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The organising committee wish to thank all those persons who helped review the submitted abstracts, judged the Residents' competitions, moved chairs, carried things and generally made the smooth running of the conference possible.

And finally many thanks to the printers and staff of the congress venue

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Message from the President

Dear colleagues and friends

It is my pleasure and honor to welcome you to the 15th Annual ESVONC congress in Frankfurt, Germany.

This is a homecoming for ESVONC since the first ESVONC meeting was held in Hofheim-am-Taunus, 15 years ago in the basement of Tierklinik Hofheim. You will enjoy German hospitality and efficiency, sauced with fun, food, beer and wine.

This year's conference will provide a variety of interesting programs and speakers. The team has worked extensively to shape a superb scientific program, in line with former years, with 3 Themed Sessions (**histiocytic tumors, inflammation and cancer and clinician-pathologist interaction**). We will have 25 oral research abstracts and as usually a poster session with 27 poster communications. The main congress will again be preceded by a resident workshop focused on supplementing the education of residents and the topic this year will be **radiation therapy and photodynamic therapy** with the participation of Carla Rohrer, Jerome Benoit and Julia Buchholz on Wednesday 23rd May.

New to this year's meeting ESVONC will have a technician's day! Thousands of vet tech's assist in treating animal cancer patients on a daily basis. We look forward meet and interact with many oncology-oriented technicians from around Europe. We feel there's a need for good education to help the many animals with cancer.

The local committee with Martin Kessler and Sandra Kuehnel has made all the efforts for this congress to be a memorable event and we would like to thank them for such a fantastic job. The congress will be celebrated with a **Welcome Reception** on a boat on the river Main and with a **Gala Dinner** and dance party at the lovely Palmengarten. We hope you will enjoy this social events. It will be an excellent opportunity for networking and to have fun!

We are very pleased that you can join us and help us to make this Congress a scientific and social success. We are looking forward to meet you (again) in Frankfurt, in May 2019!

Ana Lara
President of ESVONC

Without our sponsors it would be impossible to organise a conference like this!
On behalf of ESVONC, the Congress Committee wishes to express sincere gratitude.

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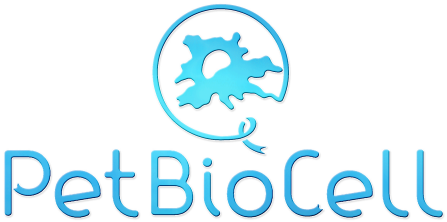

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ESVONC Congress 2019

Frankfurt, Germany, 23-25 May 2019

Radiation biology for medical oncologists

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After a radiation insult, most cells die a *reproductive cell death*, which is usually mitotic, meaning that the cells die during or after mitoses. Unlike a necrotic cell death, the mitotic cell death is not accompanied by explosively grave inflammation, swelling and necrosis. Instead, an often rather slow, continuous regression of the tumor has been observed.[1] As a result, less aggressive tumors proliferating slowly often take several months to regress after tumor therapy. This response to radiation is referred to as *"radiosensitivity"*. Since tumor cells generally divide more rapidly than the surrounding normal tissue, a local radiation dose is capable of completely destroying tumor cells while the surrounding tissue is able to recover. To maximize this effect, the entire radiation dose is not administered at once but in several fractions.
Kinetics of tissue damage: In principle, the damage of normal tissue in the radiation field depends on the tissue type and therefore the tissue-specific cell division frequency (*tissue-specific tolerance* dose). Hematopoietic and epithelial tissue, which divides and regenerates rapidly, reacts early, within days or weeks. In contrast, radiation damage to connective and supporting tissue and to neuronal tissue occurs much later with a latency of months or years.

Tolerance doses for different tissues: The 4 R's of radiobiology: The likelihood of achieving the desired local tumor control after radiotherapy treatment depends on several factors. Prof. Withers summarized these factors in the 1970s as the *"4 R's" of radiotherapy* : Repair, reoxygenation, repopulation, reassortment (cf. Table 1).[2] The radiosensitivity of tumors varies and depends on several factors. For example, hypoxic tumors (= tumors with low oxygen content) are less susceptible to radiation whereas tumors with rapid cell division, which is often associated with poor repair capacity, are somewhat more susceptible to radiation.[3]

Table 1: The 4 R's of radiation biology: Factors which occur during and/or between radiation fractions and impact tumor control and response in normal tissue. (adapted as per Gillette et al. 1995)[4]

ê reduces tumor control or normal tissue tolerance
é enhances tumor control or normal tissue tolerance
è no influence on radiation effect

	Tumor control	Normal tissue tolerance	Effect on tumor and normal tissue and consequences for therapy
Repair	ê	é	A radiation dose of 1 Gy induces about 2,500 base damages and 40 double-strand breaks per cell. The majority of the radiation-induced damages can be repaired within 6 to 24 hours. This is very desirable in normal tissue but not in tumor tissue. This constitutes the rationale for the fractionation strategy: The normal tissue is permitted to repair itself, but for the tumor cells to have as little time as possible to repair their radiation damage, the radiation series is administered within a short time (daily or twice daily).
Reoxygenation	é	è	The effect of ionizing radiation is enhanced by the presence of oxygen in the tissue: The interaction of the radiation with oxygen damages the DNA more severely due to the formation of oxygen radicals (peroxides). Hypoxic tumors are therefore less radiosensitive. The fractionation of the radiation dose improves the oxygen supply in the tumor tissue between fractions, which causes a better response to the radiation therapy.
Repopulation	ê	é	During fractionated radiotherapy, tumor cells and normal tissue cells continue to divide. This repopulation is desirable for normal tissue cells so that the acute radiation reactions can recover quickly, but not for tumor cells. In order to avoid heavy repopulation in the tumor, a series of fractionated radiotherapy treatments must not be interrupted or prolonged, if possible.
Reassortment; cell cycle effects	é	ê	The radiation sensitivity of a cell not only depends on the tissue type but also on its position in the cell cycle. Cells in the S-stage of the cell cycle are significantly less sensitive than cells in the G2/M-stage, just prior to mitosis, for example. The fractionation of the radiation dose helps here as well because the tumor cells quickly pass through the cell cycle due to their high rate of division, increasing the probability of hitting and killing them during sensitive cycle phases.

Fractionation: The DNA damage caused by radiotherapy is repaired less effectively in tumor cells than in normal cells. Since the repair (or what was still possible to repair) is usually completed within 6 to 24 hours after irradiation, a division of the total dose into individual fractions (= *fractionation*) can take advantage of the difference in reparability between tumor and normal tissue. During fractionation, the total dose can be significantly increased, consequently improving tumor control while increasing the maximum tolerable total dose of normal tissue many times over.

The calculation of the biological effect of different fractionation schemes can be performed mathematically, for example on the basis of the linear-quadratic model.[5, 6] Small fractions especially protect late-reacting normal tissue, such as bones, kidneys or spinal cord, so that protocols with high fraction numbers are mostly used in these regions (15-25 fractions, daily, administered over 4-5 weeks). The individual doses of the respective fractions are low and range from 2 to 3.5 Gy. Accordingly, the total doses are quite high and usually range from 48 to 58 Gy for curative protocols. In contrast, only a few (1-5) fractions with high single doses of 4 - 10 Gy are used in palliative protocols (= *hypofractionation*). The application frequency differs depending on the protocol but generally ranges between one and three fractions per week. Palliative protocols are usually selected if, due to advanced tumor disease, a curative approach is not promising or if life-limiting geriatric co-morbidities exist. Since only a short survival time can be expected in such cases, the long-term consequences of palliative radiation are not a matter of consideration. Logistical or financial considerations may also play a role in the choice of the protocol. However, the disadvantages of hypofractionation should be discussed with the owner during a consultation. One exception are protocols which apply high or very high fractional doses of 4 - 15 Gy, administered with extremely high precision with a curative intention (SRT, SBRT). These techniques are appealing for well-defined, non-invasive tumors because they can deliver a curative dose of radiation in a few single sessions.

Summarized and quoted from

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Techniques in radiation therapy

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In traditional 3D-CRT, target volume dose distribution is mostly created by a small number of beam portals. These can only be modified with few available parameters, such as beam direction, beam aperture and the presence of beam shaping modifiers such as wedges and collimators. Resulting absorbed dose is then computed, and the beam attributes iteratively modified by the planner. This forward-planning approach, together with the physical properties of photon radiation beams, automatically leads to more or less homogenous dose distributions. In IMRT on the contrary, sophisticated computer algorithms allow for a more complex array of multiple, individual beams that vary in intensity.[1, 2] This variation in intensity leads to the massive benefit that the dose to adjacent normal tissue structures can be minimized. Technically, this different approach uses an “inverse” treatment planning of the radiation dose. In (inverse) intensity-modulated treatment planning (IMRT), the planner chooses the number of fields and gantry/ collimator angle and sets certain prioritized goals (e.g. constraints) for target volumes and organs at risk (OAR). The optimizing planning algorithm tries to meet those goals by varying the intensity (fluence) of each beam across the target (tumor). During optimization, an iterative computer-based algorithm forces the various intensities of individual beams back into a homogenous dose distribution in the region of the tumor.[3, 4] As initial step of treatment planning, the volumes of interest are defined using cross-sectional imaging modalities such as computed tomography (CT), magnetic resonance imaging (MRI) or even functional imaging with positron emission tomography (PET). The gross tumour volume (GTV) depicts position and extent of gross tumour, i.e. what can be seen, palpated or imaged. The clinical target volume (CTV) contains the GTV, plus a margin for sub-clinical or microscopic disease spread, which cannot be fully imaged. The planning target volume (PTV) includes the former two and allows for uncertainties in positioning, planning or treatment delivery. Hence, this geometric concept ensures that the radiotherapy dose is actually delivered to the CTV. The institution’s patient position verification and correction strategies determine the extent of PTV margin for each area of treatment. The margin accounts for geometric systematic and random errors in radiation field placement, inter- and intra-fractional organ variations and hence movement of the surrounding anatomy such as variable bowel and bladder filling status. Organs at risk (OAR) are anatomically defined and often include a margin of error (movement) resulting in a planning organ-at-risk volume (PRV).

In the last two decades, radiation oncologists have embraced IG-IMRT as a worthwhile approach for the treatment of various types of cancer, such as prostate, head and neck, vertebral tumors as well as some brain cancers.[5-10] Most likely, these tumors do not respond differently to IMRT as a technique *per se*. However, the technical advantage of image-guided (IG)-IMRT enhances precision and accuracy, thereby resulting in reduced toxicity, shifting the therapeutic ratio. The therapeutic index can increase with IMRT in two ways: First, if reduced toxicity improves the patient’s quality of life and second, if the oncologist in consequence manages to escalate radiation dose (or chemotherapy dose for that matter) while maintaining acceptable toxicity. In the last decade, first original publications using IMRT emerged in veterinary medicine.[11-24] As

expected, several of these publications show marked decrease in side effects associated with tumor in complex areas e.g. canine sinonasal tumors.[15, 17, 24, 25] IMRT has been shown to provide superior dose distribution for organs at risk, compared to 3D-CRT. Tumors do not respond differently to IMRT, but a superior organ at risk distribution has the potential to increase the therapeutic ratio, possibly increasing tumor control at constant or even reduced side effects to normal tissues.[26]

Radiation therapy has always been guided by images and many aspects of the treatment process make use of imaging modalities, starting at tumour diagnosis and staging of the disease, treatment simulation and radiation therapy planning, patient positioning (setup), tumour localization and, in a last step, assessment of tumour response to treatment. Hence, image registration and data fusion are used in all stages of the patient management processes: for initial diagnosis and staging, during treatment planning and delivery and after therapy.
CT-based treatment planning and treatment verification make regular use of different types and combinations of imaging. The inclusion of modern imaging modalities incorporating functional or biological information into target delineation has been described in veterinary medicine.[27-29] While the term “image-guided radiotherapy” (IGRT) includes the functional and biological aspect of tumour tissue, the classical, daily use in veterinary medicine focuses on the use of imaging to adjust for positioning errors, adjust for target variations or motion and even, in some cases, to adapt treatment to tumour response. The goal of IGRT is to improve accuracy in radiation therapy and thereby reduce normal tissue toxicity. By improving the accuracy of the radiation field placement, the applied margins for uncertainties can be reduced resulting in lower side effects or allowing for increased radiation dose to the tumour. The reduction of the planning target volume margins is a very important area of improvement for clinical radiotherapy strategies such as 3D-conformal radiotherapy, but also for other techniques such as intensity-modulated radiotherapy (IMRT) and stereotactic (body) radiotherapy (SRT, SBRT).

To conclude, the today-used treatment delivery for radiation therapy provides improved geometric and dosimetric precision in the treatment of localized tumours. Especially for plans with sharp dose gradients or a moving target this verification technique reduces margins and hence toxicities to surrounding normal tissues. Nevertheless, an institutional protocol for correction strategies accounting for the various uncertainties in the treatment process and the individual technical specifications and limitations of the equipment must be developed in order to make best use of the technologic advantages that are provided with the various levels of IG-IMRT.

