCINES
RIGHT DISTAL HIND:
RABIES VACCINE
LEFT DISTAL HIND:
LEUKEMIA VACCINE