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Brachytherapy - Plesiotherapy « when Shorter means Better »

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It only took a couple of years after the discovery of radioactivity by Henri Becquerel (Uranium, 1896) and Pierre and Marie Curie (Polonium, Radium 1898) for Dr. Danlos to first describe the use of « curie »-therapy (brachytherapy = BT) with radium salts (Paris, 1901).
« the insertion of radioactive matierials into tumours make them shrink »
Along with external beam radiotherapy (EBRT), BT continued to develop over the years and is still routinely used in human medicine for the treatment of specific indications / locations. There is no competition between EBRT and BT, they are 2 different radiation modalities with their pros and cons. EBRT will find to be a lot more flexible (large volumes, deep inaccessible locations, not implant-dependent) and will certainly allow for the treatment of most RT indications. However, when the indication is « right », BT may bring a therapeutic benefit over EBRT by its ability to deliver high RT doses to target volumes with only little irradiation to the surrounding tissues.
Hence BT finds its indications in humans for some accessible tumours surrounded by critical organs at risk (cervix, uterus, prostate); or for radioresistant tumours as a boost to EBRT. In veterinary medicine, BT remains seldomly used due to the lack of availability of this equipment and the limited experience in small and large animals.
Brachytherapy sources are typically gamma-emitting radioactive materials including Iridium-192, and Cobalt-60. Gamma-rays are photons produced by radioactive decay. They are physically identical to X-Rays (produced by electric generators).
Most of our veterinary literature reflects the use of Low Dose Rate BT (LDR). This technique involves the use of non-complex implants (needles placed according to a specific pattern) with a LDR radioactive source being inserted for the entire treatment time (4-5 days usually). Planning is rather simple yet poorly conformal and there are radioprotection issues for the clinical staff (manual placement of the "wire" sources into the needles; radioactive patient during boarding vs. nursing).
LDR BT has now been replaced by High Dose Rate techniques in many institutions. Our literature does not currently cover much this evolution. HDR BT relies on an after- loader, which will be connected to the implants for each treatment fraction (eg. 5 to 10 fractions). A small seed-size source will then be directed in each applicator where it will progress (mm by mm) in order to cover the target volume. HDR allows for CT planning and more conformal 3D planning. Thanks to HDR techniques, the clinical staff no longer handles the radioactive sources. Finally, the patient is not radioactive during boarding.
Plesiotherapy is a form of brachytherapy using a beta-emitting radioactive material (beta only), such as Strontium-90. Beta particles are electrons produced by radioactive decay. These low energy electrons have a short range of penetration and can be used for the treatment of very superficial lesions (mm).
In the veterinary literature, reported indications of BT include equine sarcoids and other cutaneous tumours. St-90 plesiotherapy has been mainly reported for small cutaneous lesions such as nasal planum SCC, and MCT.
We can still learn a lot from the use of brachytherapy in human medicine. If the technology is available in some veterinary practices, its use could potentially be considered for wider indications than what has been published in the past at the time of simple LDR brachytherapy. Indeed, HDR allows 3D planning and the treatment of lesions in various locations. Newer indications (institution specific) may include BT as a sole RT modality for FISS (adjuvant to surgery), lower urinary tract TCC (on macroscopic disease), small cutaneous tumours (MCT, SCC). BT may also be considered for a boost to EBRT for neoplasia such as FISS, nasal tumours.

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Photodynamic therapy in veterinary oncology: principles and applications

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The principle behind PDT lies in the interaction of a photosensibilisator with light and endogenous molecular oxygen. Ideally, the photoactive drug is selectively accumulated within the tumor and will then be activated by light of a specific wavelength. The wavelength has to match the absorption maximum of the photosensitizer that is used. The combination of light, a photoactive drug and oxygen will lead to a photochemical reaction. PDT can result in tumor cell destruction through several ways: direct tumor cell destruction, destruction of tumor vessels, and modulation of the immune system. The Federal Food and Drug Administration (FDA) approved PDT as an alternative form of therapy against cancer in 1995, and since then, PDT has increasingly been used in some areas of human oncology. In addition to the photodynamic therapeutic effect that occurs after light administration, photosensitizers can also be used to detect tumors through fluorescence. In veterinary medicine preliminary results of sentinel lymph node mapping of invasive urinary bladder cancer are available (Knapp et al. 2007). In the field of neurosurgery PDT is used for diagnostic and therapeutic purposes, mainly using 5-aminolevulinic acid (5-ALA), a pro-drug of the photosensitizer protoporphyrin IX (PPIX). The topical application of 5-ALA is also approved for several therapeutic indications in dermatology. The disadvantage of topical PDT in oncology is a limited penetration of the photosensitizer until up to 3-5 mm only. In general, the poor penetration into tissue (due to photosensitizer and/or light) is one of the main limitations for treating tumors with PDT. Therefore mainly superficial tumors needing treatment up to 1.5-2 cm depth are amenable to PDT, with the exception being interstitial PDT or a combination of PDT with prior debulking surgery. With systemic PDT the penetration depth is increased compared to topical application and significant efforts have been invested in the development of sensitizers to optimize the treatment. The aim concerning light delivery is to apply the light, in general laser light, in a homogeneous way to the entire tumor. There are various application modes, with the illumination from the surface using a front lens being most common. In body cavities there are several ways of delivering the light, such as intracavitary-/intraluminal superficial or interstitial illumination. Dose planning with these variants is more complicated mainly due to the back-scattering from the opposite organ walls. Flexible applicators would facilitate homogeneous illumination in, for instance, the oral cavity, the bladder or illumination of tumor beds after debulking surgery.
Practical advantages, especially in veterinary medicine compared to other treatment options for localized tumors, such as surgery and radiation therapy are: being non-invasive, often a single treatment is sufficient and it is possible on an outpatient basis, repeatable if necessary, few side effects, little or no scar formation.

Feline superficial SCC / carcinoma in situ still represents the main indication in veterinary medicine. The established standard therapies are surgery and radiation therapy. Several studies have shown the efficacy of PDT to treat feline squamous cell carcinoma (Bexfield et al. 2008, Buchholz et al. 2005 and 2007, Stell et al. 2001, Lucroy et al. 1999, Magne et al. 1997; Peaston et al. 1993).
Bowen's disease represents an own entity defined as SCC in situ (not penetrating the basal membrane) but although being superficial they are often more difficult to control with PDT and they can cause multiple lesions.
In a study by Magne et al. 51 cats with cutaneous SCC received the photosensitizer Pyropheophorbid-alpha-hexyl-ether (HPPH-23) intravenously. Forty-nine percent of the cats showed a complete tumor remission, 12% showed partial tumor remission and 39% did not respond to PDT. There was a significant correlation between complete remission and length of local tumor control with tumor stage: the smaller and less invasive the tumors were, the better they responded to PDT (Magne et al. 1997).
Stell et al. achieved a complete remission rate of 85% using topical ALA-PDT for superficial feline

SCC. As light source they used a LED instead of a laser. After a median time of 21 weeks they had a recurrence rate of 64% though (Stell et al. 2001). In a second study using the same protocol a recurrence could be seen in 51% of the cats having a complete response after PDT with a median time to recurrence of 157 days (Bexfield et al. 2008).
In another study also using LEDs as light source and the hematoporphyrinderivate Photogem® as photosensitizer for small non-invasive tumors, satisfactory results could be obtained (Ferreira et al. 2009). Reeds et al. used the photosensitizer HPPH with a LED to treat non-invasive carcinoma in dogs and cats. Eight out of the nine treated tumors showed a complete remission and > 50% of the patients did not show tumor recurrence for the follow-up time of 68 weeks (Reeds et al. 2004).
Due to the generally unsatisfactory results while treating more invasive tumor stages, a study was conducted to find out if increasing the fluence rate would ameliorate the results treating invasive stages. Cats with invasive SCC, treated with PDT using Aluminiumphthalocyanin-Tetrasulphonat ($AlPcS_4$) as photosensitizer, showed a significantly shorter time of median remission using 100 J/cm^2 (n=8; 69 days; range 0–619 days) than using 200 J/cm^2 (n= 6; 522 days; range 151–1057 days) (Hahn et al. 1998).
A very potent photosensitizer that is approved for the treatment of head and neck cancer in humans is *m*THPC. In one study a conventional lipophilic formulation of *m*THPC (Foscan®) and a new liposomal formulation (Fospeg) was used in cats with SCC to determine their pharmacokinetic behavior. In 10 cats, *in vivo* fluorescence intensity measurements of tumor and skin were performed with a fiber spectrophotometer after intravenous injection of *m*THPC (either Foscan® or Fospeg). Fluorescence intensities, fluorescence ratios (tumor fluorescence divided by skin fluorescence) and bioavailability in the tumor were 2 to 4 times higher with Fospeg compared to Foscan®. Stage, tumor location, pre-PDT VEGF levels and presence of side effects could not be established as prognostic indicators. All cats responded to therapy, with a complete response rate of 93%. The tumor recurrence rate was 39% with a median time to recurrence of about 18 months. Local control could be achieved in 62% of patients at one year post therapy. Eighty-three percent of the tumors treated with the lipophilic formulation and 27% of the tumors treated with the liposomal formulation showed tumor recurrence. We concluded that the favorable pharmacokinetics of the liposomal drug seem to translate into superior tumor control (Buchholz et al. 2005).
In a subsequent study 20 cats were treated with the liposomal formulation only. Following the results of the pharmacokinetic study, cats were treated with laser light (wavelength of 652 nm) 4-6 hours after injection. The patients showed an initial complete response rate of 100% and tumor control in 75% after 1 year. The recurrence rate was 20% with a median time to recurrence of 172 days. The generalized light sensitivity of the patient lasts about 10-14 days (Buchholz et al. 2007). The same photosensitizer has been used in a current study to treat feline cutaneous squamous cell carcinoma as well (Flickinger et al. 2018).

Transitional cell carcinoma of the urinary bladder may represent a promising indication in veterinary medicine, In human medicine, PDT has been successfully used for bladder carcinoma (Nseyo et al. 1993, 1998). A group in the Netherlands is working on PDT for TCC in dogs (personal communication (A.Roos) and presentation at PDT meeting in Kochel, Oct 2018). Historically, five dogs with transitional cell carcinoma of the urinary bladder (Lucroy et al. 2003) and one dog with a prostatic carcinoma (Lucroy et al. 2003) showed transient improvement of clinical symptoms.

There are case studies/case series for other tumors that were treated with PDT described in the literature as well, such as hemangiopericytoma, oral and nasal tumors, esophageal SCC, scleral melanoma, mast cell and basal cell tumors, osseous tumors, and malignant STS. There are case reports/case series using PDT for cattle, birds and reptiles available as well.
Summaries of those studies can be reviewed in specific PDT literature (Buchholz and Walt 2013, Buchholz 2016, Dobson et al. 2018)

In horses, mainly periocular SCC and sarcoids have been treated with PDT. Giuliano et al. showed that the likelihood of tumor recurrence following surgery in equine PSCC was significantly reduced with local verteporfin PDT compared with cryotherapy (Giuliano et al. 2014). A case report showed good results using *m*THPC +/- surgery for an equine sarcoid (Reschke et al. 2012).

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HISTIOCYTIC PROLIFERATIVE DISEASES

Pathophysiological And Genetic Mechanisms Associated With Canine Histiocytic Malignancies

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Histiocytic sarcoma (HS) is an extremely rare cancer involving histiocytic cells (dendritic or monocytic/macrophagic lineages) with a limited response to chemotherapy and a high mortality. In the whole dog population, HS is a relatively rare cancer, but strikingly, few but popular breeds are highly predisposed to this cancer: BMD, Rottweiler, Retrievers (especially flat coated retrievers – FCRs-) (Abadie al., 2009; Affolter and Moore, 2002; Hedan et al., 2011 ; Moore et al., 2006). Most recently other breeds were reported to be at increased risk: Pembroke Welsh Corgi, miniature Schnauzer, Dobermans, Shar-Pei or Shetland sheepdogs seem to be overrepresented (Kagawa et al., 2016; Lenz et al., 2017; Mariani et al., 2015; Takahashi et al., 2014). Sporadic cases can also be found in many other breeds … (Klahn et al., 2011; Lenz et al., 2017; Mariani et al., 2015; Takahashi et al., 2014; Thongtharb et al., 2016). In the Cani-DNA BRC (Biological Resource Centre), a National collection of canine samples for biomedical research managed by the team (https://igdr.univ-rennes1.fr/en/research/research-groups/catherine-andré-group/crb-cani-dna), we have collected samples from HS predisposed breeds, and also from other breeds: Husky, Mastiff, Dachshund, sheep dog from Beauce, English setter, Boston terrier, Pyrenean shepherd, Boxer, Bichon, Doberman, Silky terrier, Yorkshire terrier, Shar-Pei, French Bulldog... This sampling suggests that HS is probably under diagnosed in many breeds, due in part to its aspecific symptoms and its aggressiveness at advanced ages.

Two main clinical presentations are distinguished: HS can be localized (LHS) or disseminated (DHS). The distinction has historically been made based upon the number of organ systems affected at the time of diagnosis: peri-articular locations, subcutaneous tissues and lungs are common primary sites for LHS, with vey common metastasis occurring in 70 to 91% of cases (Lenz et al., 2017). While external sites such as skin or joints are very common for localized HS, it could represent only 44% of LHS sites (Takahashi et al., 2014). Moreover the lung was reported to be the most common primary tumor location for LHS accounting for 31% of all cases (Skorupski et al., 2009). In DHS, tumors are diagnosed in multiple organ systems and no obvious primary site is observed. Localized and disseminated forms of HS present with identical histopathological features; moreover, due to the aspecific nature of the clinical signs and the strong ability to metastasize, it is currently not possible to determine the onset of the disease in these patients (Kennedy et al., 2016). Thus, the current belief is that these variants represent the early and late stages of a single disease rather than two unique pathological processes (Dervisis et al., 2016; Kennedy et al., 2016). Concordant with this hypothesis, we showed the clonal link between several tumors in Bernese Mountain DHS cases. In such a situation, it is not yet possible to distinguish LHS, with regional lymph node metastases, from DHS.
Nevertheless, breed predispositions influence the clinical presentation with Retrievers developing more localized/external HS (ie affecting skin/soft tissue, joints or lymph node), while BMDs and Rottweilers are mostly affected by internal/disseminated HS. Indeed, in a previous study, we showed that 87% of BMDs with HS present internal organs tumors compared to 48% of FCRs

(Hedan et al., 2011). The Pembroke Welsh corgi has unique anatomical localizations for HS with a predisposition to localized HS to lung or dural sites (Kagawa et al., 2016; Takahashi et al., 2014; Thongtharb et al., 2016). In DHS, the internal masses, such as mediastinal masses, could be challenging to detect and to sample for a histological/cytological diagnosis. This issue plus none specific clinical symptoms could, in part, explain why HS is mainly diagnosed at latter stages.

For several years, the goal of our team is to decipher the genetic bases of this cancer for the benefit of dogs but also for human patients affected by this cancer. In humans, this cancer is extremely rare and is characterized by proliferation of cells with the phenotype of mature tissue histiocytes. This aggressive tumor leads to a high mortality and nowadays there is no consensus on prognostic factors and standard treatment (Emile et al. 2016). Faced to such rare cancers, the challenging problem is the small number of patients and the difficulty to point out genetic recurrent and driver alterations to select targeted therapies. On the opposite, this rare cancer in humans is very frequent (up to 20%) in some popular predisposed dog breeds; thus offering a unique opportunity to better characterize the genetics of SH in such canine natural models of HS.

Generally speaking, cancer is a genetic multifactorial disease leading after a multiple step process to dysregulation of genes involved in cell growth and differentiation. Some gene alterations/dysregulations –called germline or predisposing alterations- occurring in all cells of the body, are transmitted from parents to siblings; these predisposing mutations explain the high risk of some dog breeds to develop HS, but they are very difficult to identify in humans. On other hand, other genetic alterations–called somatic alterations- occur during life in the affected tissue only and participate to cancer progression. Environmental or health-related factors can also influence the occurrence of these somatic alterations and cancer progression, severity, age of onset …. In HS, inflammation has been identified as a modifiable risk factor for cancer development in BMD (Klopfenstein et al., 2016; Ruple and Morley, 2016). These environmental factors are suspected to promote the occurrence and selection of somatic alterations, which boost histiocytic proliferation and tumorigenic transformation.
Concerning the identification of the genetic bases of HS in dog predisposed breeds, we have collected over 3000 blood samples from BMD, including 300 HS affected dogs, for which we also collected tissue samples. We focused on the 2 genetic aspects :

- The Identification of prediposing alterations, in collaboration with Dr. E. Ostrander (NIH, NHGRI, Bethesda, USA) (Shearin, Hédan et al., 2012) ;
- The identification of somatic alterations, using CHG arrays in collaboration with Dr. M. Breen (NCU, Raleigh, USA) (Hédan et al, 2011) and using RNAseq and exome sequencing

1. The search of predisposing genomic regions led us to the identification of several loci, including 2 major HS significantly associated loci on canine chromosome 11 (CFA11) and on CFA14. The sequencing and analyses of these regions are still under investigation. However, in the meantime, in order to help breeders to diminish the frequency of HS in their breed through the selection of BMDs with lower risk to develop and transmit HS, we developed a genetic risk test, based on our first results. Through collaboration between the French bernese breed club (AFBS) and the Antagene Company, we developed a genetic risk test based on 9 markers located in the predisposing loci, which alleles significantly discriminate at risk and protected individuals. We confirmed the reliability and significance of this set of markers in a population of over 1000 BMD form France. This genetic risk test has been set up for BMD breeders to help their selection, based on A, B and C indexes: A dogs present 4 times less risk to develop and transmit HS, while C dogs present 4 times more risk to develop and transmit HS. This genetic risk test has been first used in a pilot study made of French breeders (2012), then has undergo a validation phase on BMDs from Europe and USA (2013-2014), then is now available for breeders to adapt matings in order to reduce the prevalence of this devastating cancer.

2. The search somatic alterations has first been performed through CGH analyses of 104 dogs from BMDs and FCRs, and allowed to identify chromosomal regions recurrently duplicated or deleted in the tissues affected by HS (Hédan et al., 2011). Second, we performed RNA-Seq on three canine HS cases and out of 200 somatic mutations, we selected relevant mutations in

oncogenes of the MAP kinase pathway, recently confirmed by Thaiwong et al., 2017, Takada et al., 2018). To check for the frequency of such mutations, we tested 111 canine MH cases for 8 oncogenes of the MAP Kinase pathway. We showed that all cases were *BRAF* wild-type but somatic mutations were found in 2 interesting oncogenes in 64% (57% and 7.2% respectively), these mutations being mutually exclusive (B. Hédan, presentation at ESVONC, Brazil, 2016). We anticipated these mutations as targetable for therapies and owing to canine HS cell lines that we developed; we tested the efficacy of several drugs on cell proliferation. Altogether, these results, not only provide a biomarker to HS in dogs, but also provide a pertinent model to human medicine.

We will detail the state of art on these 2 aspects and the consequences for breeders and veterinary medicine of these scientific advances, on the selection, diagnosis and treatment of Histiocytic sarcoma

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Periarticular histiocytic sarcoma (PAHS) – Epidemiology, clinical characteristics and prognosis in 70 dogs

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Introduction

PAHS is a localized aggressive neoplasia arising from interstitial dendritic cells associated with the synovial membrane. The aim of this study was to describe clinical characteristics and therapy outcome in dogs with PAHS, and to identify possible prognostic factors.

Materials and methods

70 dogs diagnosed between 2005 and 2018 with PAHS were analysed retrospectively. Treatments consisted of limb amputation (LA), hypofractionated radiation therapy (RT), Lomustine chemotherapy (CeCeNu), or combinations thereof.

Results

Bernese Mountain Dogs (n=21; 30%) and Flat-Coated Retrievers (n=16; 23%) were significantly overrepresented (p

Conclusions

This study confirms known breed and tumor location predispositions for PAHS. Even in metastatic cases multimodal therapy can significantly prolong survival time.

Comparison of computed tomographic (CT) appearance of pulmonary involvement in localized and systemic histiocytic sarcoma in dogs

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Introduction

Periarticular and systemic forms of histiocytic sarcoma are recognised and both show potential pulmonic involvement. The aim of this retrospective study was to describe and compare the CT appearance of both forms.

Materials and methods

Thoracic CT studies of 17 dogs with systemic histiocytic sarcoma (SHS) with intrathoracic manifestation and 12 dogs with periarticular histiocytic sarcoma (PHS) with pulmonic metastasis were reviewed.

Results

In SHS pulmonary lesions were characterized by a solitary, large (>4 cm) (53%) or multifocal large masses >4cm accompanied by additional smaller nodules (47%). Pulmonary metastases in all cases of PHS were characterized by multifocal lesions with 10 of 12 (83%) dogs having nodules

Conclusions

These diagnostic imaging findings support the notion that PHS constitutes a different disease entity within the histiocytic proliferative diseases. The intrathoracic manifestation of SHS seems to constitute a primary multicentric tumour whereas multifocal pulmonary metastasis of PHS resembles the nodular interstitial distribution of other high grade sarcomas.

Clinical Management Of Canine Histiocytic Malignancies, Past, Present And What Is On The Horizon.

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Canine proliferative histiocytic diseases represent a range of disorders with marked differences in pathological features and clinical behaviour. As discussed by previous speakers, our understanding of the pathology of these diseases has evolved over the past 20+ years leading to changes in classification and terminology. The diseases that I propose to discuss are currently referred to as localised and disseminated histiocytic sarcoma (the latter formerly malignant histiocytosis), although it is recognised that these are likely two ends of a spectrum of malignant histiocytic disease, with different manifestations in different breeds of dog. Although the localised form presents as a single mass lesion it is locally invasive with a high rate of distant metastasis, initially to the loco-regional lymph node and latterly to multiple visceral sites. Disseminated histiocytic sarcoma is an aggressive multisystem disease with multiple visceral tumour masses, often including spleen. liver, lung and bone marrow.

The clinical management of these conditions both in terms of diagnosis and treatment is challenging. Whilst the localised form usually presents with a palpable limb mass which may or may not be associated with lameness, some lesions sited deeply in the axilla may cause severe lameness or even paresis due to their proximity to the brachial plexus, without an obvious mass. The disseminated form presents with more vague systemic signs of malaise, with clinical signs referable to the organs affected, whilst the haemophagocytic variant presents with a regenerative anaemia often accompanied by hypoalbuminaemia, and thus easily confused with conditions causing gastro-intestinal blood loss. Establishing a definitive diagnosis can be challenging. Typically histological findings include diffuse proliferation of neoplastic histiocytes, multinucleated histiocytic giant cells, spindle cells, anaplastic cells and in some cases presence of erythrophagocytic cells. Lymphocytic infiltrates in HS have also been reported and shown to have a significant regulatory T cell component (Marcinowska et al, 2017). Immunohistochemical staining is an increasingly important technique to accurately identify the cell of origin in such poorly differentiated tumours. Identification of histiocytes can be achieved with molecules involved in antigen presentation such as MHC class II molecules and the b2 integrins CD11d/CD18. On the basis of immunohistochemistry, HS is MHCII and CD18 positive, and the use of these markers has enabled HS to be differentiated from synovial sarcomas of the joint, and poorly differentiated sarcomas elsewhere in the body (Craig et al., 2002). More recently the ionized calcium-binding adapter molecule 1 (Iba1) has been shown to be a useful marker of cells of the monocyte-macrophage lineaqge in canine and feline inflammatory, proliferative and neoplastic conditions but expression does not allow classification of these histiocytic disorders (Pierezan et al, 2014).

Although termed ‘sarcoma’, histiocytic sarcoma is derived from the monocyte-macrophage lineage and is not a mesenchymal tumour. As such its behaviour differs from other soft tissue sarcomas, even the localised presentation is usually a rapidly growing mass often arising in periarticular sites or deep in the musculature of limbs, often precluding effective local surgical excision short of amputation. Metastasis to local and regional lymph nodes is common with subsequent widespread dissemination to other viscera. The skin and CNS may also be affected, we have documented diffuse infiltration of the leptomeninges in one dog that initially presented with a limb mass (Marcinowska et al, 2014), and other reports of CNS involvement may be found in the literature. Clinical management of this disease is therefore challenging and no one treatment has proven to be effective. Local surgical resection is often not possible, although splenectomy may provide short term resolution in cases where the lesion is primarily splenic (Dobson et al, 2006). In keeping with its haematological origin, we have found the primary localised lesions to be highly radiosensitive in flatcoated retrievers with appendicular histiocytic sarcoma. Thirty dogs with appendicular histiocytic sarcoma that were treated with radiotherapy were included in this study.

Twenty-five dogs presented with gross measurable disease and this was seen to resolve or reduce in size following coarse fraction radiotherapy (4 x 8.5 Gy) in 23 dogs. Of the twenty-six dogs presenting with lameness, 22 dogs (85%) had an improvement following treatment with radiotherapy. All 29 dogs that had follow-up were assessed as showing clinical benefit, either a reduction in the severity of lameness and/or size of gross disease, following treatment with radiotherapy. However, the median overall survival time achieved in this study was 147 days, which is similar to the 182 days for dogs that received radiotherapy previously reported by Fidel et al, 2006, with most dogs succumbing to metastatic disease in both studies, which underpins the malignant nature of this tumour.

Based on my personal experience, and some of the evidence now emerging from genetic studies, I believe that there are subsets of histiocytic sarcoma which are probably breed related, and that treatment response may vary between these subsets. If this is indeed the case it may confound our interpretation of clinical reports of response of HS to chemotherapy. Lomustine CCNU was first reported to have some efficacy in treatment of HS, with a documented response rate of 46% in 56 dogs with gross disease (including a variety of breeds) (Skorupski et al, 2007), but for all 59 dogs included in this study the MST was only 106 days (range 2 – 884 days). Other small scale clinical studies have used anthracyclines (doxorubicin or epirubicin) alongside lomustine with only modest improvements in survival (Mason et al, 2017, Moore et al, 2017). Dacarbazine has shown very limited benefit in the rescue setting for dogs with disseminated disease (Kezer et al, 2017). The haemophagocytic variant appears to be particularly refractory to lomustine treatment. So it is clear that conventional chemotherapeutic agents offer modest and only short term benefit in the management of this disease.

So is there any hope for the future? A number of possibilities lie on the horizon. Biphosphonates have been shown to significantly increase the activity of doxorubicin or vincristine against canine malignant histiocytosis cells in vitro (Hafeman et al, 2011), possibly by depleting macrophages. Liposomal clodronate has been shown to effectively deplete macrophages and dendritic cells in mice, and liposomal clodronate was reported to elicit significant tumour regression in 2 of 5 treated dogs with HS (Hafeman et al, 2010). Since the development of imatinib (Gleevec) a large number of small molecule kinase inhibitors have been developed and screened against various receptor targets. The kinase inhibitor dasatinib has been shown to have potent growth inhibitory activity against HS cells in vivo, possibly targeting the EPHA2 receptor (Ito et al, 2017). Finally we have recently completed a study evaluating the tumour microenvironment in HS from flatcoated retrievers confirming a significant infiltration of regulatory T cells within the microenvironment of these tumorus and identifying a possible role for the PD-1/PD-L1 pathway in HS immune evasion, which might suggest a future role for check point inhibitors in the management of this disease (Talamonti – University of Cambridge,MPhil 2018)

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Inflammation and Cancer Initiation and Maintenance

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Introduction

The link between cancer and inflammation is well known. Inflammation was initially believed to be a host response against tumors, leading to tumor suppression and favorable prognosis; however, evidence increasingly suggests that inflammation can also be associated with unfavorable clinical prognosis in cancer patients. In human and mouse models, tumor cells secrete pro-inflammatory cytokines such as TNF and IL-1, chemokines including CXCL8 (also known as IL-8), and other soluble factors which promote tumor development and progression. While cytokines are known to have an important role in cancer pathogenesis, many cytokines have both tumor- inhibitory and tumor-promoting activities.

For decades it has been recognized that tumors contain inflammatory and immune cell infiltrates that have classically been considered to be an attempt by the immune system to eradicate the tumor. However, recent evidence suggests that tumor-associated inflammation may paradoxically have a tumor-promoting effect. Inflammation can contribute to neoplastic progression through of the following various mechanisms.

- The supply of growth factors and growth signals to the microenvironment, which promote angiogenesis, cell proliferation, and invasion.
- Induction signals that support the process of Epithelial to Mesenchymal Transition (EMT). EMT is a key pathway in invasion and metastasis.
- Fostering the progression of premalignant lesions to fully blown cancer
- The production of reactive oxygen species that are mutagenic

Many of the cells that contribute to this are components of the innate immune system, particularly macrophages with a specific, cancer-promoting phenotype. Specifically, they form part of the tumor microenvironment that supports the maintenance of the cancer phenotype.

Tumour-promoting Inflammation

Inflammation normally functions to maintain tissue homeostasis in response to tissue stressors such as infection or tissue damage. Clinical studies suggest a close association between inflammation and tumorigenesis. **Acute inflammation** frequently precedes the development of protective adaptive immune responses to pathogens and cancer. Enormous efforts have been made to elucidate the contribution of chronic inflammation at different stages of tumour development, including initiation, growth and metastasis. **Chronic inflammation**, by contrast, has been shown to contribute to tumorigenesis at all stages. It contributes to cancer initiation by generating genotoxic stress; to cancer promotion by inducing cellular proliferation and to cancer progression by enhancing angiogenesis and tissue invasion. We now appreciate that chronic inflammation orchestrates the tumour-promoting microenvironment that is intimately linked with tumorigenesis. Based on these observations, it has been proposed that inflammation and tumour immunity are mutually exclusive processes.

Key Points:

- Chronic inflammation increases cancer risk.

- Subclinical, often undetectable inflammation may be as important in increasing cancer risk (for instance, obesity-induced inflammation).
- Various types of immune and inflammatory cells are frequently present within tumors.
- Immune cells affect malignant cells through production of cytokines, chemokines, growth factors, prostaglandins, and reactive oxygen and nitrogen species.
- Inflammation impacts every single step of tumorigenesis, from initiation through tumour promotion, all the way to metastatic progression.
- In developing tumours anti-tumorigenic and pro-tumorigenic immune and inflammatory mechanisms coexist, but if the tumour is not rejected, the pro-tumorigenic effect dominates.
- Signaling pathways that mediate the pro-tumorigenic effects of inflammation are often subject to a feed-forward loop (for example, activation of NF-**k**B in immune cells induces production of cytokines that activate NF-**k**B in cancer cells to induce chemokines that attract more inflammatory cells into the tumour).
- Certain immune and inflammatory components may be dispensable during one stage of tumorigenesis but absolutely critical in another stage.

IL-1b

IL-1**b** is a key pleiotropic pro-inflammatory cytokine produced by antigen presenting cells and known to mediate acute immune responses, providing a link between the innate and adaptive immune responses. Excessive IL-1 **b** production has been implicated in chronic inflammatory diseases and malignancies. Several studies support the finding that production of IL-1**b** pro-inflammatory cytokines in the pathogenesis of chemically-initiated skin cancers. It would seem that both IL-1 **b** and IL-17 act to either promote tumour initiation or suppress tumour growth in the context of inflammation caused by therapeutic intervention. This paradox might be explained by the type of cell making the cytokine, the stimuli that cell is receiving, and its relationship to the tumour itself. These complexities are yet to be unravelled.

The Central Role for NF-kB

NF-κB plays a key role in regulating the immune response to infection and dysregulation has been linked to conditions such as autoimmunity and cancer. The key role that this molecule plays in cancer can be summarised as follows:

- Constitutive NF-κB expression has been identified in many cancer types, supporting its role in promoting the cancer phenotype
- Dysregulation of the NF-κB pathway has been extensively documented in haematological malignancies lymphoma. These lymphoid malignancies feature aberrant NF-κB activation, which promotes tumourigenic cell survival, protects from apoptotic stimuli and favours oncogenesis (Karin M., 2006; Escárcega et al, 2007).
- Constitutive activation of NF-κB is a hallmark of chronic inflammatory disease, the latter also being associated with an increased incidence of cancer.
- A significant impediment to current cancer treatment regimes is the acquisition of resistance to the cytotoxic effects of chemotherapeutic agents. Ironically, many chemotherapeutic agents that trigger p53-mediated apoptosis also activate NF-κB (Das, 1997). In various *in vivo* and *in vitro* models, NF-κB inhibition was shown to increase the efficacy of anticancer agents and reduce the incidence of resistance to these agents making NF-κB an interesting drug target in oncology.

IL-6 in Cancer-Related Inflammation

L-6 mediated signalling is implicated as a key cytokine linking inflammation with carcinogenesis. Overexpressed across several cancer types, multifunctional IL-6 is highlighted as a key player in pro-inflammatory conditioning of carcinogenesis:

- Detailed cancer mechanistic studies involving IL-6 are yet to be carried out.in canine tumours, However, studies indicate that increased IL-6 protein accompanies tumoural progression.
- IL-6 also acts as a major modulator of the immune system, particularly of dendritic cell maturation and other antigen-presenting cells. IL-6 signalling can be polarized towards anti

or pro-inflammatory functions, with the activation of JAK1–STAT3, RAS–MAPK, and, PI3K–AKT (Yao et al., 2014).

The variety of roles that IL-6 plays and its activation states, allied to the complexity of the tumour microenvironment, highlights this cytokine can act as either a friend or a foe at the interface between cancer and inflammation. In addition, cytokines and chemokines also interact with many other proteins within the cell and within the tumour microenvironment. IL-6 and NF-κB can interact in an amplifying loop of inflammation that promotes carcinogensis. Simultaneous activation of NF-κB and STAT3 in non-immune cells occurs by secretions of inflammatory and non-inflammatory cell types in the tumour microenvironment and is capable of up-regulating IL-6 expression, which is followed by accumulation of immune cells, inducing inflammation and deregulation of local homeostasis. This mechanism is designated as an "*inflammation amplifier*" , and seems to be responsible for the transition between acute and chronic inflammation, perpetuating genetic instability and the diversity of mutations in the cancer cell genome.

Within the tumour microenvironment, diverse cytokines and chemokines are responsible for the mediation of cell signalling among the different cell types: tumour-associated macrophages and fibroblasts, regulatory T-lymphocytes and cancer cells themselves, mediating paracrine and autocrine signalling (Table 3). To avoid listing all the involved cell types, cytokines and chemokines, here we will focus on tumour-associated macrophages (TAMs). TAMs have been considered key orchestrators of the tumour microenvironment, directly affecting neoplastic cell growth, neoangiogenesis, and extracellular matrix remodelling.

The Inflammasome

The inflammasome is a multi-protein complex that mediates immune responses to microbial, host, and environmental signals. When active, inflammasomes regulate caspase-1 activation and IL-1b secretion. There is a strong link between inflammation and cancer, and IL-1b is one of the major molecules involved in both of these disease processes. In addition to IL-1b, other members of IL-1 family such as IL-18 (IL-1F4) and IL-33 (IL-1F11) are processed and activated by caspase-1 and inflammasomes. Experiments using mice deficient in inflammasome termed NLRP3 have shown a protective role for inflammasomes in colorectal tumorigenesis through IL-18 that induces tumour suppressors. Inflammasome-mediated IL-1b secretion and its downstream mediators enhance macrophage chemotaxis, angiogenesis, and resistance to chemotherapy. Furthermore, it has been reported that expression of NLRP3 inflammasomes in the melanoma tumour microenvironment diminishes anti-tumour immune response by facilitating the migration of MDSCs to the tumour site, indicating a critical role for NLRP3 inflammasome in suppressing the anti-melanoma T-cell response.

Tumour Macrophages

Recently, it has been recognized that tumour-associated macrophages (TAMs) are not homogenous. Increased number of macrophages within the tumour islets confers a marked survival advantage, whereas increased number of macrophages in the tumour stroma is associated with poor prognosis in in certain tumours. In addition, macrophages are polarized into two functionally distinct forms **M1 and M2**, mirroring the Th1 and Th2 nomenclature of T cells. Differentiation of the M1 macrophages is induced by interferon-γ, lipopolysaccharides, tumour necrosis factor (TNF) α, and granulocyte-monocyte colony-stimulating factor. The M1 macrophages produce high levels of interleukin (IL)-12, IL-23, TNFα, IL-1, IL-6, CXC ligand 10 (CXCL10), inducible nitric oxide synthase (iNOS), and reactive oxygen and nitrogen intermediates. Differentiation of the M2 macrophages is induced by IL-4, IL-10, IL-13, IL-21, activin A, immune complexes, and glucocorticoid. The M2 macrophages express high levels of IL-10, IL-1 receptor antagonist and are considered tumour promoting. In contrast, the presence of M1 macrophages has been associated with a positive prognosis in certain tumours. The manipulation of macrophages through the activity of the CSF-1 receptor may prove important in cancer therapeutics.

Infectious Agents

As well as tumour-causing viruses that have a direct link to carcinogenesis through manipulation of the cellular genome, other pathogens have the ability to promote tumorogenesis through inflammation. Several types of inflammation—differing by cause, mechanism, outcome, and intensity—can promote cancer development and progression. Persistent Helicobacter pylori infection is associated with gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphoma. Infections with hepatitis B (HBV) or C (HCV) viruses increase the risk of hepatocellular carcinoma (HCC), and infections with Schistosoma or Bacteroides species are linked to bladder and colon cancer, respectively. The inflammatory response triggered by infection precedes tumour development and is a part of normal host defence, whose goal is pathogen elimination. However, tumorigenic pathogens subvert host immunity and establish persistent infections associated with low-grade but chronic inflammation. By contrast, acute inflammation induced by certain microbial preparations was used by Coley with some success to treat cancer in the 1890s, and one such preparation is currently used in the treatment of bladder cancer in man. What makes bladder carcinoma uniquely sensitive to acute inflammation, even though it is promoted by chronic inflammation, is currently unknown.

Cancer Stem Cells and Inflammation.

The mechanisms underlying tumour promotion by cancer-related inflammation are not yet fully understood, but both CSCs and inflammatory cells in the surroundings would appear to have a central role. Cancer stem cells evolvability - the capacity to adapt to new, harsh microenvironments - can be influenced by states of inflammation, with coexistent hypoxia. Using network models at the system-level.

The concept of CSCs evolvability, suggests that one of the main reasons for cancer recurrence after treatment could be the generation of more CSCs with the application of selective pressure by sudden changes on the microenvironment after radiation or chemotherapy. These CSCs would be again active on the restoration of a resistant tumour, re-establishing cytokine and vascular networks, at the same type as inflammatory cytokines can also regulate CSCs and EMT.

Awareness to the evolvability and potential of CSCs to recreate the tumour microenvironment should point researchers to develop new therapeutic options targeting the roots of malignancy and not only cancer cells or cancer stem cells by themselves, as it has been hypothesized before (Argyle and Blacking, 2008). Hereby we advocate that targeting inflammation as the continuous drive of CSCs generation could be a good alternative for typically radioresistant and chemo-resistant cancers and also those where cancer-related inflammation prevails.

Conclusions

The influence of inflammation on tumorigenesis, and even on cancer therapy, is evidently significant. However, as mentioned above, many of the inflammatory mediators can benefit the host in therapy of tumours despite also having a critical role in cancer development and progression. The dual role of certain molecules is far from being completely understood. Currently, the markers that we use for immune cell phenotyping might not be very useful in functionally differentiating these immune cells in a tumour microenvironment. Some inflammatory cytokines have been shown to be required for the efficacy of cancer therapy, but many inflammatory mediators can abrogate the effect of therapeutics, even promoting tumour resistance to the therapy. Clearly, a better insight into tumour-associated inflammation will help us to develop the effective cancer therapy or even prevention.

Inflammation in tumor progression and metastasis

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The tumor microenvironment potentially impacts on different aspects of tumor stem cell biology. Different subpopulations of tumor stem cells might be able to interconvert in response to environmental triggers. Microenvironmental signals influence the de-differentiation process of transformed cells into cancer stem cells. Furthermore stromal-derived factors can lead to the activation of a transdifferentiation program resulting in epithelial-to-mesenchymal transition (EMT) and metastatic spread. We have recently developed a new mouse model of invasive colon cancer that closely recapitulates late stage human CRC [1]. Mice with an IEC-restricted deletion of p53 (*Tp53*$^{\Delta IEC}$ mice) that are repetitively challenged with the pro-carcinogen azoxymethane (AOM) develop invasive cancer in the distal colon within three months. In about 30 % of *Tp53*$^{\Delta IEC}$ mice lymph node metastases can be detected after 5-6 months. Interestingly, p53 controlled invasion does not depend on well-described downstream functions of p53 such as apoptosis, cell cycle control or genomic stability but rather on the development of an NF-κB dependent inflammatory microenvironment supporting the induction of EMT. Loss of IKKβ in IEC significantly blocks tumor invasion in this model. Interestingly, loss of IKKβ in myeloid cells does not decrease the incidence of p53-deficient invasive tumors, but instead it reduces the size of invasive lesions and decreases the proliferation rate of invading epithelia. This is paralleled by a diminished paracrine activation of STAT3 in invading tumor cells due to lower expression levels of STAT3 activating cytokines in recruited IKKβ-deficient myeloid cells. Strikingly, mice with myeloid specific IKKβ deletion are completely protected from lymph node metastases. The interleukin (IL)-6 family of cytokines is defined by the shared use of the gp130 receptor β-subunit. Engagement of the gp130 receptor by either IL-6 or IL-11 induces transient activation of Janus kinases (JAK) and the latent transcription factor STAT3. To determine the contribution of Stat3 signaling to the development of lymph node metastases in AOM-challenged *Tp53*$^{\Delta IEC}$ mice, we generated intestinal epithelial cell (IEC) specific knockout mice of the common IL-6 cytokine family receptor β subunit gp130 (*gp130*$^{\Delta IEC}$) and crossed these to *Tp53*$^{\Delta IEC}$ mice. Expectedly, the loss of gp130 in IEC substantially decreased the formation of lymph node metastases. However, this was not accompanied by a loss of Stat3 activation in p53 deficient IEC, suggesting alternative gp130 independent activation of Stat3 in these tumor cells.

Over the past 30-40 years basically every review on the role of reactive oxygen species (ROS) in cancer states that ROS can induce DNA damage and mutations. It had been suggested that during chronic inflammation increased ROS production may cause mutations. Myeloid cells are a major source of ROS in acute and chronic inflammation as well as in tumor induced inflammation. Surprisingly - and despite the firm statement in a huge number of reviews - nobody has ever shown and formally proven using a genetic model that myeloid derived ROS indeed can cause mutations in neighboring epithelial cells *in vivo*. Moreover, it has even been speculated that mutagenesis is not a direct consequence of myeloid derived ROS but rather an effect of ROS production in epithelial cells in response to pro-inflammatory cytokines such as TNFα. Using relevant genetic models we have now systematically addressed this question.

We used a conditional *Gpx4* knockout in myeloid cells which causes spontaneously increased ROS production in myeloid cells that is comparable to *wt* cells during an inflammatory condition. Importantly, loss of Gpx4 does not affect myeloid cell survival and function allowing us to use these mice in tumor models. We intended to use these mice as a genetic tool to increase ROS production in myeloid cells rather than to decipher GPx4-dependent functions in macrophages during tumor development. We were then indeed able to demonstrate that these mice

develop invasive cancer in a model of AOM-initiated intestinal tumorigenesis, which is not detected in *wt* animals. Invasive cancer can even be found when *Gpx4* is deleted from myeloid cells in established tumors using a newly developed tamoxifen-inducible LysM-CreERT2 mouse. Mechanistically we could show that increased mutational load in IEC is dependent on myeloid derived H_2O_2 rather than cytokine mediated effects in IEC. Nevertheless, we provide evidence that also cytokines contribute to enhanced tumor progression. TNFα induces a feed-forward loop that culminates in enhanced macrophage recruitment and therefore increased ROS levels in tumors. Importantly, however, TNFα is not directly involved in DNA damage. We complemented our *in vivo* data with extensive *ex vivo* analysis of intestinal organoids and we can impressively demonstrate that repetitive treatment of *wt* organoids with H_2O_2 (but not TNFα) leads to the formation of a large number of mutations (verified by whole exome sequencing) closely mimicking the *in vivo* situation. Importantly, we could further demonstrate that *Gpx4*-deleted mice develop tumors in a model of chronic inflammation whereas *wt* animals do not in the same time and most importantly that *Gpx4* ko mice spontaneously develop tumors at various locations, lung being the most frequently affected organ.

In summary, our study [2] actually proves for the very first time that chronic inflammation (=increased ROS production in myeloid cells) can initiate cancer and does not only contribute to tumor promotion and progression by shaping a pro-tumorigenic cytokine milieu in the tumor microenvironment. Moreover, we provided a detailed molecular mechanism and could distinguish ROS-dependent events from cytokine mediated effects: we could rule out a direct role of TNFR-dependent signaling, MyD88-dependent signaling as well as RAGE-dependent signaling for epithelial mutagenesis.

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The role of cyclooxygenase-2 in cancer stem cell survival and repopulation during anticancer therapy

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Cancer is now considered a stem cell disease, where tumours are composed of a mixture of genetically and functionally distinctive cells that contribute to tumour outgrowth, and a small population of cancer stem cells (CSCs) that can drive tumour initiation, therapy resistance, tumour repopulation and metastasis. The CSC model posits that tumours are organised hierarchically in a similar, albeit distorted, manner as normal tissues. In a normal tissue, stem cells at the apex of this hierarchy give rise to transit amplifying cells, which proliferate rapidly and finally enter a post-mitotic, differentiated state, in which the cells fulfil the various functions of the specific organ. CSCs share important properties with normal stem cells, including self-renewal and multi-lineage differentiation potential, and can drive tumour progression as they have the exclusive ability to perpetuate indefinitely the growth of the tumour, and give rise to a diverse array of differentiated progeny that make up the bulk of the tumour mass. CSCs, similar to normal stem cells, are highly resistant to the cytotoxic effects of chemotherapy and radiotherapy, and are able to reinitiate tumour growth. This is seen clinically where these therapies do shrink the bulk of the tumour but, after a remission period of variable length, most patients do relapse with frequent development of drug resistance and metastatic dissemination. It is therefore essential that CSCs are targeted and destroyed for cancer to be completely cured.

Tumours are not only clonal outgrowths of deregulated cancer cells but potentiate their own progression and survival by fostering a complex and highly dynamic microenvironment, consisting of the extracellular matrix, endothelial cells, immune cells and a plethora of cytokines and growth factors. Importantly, inflammatory cells and the cellular mediators of inflammation are prominent constituents of the microenvironment of all tumours. In some cancers, the inflammatory conditions precede the development of malignancy, for example inflammatory bowel disease is associated with colon cancer. Alternatively, an oncogenic change can drive tumour-promoting inflammation in tumours that are epidemiologically unrelated to overt inflammatory conditions. This 'smouldering' inflammation in the microenvironment has many tumour-promoting effects including tissue remodelling, angiogenesis, cancer cell survival, metastasis, and immune evasion. One key inflammatory mediator deregulated in many cancers is cyclooxygenase-2 (COX-2).

COX-2 is an inducible form of the enzyme that catalyses the synthesis of prostanoids, including prostaglandin E2 (PGE_2), a major mediator of inflammation and angiogenesis. Under normal conditions, acute inflammation is a tightly controlled self-limiting response, where upon abatement of the inflammatory stimulus, specific cytokines, including interleukin-1 (IL-1) and IL-6, exert feedback inhibition causing COX-2 expression and PGE_2 production to cease and the inflammatory response to subside. However, with sustained exposure to pro-inflammatory stimuli, continued expression of COX-2 leads to the transition from acute to chronic inflammation. In recent decades, COX-2 overexpression has been reported in several human cancers including breast, lung, skin, colon, bone, cervical, oesophageal, pancreatic, prostate and bladder cancer, and is inversely associated with patient survival.

PGE_2 also has a role in stem cell biology, and is heralded as an evolutionarily conserved regulator of haematopoietic stem cells (HSCs) where the COX-2/PGE_2 axis is required for HSC formation, proliferation, maintenance of the haematopoietic lineage, and bone marrow recovery following irradiation injury. Therefore, it is unsurprising that up-regulation of COX-2 is associated with CSCs isolated from several cancer types. A functional marker of CSCs is the ability to grow as spheroid colonies in defined serum-free culture conditions that supports the proliferation of undifferentiated cells. Previously, we have shown that COX-2 expression is elevated 141-fold in the CSC

population compared to the non-CSC population of canine osteosarcoma cells, and that COX-2 inhibition induced a dose-dependent decrease in sphere forming ability, indicating that COX-2 plays a major role in tumour initiation. Furthermore, CSCs isolated from human glioma cell lines, express constitutively high levels of COX-2 protein that correlates positively with radioresistance. Inhibition of COX-2 enhanced radiosensitivity of glioma CSCs and suppressed the expression of angiogenic and stemness-related genes. Together, this data suggests that inhibiting COX-2 in CSCs reduces stem cell characteristics, and that COX-2 plays a vital role in the maintenance and function of the CSC population.

COX-2 overexpression is also associated with resistance of cancer cells to conventional chemotherapy and radiotherapy. These therapies are often delivered in multiple doses, which are spaced out to allow the recovery of normal tissues between treatments. However, surviving cancer cells also proliferate during treatment intervals, leading to repopulation of the tumour, and limiting the effectiveness of the treatment. Tumour cell repopulation is a major cause of treatment failure. Resistance can be divided into two broad groups: intrinsic or acquired. Intrinsic resistance indicates that prior to receiving the therapy, resistance-mediating factors pre-exist in a subset of cancer cells that make the therapy ineffective, including increased drug efflux, and aberrant DNA damage repair pathways. Acquired resistance can develop during treatment of tumours that were initially sensitive and can be caused by mutations arising during treatment, as well as through other adaptive responses, including activation of alternative compensatory signalling pathways and evasion of cell death. Tumours also contain a high degree of molecular heterogeneity; thus, drug resistance can arise through therapy-induced selection of a resistant population of cells that was present in the original tumour.

However, there is compelling evidence of an active proliferative response akin to how normal tissue stem cells mobilise to the site of a wound during tissue repair: here, injury to the tissue induces apoptosis. Apoptotic cells then signal their presence to the surrounding tissues and, in doing so, elicit tissue repair and regeneration that compensates for any loss of function caused by cell death. The first evidence of this in a mammalian model was provided by Li *et al.* (2010) and coined the phrase “phoenix rising pathway”, in which tissue damage initiates tissue repair. This study revealed that mice deficient in caspase-3 and caspase-7, which are essential apoptotic proteases, exhibited reduced rates of tissue repair in dorsal skin wounds, and defects in liver regeneration following partial hepatectomy. Mechanistically, apoptotic cells released PGE_2 in a caspase-dependent manner, and this in turn stimulated stem cell proliferation and tissue regeneration. Given that aberrant apoptosis is a hallmark of cancer, and that activation of caspases to induce apoptosis is the prevailing ideology of most cancer treatments: what is the role of apoptosis-induced compensatory proliferation in cancer development? Is the phoenix-rising pathway clinically relevant? Can repopulation be abrogated by COX-2 inhibition of PGE_2 signalling?

Subsequently, we have investigated the role of COX-2 in tumour repopulation after irradiation *in vitro*. We showed that conditioned media isolated from irradiated cells could stimulate the growth of canine osteosarcoma cells and increase the size of the CSC-pool. This effect was abrogated by pre-treatment with the specific COX-2 inhibitor, Mavacoxib. Further work is required to dissect the molecular mechanisms of this pathway and to determine if we can uncouple apoptotic cell signalling to CSCs from apoptotic cell death, to ultimately inhibit tumour repopulation during therapy, and improve clinical outcomes.

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COX-2-independent anticarcinogenic effects of selective COX-2 inhibitors

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Non-steroidal anti-inflammatory drugs (NSAIDs) reduce the risk of tumor development. One mechanism by which NSAIDs exert their anti-carcinogenic effect is through inhibition of cyclooxygenase-2 (COX-2), which is overexpressed in various tumor tissues, thereby enhancing cell proliferation and inhibiting apoptosis. However, as a result of numerous studies in recent years, it has become apparent that COX-independent mechanisms also play a decisive role in the anti-carcinogenic effects of selective COX-2 inhibitors (coxibs). Today the anti-carcinogenic effect of celecoxib and other NSAIDs have been investigated extensively with variable results. Especially the results to rofecoxib and celecoxib demonstrated that the anti-carcinogenic effect of coxibs is not a class effect and each substance has its particular molecular mechanism [1].The anti-carcinogenic effect of coxibs is based on their influence on proteins that regulate cell cylcle progression, apoptosis, angiogenesis and metastasis. Most *in vitro* and *in vivo* studies used rofecoxib or celecoxib to inhibit tumor growth. One of the first published clinical trials showed a significant reduction in duodenal polyposis after six months of treatment with celecoxib 400 mg twice daily in patients with familial adenomatous polyposis (FAP) [2]. This study led to the approval of celecoxib by the FDA (in December 1999) for adjuvant treatment of patients with familial adenomatous polyposis.
Because tumor therapy is usually carried out over long periods of time (months to years), a reasonable benefit-risk ratio of the substances used is of vital importance. The long-term use of tNSAIDs, which inhibit both COX-1 and COX-2, is associated with serious gastrointestinal side effects such as ulcerations and perforations of the gastric mucosa. Selective COX-2 inhibitors, such as celecoxib, rofecoxib and other were supposed to exhibit reduce gastrointestinal side effects in comparison to tNSAIDs. Patient studies indeed demonstrated a lower incidence of gastrointestinal complications but simultaneously illustrated other critical side effects like an increased risk of cardiovascular events, skin or liver toxicity. Also these side effects occur substance specific and are in part independent of COX-inhibition. Today only celecoxib and etoricoxib are still on the market. 2011 Pfizer has withdrawn the high dose application of celecoxib for adjuvant treatment of FAP patients due to the lack of further supporting data.

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The use of COX-2 inhibitors in treating cancer: what's the evidence?

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Introduction
cyclooxygenase-2 or **COX-2**, is an enzyme that is encoded by the *PTGS2* gene. It is one of two cyclooxygenases and is involved in the conversion of arachidonic acid to prostaglandin H2, an important precursor of prostacyclin, which is expressed in inflammation.

It is accepted that COX-2 contribution to carcinogenesis is mediated through overproduction of Prostaglandins (PGs). PGE2 is a cardinal mediator of inflammation playing a key role in carcinogenesis and there is a positive-feedback loop between COX-2 expression and PGE2. COX-2 action on PGs especially PGE2 accounts for diverse functions that promote cancer initiation and progression.

- Apoptosis resistance
- Angiogenesis through upregulation of vascular endothelial growth factor.
- Metastasis by upregulation of matrix metalloproteinases.
- Tumour growth via upregulation of epidermal growth factor receptor (EGFR;
- Maintenance of cancer stem cell (CSC)-like activity via activation of WNT/β-catenin/TCF
- Cancer cell survival
- Cancer cell invasion via upregulation of extracellular signal-regulated kinase (ERK)
- Modulation of the immune system through increase of the activity for regulatory T cells (Tregs)

Cox-2 Inhibitors in the Treatment of Human Cancer
Inhibition of COX-2 can provide positive therapeutic outcomes in cancer treatments as demonstrated from certain human studies.

- COX-2 inhibitors are relatively inexpensive in comparison with the standard cancer therapies, having tolerable side effects, and that they are able to sensitize cancer cells to treatments like radiotherapy and chemotherapy both of which are recognized to induce COX-2 expression in cancer cells.
- COX-2 inhibitors have been shown to relieve COX-2-mediated expression of multidrug resistance proteins in cancer cells
- In the perioperative setting, inhibitors have been shown to reduce the risk of surgical related metastasis.
- In cancer prevention, administration of COX-2 inhibitors for organs like lung, breast, colon, and prostate have been shown to cause a reduction of cancer risk by about 70%.
- Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the drugs used for prevention of cancer. COX-2 inhibition by NASIDs is related to the reduction of cancer recurrence and increase of patient survival.
- Elevation of ROS concentration by NSAIDS is possibly accounted for inhibition of COX-2 in cancer cells. Conventional NSAIDs are not specific for inhibition of COX-2, as they are also an inhibitor for COX-1, and can cause GI irritation.
- Aspirin may also suppress growth of cancer cells via mechanisms independent on COX-2 but has also shown to have a protective effect on cancer.
- COX-2 inhibitors provide a common target for exerting synergistic therapeutic effects after combination with either chemotherapeutic drugs or chemoradiation therapy.
- Chemotherapeutic agents could adversely cause cancer cell repopulation through induction of caspase-3-mediated COX-2 activity. Irradiation is also an inducer of COX-2-

mediated angiogenesis through activation of the same pathway. Some chemotherapeutic agents adversely induce COX-2 expression indirectly via promotion of an inflammatory setting probably through developing CAFs into senescence CAFs by acquiring a proinflammatory phenotype. Therefore, it would be advisable to use COX-2 inhibitors as an adjuvant with chemotherapy and/or radiotherapy. Such combination has been reported to synergistically increases the antitumoral activity for chemotherapeutic agents like sorafenib, 5-fluorouracil, bleomycin, irinotecan, cisplatin etc.

- This combination also improves the rate of tolerance to chemoradiation and overall response rate for advance stages of cancers), especially when it is administered before radiotherapy

Cox-2 Inhibition in Veterinary Patients

The most obvious treatment for cox-2-dependent cancers in veterinary patients would be the use of non-steroidal anti-inflammatory drugs (NSAIDs), which have been widely used as a therapeutic option in dual modality treatment of cancer patients with colorectal (Thun et al., 2002; Din et al., 2010) and prostatic cancer (Liu et al., 2014a). The success of NSAIDs therapy for human cancers has not yet been replicated for the canine counterpart.

The therapeutic value of NSAIDs for canine patients with transitional cell carcinoma of the bladder (TCC) was first investigated more than two decades ago (Knapp et al., 1992; Knapp et al., 1994; Knapp et al., 1995). More recently, published literature on TCC treatment advocates the benefit of combining NSAIDs, either specific or non-specific COX-2 inhibitors, single use or combined with chemotherapeutic drugs (Henry et al., 2003;McMillan et al., 2011; Robat et al., 2013; Knapp et al., 2013). Even though, there is a concern to the potential side-effects when using non-specific cox-2 inhibitors, and also that not all chemotherapeutic drugs benefit by combining adjuvant cox-2 inhibition (Greene et al., 2007; Marconato et al., 2011).

Excluding malignancies of the urinary tract, other canine cancer types have been tested in vitro for COX-2 inhibitor sensitivity. Osteosarcoma, glioma, haemangiosarcoma and lymphoma cell lines have shown sensitivity for mavacoxib and carprofen treatment, presenting reduced proliferation and increased caspase-independent apoptosis. (Pang et al. 2014) The activity of two non-specific but preferential COX-2 inhibitors, etodolac and celecoxib, has also been studied in a canine mammary carcinoma cell line. Celecoxib, in particular, was able to activate the mitochondrial apoptosis pathway and cause cell cycle arrest (Saito et al., 2014).

Several retrospective studies and in vivo clinical trials have interrogated the ratio of therapeutic value:toxicity of COX-2 inhibition, but the opinions are divergent for non-urinary tract malignancies (Schmidt et al., 2001;Mutsaers et al., 2002;Sorenmo et al., 2004;Elmslie et al., 2008;Chon et al., 2012;de Vos et al., 2012)

The mechanisms of action of different NSAIDs seem to be different according to the type of cyclooxygenase inhibition, and the success of anti-inflammatory drugs seems to derive from their multifunctional actions on different fronts (Liggett et al., 2014).

Pang et al., suggested a central role for Cox-2 in tumour initiation, having identified COX-2 upregulation in cancer stem cells. Even though Cox-2 inhibition treatment did not result in reduced viability or chemoresistance in CSCs, tumoursphere formation was inhibited in both human and canine osteosarcoma cell lines (Pang et al., 2014). Although the molecular pathways for COX-2-in cancer are not clearly elucidated, there is a rationale for targeting inflammation based on this evidence. Within the tumour microenvironment COX-2 can be also be produced and amplified by tumour-associated macrophages, promoting tumour progression to advanced metastatic states (Nakao et al., 2005; Hou et al., 2011). It is therefore relevant to target inflammation and its roots, by using NSAIDs and affecting multiple target genes at once (Liggett et al., 2014), or by targeting specific multifunctional cell types such a TAMs (Mitchem et al., 2013).

Factors Which May Influence the Success of Targeting Cox-2

- The type of cancer: Some types of cancers like schwannoma do not respond to COX-2 inhibitors despite its high rate of expression of Cox-2.
- The type of COX-2 inhibitor: As for NSAIDSs, the response would be more potent after application of selective drugs compared to the non-specific agents.
- The dose for COX-2 inhibitors: High doses of COX-2 inhibitors exert opposite effects and can promote activity. This might be due to the effects of a negative feedback loop or probably upregulation of COX-2 isoforms.
- The type of chemotherapy (a conventional antitumor drug received by patient) used in combination with COX-2 inhibitors. Choosing a right type of chemotherapy as an adjuvant with COX-2 inhibitor is also important.
- The grade of tumour. However the correlation between cox-2 expression and grade is controversial in human medicine.

Key Points

- Both activation and suppression of COX-2 can be linked to cancer progression. COX-2 activation could be both pro- or antitumorigenic, but the evidence for its pro-tumour activity is far more evident.
- For most type of cancers, uncontrolled activation of COX-2 is related to a poorer prognosis and its blockade favours cancer treatment.
- Most of the functions of COX-2 on tumour cells are exerted through engagement of PGE2/EP4, so other targets are a potential.
- Members of MAPK family, EGFR, and p65 NF-κβ are major regulators of COX-2 in cancer cells. ERK acting on COX-2 is contributed to both pro- and antitumorigenic functions of COX-2.
- Tumour Microenvironment (TME) is central for the promotion of COX-2-mediated tumorigenesis. Macrophage (M2) Tregs, and cancer cells promote the expression of COX-2.
- Cox-2 Inhibition may be synergistic with chemotherapy and radiotherapy.

How do you get what you need? Problems & pitfalls in the pathologist-clinician interaction

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Veterinary patient care has moved towards an integrated approach with a healthcare team tailoring the treatments/decisions to the individual patient. This team not only includes the medical, surgical and radiation oncologist, but also the radiologist and pathologist.

One of the areas where good communication is needed is the interpretation of the surgery that was performed. This starts with planning the extent of surgery, often times based on palpation and imaging in light of the location & type of the tumor, and the overall treatment plan. Assessing the success of surgery is often times based on histological assessment of the margins, including the deep and lateral margins, which is the second area where communication regarding the findings is crucial. In the ideal situation the area of most concern (margin wise) would be targeted specifically, and both the pathologist and medical oncologist would have direct knowledge of the amount & type of tissue removed during surgery.

Communication (and potential for breakdown in communication) starts with something as simple as the terminology used. The surgical definitions/terminology might differ between surgeons ↔ non-surgeons, pathologists ↔ non-pathologists, as well as other specialists, but also amongst surgeons or pathologists. Similarly, the methods of reporting the histological findings (margins, mitotic index) varies as well.

Understanding the intent and definitions as used by different groups will lay the groundwork for better outcome studies, as they pertain to specific tumor types. Several definitions are commonly used by surgeons, such as: down to macroscopic disease, incisional biopsy, debulking surgery, and excisional biopsy, marginal excision, down to microscopic disease, excision with margins, wide excision, radical excision, and compartmental excision – some of which might have a similar meaning. This abundance of terminology makes communication between clinicians difficult, as some terms might mean something different to different people. In outcome literature reported by non-surgeons, other terms have been used, such as biopsy (ranging from complete excision with margins to leaving macroscopic disease behind). (See also “On the cutting edge: A surgeon’s perspective on resection margins”).

Regardless, margin planning can be loosely broken down in groups that can be easily excised with large margins, those where margins are not possible, and a third group where margins will be small. The third group becomes the most interesting, and of importance to communicate and pin point the areas where the surgeon is most concerned about.

Preoperative communication: what is the goal/what is the intended surgical dose?
Preoperative discussion and further diagnostics might play a role in further developing a plan specific to this patient – are any other prognostic indicators present, such as metastasis. Should we take a biopsy prior to surgery in these cases to test for markers ahead of surgery? Would knowing negative indicators for survival alter the surgical aggressiveness or surgical dose?

Perioperative communication: What did it look like/what areas is the surgeon worried about?
Perioperative planning could revolve around specifically identifying areas of concern, marking the edges of the wound bed with hemoclips to assist in postoperative RT planning. A good understanding of each other’s language, as well as the limitations of what information both parties can provide, will help in getting the most information out of the specimen obtained.

A close working relationship between the surgeon and pathologist will help in identifying the preferences in inking, labeling and identifying specimens. Being present while the specimen is

trimmed will help in pin pointing the areas identified as concerning during surgery. However, this is only possible with when the surgeon and pathologist work in close proximity to each other, such as academic hospitals. Inking will help with orientation and identifying specific margins, and is instrumental in identifying specific areas and planes during and after trimming of the specimen.

Postoperative communication: what does it mean? And how do we interpret the findings?
Amongst surgeons, there is no consensus or agreement regarding the extent of margins that needs to be taken for specific tumor type, or what constitutes a completely removed tumor, and more importantly, when a scar revision is indicated.
Margins have been described as: dirty ↔ clean, complete ↔ incomplete, clean but close, narrow.[1-5] Clean has been described as >1mm in one paper[6], but as >2mm,[4] >3mm,[7] or >5mm^2 in other papers. Specific long term follow up & outcome studies, regarding recurrence rates based on margins for specific tumors, grouped by prognostic indicators are lacking. Furthermore, margins might only be one parameter in the prognosis for that patient.
Another factor might be a variation between pathologists, which is not only important in the daily management of your patients, but also with interpretation of the existing literature and conclusions drawn. For example, one study looked at inter & intra observer agreement in assessment of Soft Tissue Sarcomas. While there was a strong intra-observer correlation for diagnosis and tumor grading (κ0.91), there was only a moderate correlation for diagnosis (κ0.6) and grading (κ0.43) between observers.[8] A recent opinion paper raised a concern regarding the reporting of mitoses: mitotic count is typically reported but might be mislabeled as mitotic index (MI). Furthermore the MC might differ depending on the field of view (by virtue of the ocular used).[9]

The above highlights the fact that understanding each specialty's language, preferences and difficulties will aid in getting the optimal diagnostic outcome for our patients, and will aid towards translating this to clinical outcome as well.

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Determining resection margins as a pathologist – clean but close or too close to be clean?

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Assessment and interpretation of tumour margins is controversial for both clinicians and pathologists. This talk aims on giving an overview of current status of margin assessment in veterinary oncology from a pathologist's point of view.

As a starting point, methods and techniques to determine tumour margins histologically and potential pitfalls and artefacts introduced by handling, fixation and processing are summarised (Kamstock et al. 2011, Milovancev and Russell 2017, Risselada et al. 2015 and 2016).
Inking/marking of tumour margins by the surgeon vs. the pathology lab are discussed. Different trimming techniques for tumour margin assessment (transverse and cruciate sections, bread-loafing and shaving of tumour margins) and trimming of more complex tissue, such as toes, legs or intestinal samples are outlined, and the advantages and disadvantages of the methods are reviewed. For example, shaving the surgeon-cut edge is a good method for relatively small masses, for which clinically, a wide margin was aimed for, but the tumour is poorly demarcated or irregularly shaped. Shaving the surgeon-cut edge is, however, unsuitable for well-demarcated tumours excised with minimal margins, as the minimum tissue thickness of shaved margins is 1-2mm and tumour tissue will inevitably be present in the shaved margins.

Overall, the tissue trimming techniques to assess tumour margins are not standardised in veterinary medicine. An attempt has been made by Kamstock et al. (2011) but they conclude that 'a consensus regarding an optimal technique for margin evaluation could not be reached, despite much debate, given the absence of scientific support in this field.'
In conclusion, there is neither a standardised nor a 'one fits all' approach for tissue trimming for tumour margin assessment. Furthermore, in veterinary medicine the extent of margin assessment is clearly limited by cost.

An overview of the confusing terminology (positive, negative, clean, close but clean, marginal, wide etc.) used in tumour margin assessment in the veterinary (and human) literature is given.
For example, a 'marginal excision' can be anything between ≤1mm of the surgeon-cut edge or no tissue outside the pseudocapsule (McSporran et al. 2009) and '<1-3 cm margin or not including a fascial plane (Stefanello et al. 2008)'.
Most literature in veterinary medicine on the relation of tumour margins and local recurrence is published for canine soft tissue sarcomas and canine cutaneous (and subcutaneous) mast cell tumours, which are discussed as examples.
For both, canine soft tissue sarcomas and mast cell tumours, traditionally, a wide excision within 3cm lateral margins and one intact fascial plane deep are recommended (Dernell et al. 1998, Schultheiss et al. 20011, Simpson et al. 2004). However, more recent literature suggests that the width of the margin is not predictive for local recurrence and that the percentage of tumour recurrence is more dependent on the histologic grade than on the extent of the margin, suggesting that narrower margins could be as effective to prevent local recurrence for low grade soft tissue sarcomas and mast cell tumours (Bray et al 2014, Dennis et al 2011, Donnelly et al 2013, McSporran et al 2009, Stefanello et al 2008, Schultheiss et al. 2011, Simpson et al. 2004 and Weisse et al. 2002).

Limited information regarding the relation between tumour margins and clinical outcome is published for a number of other tumours, e.g. canine squamous cell carcinoma and feline injection site sarcoma (Milovancev and Russell 2017). However, for the majority of tumours in domestic animals, no grading schemes and no published data regarding the relation of local recurrence and tumour margins are available. For those tumours, description and interpretation of histologic features of malignancy and growth pattern (e.g. expansile, pushing, bluntly infiltrative, diffusely infiltrative), demarcation and encapsulation are especially important and need to be reported together with the width of the margin.

In summary, the assessment and interpretation of tumour margins in veterinary oncology is poorly standardised and challenging for both, the clinician, as well as the pathologist.
A meaningful tumour margin assessment depends on clear communication of all relevant information between the clinician and pathologist, starting with a concise clinical history and marking/ inking of (important) surgeon-cut surfaces by the clinician. From the pathologist's side, margin assessment should not equal a number, but should include the tumour grade (if applicable), as well as the behaviour of the tumour at its borders (expansile/ infiltrative, well-demarcated/ poorly demarcated, contiguous outline/ satellite tumour nests) and the quality of the margin (e.g. few strands of fibrous connective tissue vs. an intact fascial plane) in an integrated statement.
Controversial terms, such as 'clear', 'marginal' or 'wide' are best avoided, unless a definition is provided.
In conclusion, tumour margin assessment and interpretation need to be understood as an integrated safety assessment conducted by the clinician and pathologist as a concerted effort.

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Oral Presentations

Adjuvant dose-intense versus metronomic chemotherapy for dogs with metastatic splenic hemangiosarcoma: a multi-institutional retrospective study

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Introduction

Treatment options for dogs with metastatic (stage III) splenic hemangiosarcoma are limited. A doxorubicin-based chemotherapy regimen is commonly administered; however, there are no published data to support this practice. The aim of this study was to investigate the impact of maximum-tolerated dose chemotherapy (MTD), metronomic chemotherapy (MC) and no adjuvant treatment in terms of outcome in dogs with stage III splenic hemangiosarcoma undergoing splenectomy.

Materials and methods

Medical records of dogs with stage III splenic hemangiosarcoma that underwent splenectomy followed by MTD chemotherapy, MT or no adjuvant treatment were retrieved. Time to progression (TTP), survival time (ST) and toxicity were evaluated.

Results

103 dogs were identified: 23 received adjuvant MTD, 38 MC, and 42 were not medically treated. Overall median TTP and median ST were 50 days (95% CI, 39-61) and 55 days (95% CI, 43-66), respectively. Dogs treated with adjuvant MTD had a significantly longer TTP and ST compared with dogs treated with MC (median, 134 versus 52 days, p=0.025; and median, 140 versus 58 days, p=0.023, respectively). Dogs treated by splenectomy only had the shortest median TTP (28 days) and ST (40 days). However, treatment-related adverse events (AEs) were significantly higher in the MTD group (p=0.017).

Conclusions

The outcome of dogs with metastatic splenic hemangiosarcoma is poor. While MTD showed greater efficacy compared to MC, toxicity was higher. Treatment-related AEs need to be carefully balanced against a modest survival prolongation. When efficacious treatments are no longer options for dogs with terminal cancer, the focus should shift from prolonging life to maintaining quality-of-life.

Is radiation therapy a useful treatment option for meningoencephalomyelitis of unknown origin in dogs?

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Introduction

The term meningoencephalomyelitis of unknown origin (MUO) summarizes inflammatory cerebral disease with focal or multifocal lesions and abnormal cerebrospinal fluid, free of infectious causes. Treatment options include immunosuppression with various drugs such as corticosteroids, calcineurin inhibitors or cytotoxic agents. A gold standard for appropriate treatment has not been established.
Low dose radiation therapy has been described to suppress inflammation. Our objective was to retrospectively investigate long-term outcome after combined radiation therapy with corticosteroids.

Materials and methods

We treated dogs with focal or multifocal MUO diagnosed on diagnostic imaging. Radiation consisted of 10x3Gy to the whole brain. Focus was set on the clinical response to radiotherapy, time to symptomatic progression (TTP) and overall survival time (OS).

Results

Fourteen dogs (1 with focal, 13 with multifocal lesions) were included. At time of radiation, 11 patients (78.6%) were fully or partially refractory to medical treatment.
Median OS was 732 days (95%CI:0;2556). Of the 9/14 (64.3%) dogs that died, 4/14 (28.6%) died within 3 months post radiotherapy. Six dogs showed clinical complete and five dogs partial resolution of symptoms after radiation. Eight dogs showed progression after a median TTP of 631 days (95%CI:0;2130). Median follow-up time was 2022 days (95%CI:1160;2885).

Conclusions

Radiation therapy appears as a viable treatment option for dogs with MUO in medically refractory cases. Comparable to medical treatment, about 30% died within 3 months after radiotherapy but there is a 40% fraction of long-term survivors with OS >48.4 months. For the future we plan to compare radiation therapy to cytotoxic treatment in a randomized setting.

DNA double-strand breaks quantification in U87 cell-line exposed to 165Holmium, combined with ionizing radiation

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Introduction

Radioactive microspheres hold promise for the treatment of various tumors, allowing delivery of high concentration of radioactivity to the target area without causing damage to the surrounding tissues. Among other radionucleotide, Holmium (166Ho) is recently sparking interest. Its unique properties make it visible with medical imaging, enabling easier treatment monitoring. However, few data exist regarding toxicity of its stable isotope : 165Ho. The objective of this study was to characterize the impacts of 165Ho on DNA-damage induction, recognition and repair.

Materials and methods

Endpoint measured consisted of quantitation of DNA double-strand break (DSB) formation, based on immunofluorescence-recognition of phosphorylated-H2AX foci. U87 glioblastoma cells, known for their resistance to radiation, were first exposed to a solution of 165Ho microspheres at concentrations ranging from 0mM to 130mM, and the mean number of H2AX-foci par cell was determined for each concentration. 165Ho was then combined to a physical stressor (2 Greys of ionizing-radiation) and the DSB-recognition kinetics was established.

Results

The dose-effect curve was non-linear, fitting a curvilinear model, as reported with other metals. An increament of 100mM of 165Ho led to the recognition of three H2AX-foci after one hour repair time and two foci after 24 hours repair time. Combined action of ionizing radiation and 165Ho led to impaired DNA-damage recognition compared to ionizing radiation only.

Conclusions

Our data strongly suggest that 165Ho is able to induce a low amount of DNA-damages in a dose-dependent manner, and to impair recognition of DSB. The mechanism underlying these effect may include interaction with the ATM-protein, major determinant of DSB recognition and repair.

Low dose metronomic cyclophosphamide as adjuvant treatment in feline mammary carcinomas – A preliminary evaluation

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Introduction

Feline mammary tumors (FMT) are highly malignant neoplasms, with elevated metastatic rates. This study aims to evaluate the efficacy and side effects of low dose cyclophosphamide chemotherapy (LDM) as adjuvant treatment vs high dose doxorubicin or surgery alone in cats with mammary cancer.

Materials and methods

Medical records of 109 female cats treated between 2008 and 2018 for mammary cancer were reviewed. Cats were included in the study if they underwent a complete tumor staging (classified according to WHO) after performing radical surgical excision. The cats were divided into 3 different treatment groups: Group1 (n=66) included cats treated only with surgery, Group2 (n=26) cats that had surgery and adjuvant treatment with doxorubicin and Group3 (n=17) cats with surgery and adjuvant treatment with LDM. The study end points were time to progression (TTP) and tumor specific survival (STS). Toxicity was evaluated according to the VCOG-CTCAE criteria.

Results

Median TTP was 270, 238 and 408 days in groups 1, 2 and 3 respectively. Median STS was 414 (Group1), 447 (Group2), and 708 (Group3) days. Differences in TTP and STS between the groups were not statistically significant (p= 0,431 and p=0,172 respectively). Toxicity was observed in 65,3% of the cats in group 2 and in 30% of the cats in group 3, with mild to moderate intensity.

Conclusions

Adjuvant LDM may be an option for FMT with median TTP and STS similar than previous reported, with low toxicity associated. Randomized prospective trials are necessary to determine if LDM is superior than Doxorubicin.

Evaluation of a double deleted oncolytic Vaccinia virus encoding FCU1 protein in dogs diagnosed with malignant solid tumors

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Introduction

Oncolytic virotherapy offers a promising treatment modality for cancer. TG6002 is a double deleted oncolytic Vaccinia virus (VV) expressing the FCU1 protein which converts 5-fluorocytosine (5-FC) into 5-fluorouracil (5-FU). A pre-clinical study in healthy dogs has confirmed safety of TG6002 administration at a dose of 5.10^7pfu/kg with 5-FC. Objectives were to: 1/ assess tolerance, 2/ evaluate intratumoral synthesis of 5-FU and response to treatment in dogs with malignant tumors.

Materials and methods

14 dogs with malignant, non-operable, solid tumors were included. Three weekly intratumoral injections of TG6002 (5.10^7pfu/kg) were performed with oral administration of 5-FC.Tolerance was evaluated by clinical exams, complete blood count and biochemistry. Production of 5-FU was measured on biopsies by liquid chromatography and high-resolution mass spectroscopy. Response to treatment was evaluated by computed tomography scan at one month.

Results

Main adverse events consisted in reversible ulcerative and crusted skin lesions (n=7/14). qPCR results for VV detection were negative in cutaneous lesions. Histological exams were diagnostic of toxic epidermal necrolysis suspected to be induced by 5-FC. Hepatic parameters were elevated in one dog. No hematological toxicity was observed. 5-FU was detected in tumor biopsies (n=3/3). Of the 9 dogs included, one achieved a partial response, four showed a stable disease and four had a progressive disease.

Conclusions

Taken together, these results indicate a satisfactory safety profile of TG6002. Intratumoral synthesis of 5-FU is observed, supporting the interest of TG6002 treatment in cancer therapeutic approach. However, the cutaneous toxicity probably induced by 5-FC will require dose adjustment.

Canine anal sac adenocarcinoma with lymph node metastases treated with anal sacculectomy and lymphadenectomy: is there a need for a new staging system?

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Introduction

Surgical debulking is a common strategy in the management of canine anal sac adenocarcinoma (ASAC) with lymph nodes metastases. The size of the largest lymph node has been associated with survival and plays a major role in the currently used staging system.

The purpose of this study was to evaluate the prognostic significance of the number of metastatic lymph nodes in dogs with metastatic ASAC treated with anal sacculectomy and lymphadenectomy; lymph node size was also revaluated.

Materials and methods

Medical records of dogs with ASAC and metastatic disease confined to the lymph nodes, from 2008 to 2018, were reviewed. Only dogs that underwent anal sacculectomy and lymphadenectomy were included. Histopathological reports and pre-operative images were reviewed. The absolute lymph node sizes and normalised lymph node ratios (lymph node/aorta, lymph node/weight) were assessed. Disease-free interval (DFI) and overall survival (OS) were evaluated.

Results

Thirty-three dogs were included. The median OS was 482 days (range 0-1777 days). The median DFI was 200 days (range 0-711 days). Lymph node size was associated with DFI and OS on univariable analysis but not on multivariable analysis. On multivariable analysis, a primary ASAC larger than 25mm ($p=0.02$), and the presence of two or more metastatic lymph nodes ($p=0.02$) were associated with decreased DFI. No factors were associated with OS.

Conclusions

The size of the metastatic lymph nodes may not be a reliable prognostic factor in canine ASAC. The number of metastatic lymph nodes may have a more valuable role in predicting prognosis in canine ASAC with locoregional metastases.

Definitive radiation therapy for dogs with oral papillary squamous cell carcinoma.

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Introduction

Canine oral papillary squamous cell carcinoma (COPSCC) is a rare and locally invasive neoplasm that carries a favorable prognosis following radical excision. This retrospective case-series study describes dogs diagnosed with a macroscopic COPSCC treated with definitive radiation therapy (DRT) as sole treatment.

Materials and methods

Medical records of dogs diagnosed with COPSCC treated with DRT between May 2010 and January 2019 at UUACC were reviewed. Data collected included signalment, tumor location, tumor size, staging results, DRT protocol, treatment response (RECIST criteria), toxicity (VRTOG) and outcome.

Results

Nine dogs (F:M = 2:7) with histologically diagnosed COPSCC were included. The median age was 5-years (range 0.4-9.6 years). The rostral oral cavity was affected in all dogs, the median tumor size was 24 mm (range 8-68 mm) and no local or distant metastases were identified. Dogs were treated with electron beam DRT (>32 Gy, 10-16 daily fractions of 3.2Gy). Median follow-up was 19.5 months (range 1-103 months). Seven dogs achieved a complete response and two dogs a partial response (remnant lesion was histologically free of neoplasia). Two dogs died from non-tumor-related causes, 2 and 5 years after DRT respectively. The remaining 7 dogs were still alive at time of this analysis. Median progression-free survival time and median survival time were not reached. The DRT was generally well tolerated. All dogs experienced self-limiting grade 2 or 3 oral mucositis. No late adverse effects have been reported.

Conclusions

Macroscopic COPSCC is a highly radiosensitive tumor that can be successfully treated with DRT, eliminating the need for aggressive surgery.

Expression of HIF-1 alpha and hypoxia-related markers correlate with prognosis in canine appendicular osteosarcoma

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Introduction

Cellular adaptation to hypoxic microenvironment is essential for tumor progression and largely mediated by HIF-1? and other hypoxia regulated markers, such as CXCR4, VEGF-A and GLUT-1. In human osteosarcoma, these proteins are correlated to aggressive behavior, chemoresistance and shorter survival. The aims of this study were to evaluate the potential prognostic role of these markers in canine osteosarcoma and to investigate their regulation under hypoxic conditions.

Materials and methods

Immunohistochemical analysis and quantification for HIF-1?, CXCR4, VEGF-A and GLUT-1 were performed on 56 canine appendicular osteosarcomas. The correlation with the histological grade, overall survival and disease-free interval were investigated. Two primary canine osteosarcoma cell lines were cultured and exposed to hypoxia (CoCl2) to highlight the modifications of CXCR4, VEGF-A and GLUT-1 genes and proteins by q-RT-PCR and western blot assay.

Results

Immunohistochemistry showed that samples with a higher histological grade (35.7%) were associated with increased HIF-1? and GLUT-1 expression (p

Conclusions

These results show that in canine osteosarcoma a more aggressive behavior and progression is associated with a significantly higher protein expression of hypoxia-induced markers. The in vitro results confirm that hypoxic conditions cause accumulation of HIF-1? in osteosarcoma cells reflecting a higher transcriptional activity on the hypoxia related markers investigated.

Non-Injection Site Soft Tissue Sarcoma In Cats: Outcome Following Adjuvant Radiotherapy

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Introduction

Biological behaviour and treatment options of non-injection site soft tissue sarcomas (STS) in cats are less well understood than in dogs. The aim of this retrospective study was to assess the outcomes of cats with non-injection site sarcomas following treatment with adjuvant radiotherapy.

Materials and methods

The medical records of cats with soft tissue sarcomas in locations not associated with and histology reports not consistent with injection site sarcomas were reviewed. All cats underwent adjuvant radiotherapy, either fractionated (48-54Gy delivered in 16-18 3Gy fractions) or hypofractionated (32-36Gy delivered in weekly 8-9Gy fractions) to microscopic disease.

Results

Eighteen cats were included in the study, seventeen with extremity STS and one facial STS. Nine received radiation after a single surgical procedure and nine after multiple surgeries for recurrent STS. Median follow up time was 540 days (range 51-3317 days). Sarcoma recurred in 6/18 cats following adjuvant radiation therapy, of which three cats had previously recurrent STS following surgery. 3/8 recurred in the hypofractionated group and 3/10 in the fractionated group. The median PFI for 5/6 cats with recurrence was 121 days (range 58-240 days). The median PFI for 17/18 cats was 362 days (range 51-3317 days) and the median PFI for 12/18 cats without recurrence was 602 days (range 51-3317 days).

Conclusions

Adjunctive radiation resulted in good long-term tumour control in 12/18 cats with non-injection site STS. Further studies in larger populations are required to assess the significance of radiation dose and fractionation on tumour control and the effect of multiple surgeries on outcome.

Using the dog's definitive-intent radiotherapy protocol for sinonasal carcinomas in cats: a multicenter retrospective assessment

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Introduction

Treatment of epithelial sinonasal tumors in cats is not well established and palliative protocols are often used. Limited data regarding prognosis and prognostic factors after definitive-intent radiation therapy exists. In this multicenter retrospective study, we (1) describe the outcome after radiation therapy, and (2) investigate the influence of variables on outcome.

Materials and methods

We included cats with local or locoregional sinonasal epithelial tumors treated with definitive-intent 10x4.2Gy radiation therapy. Cats with distant metastasis or previous treatment were excluded. We investigated the influence of presumed prognostic factors such as inappetence or epistaxis at diagnosis, tumor size, location, histology, modified canine Adam's stage, locoregional metastases on time to progression and overall survival.

Results

We collected 25 cats with sinonasal carcinomas from 3 institutions, all treated with the same radiation protocol. 14/25 cats (56%) had advanced (Adam's stage IV) disease and 4/25 (16%) had locoregional metastases. 2/25 (8%) received an adjuvant chemotherapy. Median time to progression was 269 days (95%CI:221;318), with only 21.9% of cats free of progression at 1 year. 80% of cats had local tumor recurrence. Overall survival was 452 days (95%CI:318;587), with 58.5% and 27.4% of cats alive at 1 and 2 years, respectively. None of the variables had a significant infuence on the outcome.

Conclusions

Outcome after treatment with this definitive-intent radiation protocol remains guarded and seems to be similar to palliative protocols. No prognostic factor was identified for sinonasal carcinoma in cats.

High-Resolution Transcriptome Analysis of Feline Mammary Carcinomas and derived cell lines

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Introduction

Feline mammary carcinomas (FMCs) heterogeneity has been realised through histopathology and immunohistochemistry. Nonetheless, the actual extent of diversity can be appreciated only through detailed molecular approaches. Next-generation sequencing (NGS) allows characterisation of differentially-expressed genes (DEGs) modulating the FMCs dysregulation. This study aimed to identify DEGs, dysregulated molecular pathways, and possible biomarkers and therapeutic targets in FMCs and derived cell lines.

Materials and Methods

Transcriptomic analysis was performed on RNA isolated from tumour and healthy mammary samples (paraffin-embedded and frozen-tissue) from 33 female cats with FMCs, and two FMCs-derived cell lines. Additionally, the immunoexpression of epithelial, mesenchymal, and hormonal markers was assessed.

Results

At the transcriptomic level, immunohistochemical groups were not separated. However, common DEGs in human triple-negative and claudin-low breast cancers were identified (e.g. *FOXM1, MYBL2,* and *HSPB7)*. Upregulated genes influenced cell-growth and death regulation (e.g. *CDK1, ESPL1, CHEK1, MCM3,* and *CCNB1*). Downregulated genes were involved in pathways that prevent tumour spreading including tight-junction components (e.g. *CLDN4, CLDN5, CLDN8* and *CLDN23*) and cell adhesion molecules (e.g. *CD40, CDH1, ICAM2, ITGAM* and *ITGB2*). DEGs participating in the PI3K-Akt (e.g. *FLT4, PDGFD* and *BRCA1*) and p53 (e.g. *CCNB2, CDK1* and *RRM2*) signalling pathways were identified. Cellular models shared many similarities with original tumours; however, alterations correlated with endocrine regulation, circadian rhythm and metabolic pathways showed important differences.

Conclusions

NGS can be used to identify pivotal biological processes in FMCs. Furthermore, transcriptomic profile comparison of FMCs and cell lines provide information about which aspects of the neoplastic change can be modelled *in vitro* and also denote important constraints.

"Outcomes of canine subcutaneous mast cell tumours treated with adjunctive radiation therapy"

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Introduction

Subcutaneous mast cell tumours (SCMCT) generally behave in a benign fashion however, around 9% will recur locally and histopathological features such as MI and Ki67 can predict local recurrence and metastasis. Our aim is to describe the outcomes of a population of dogs with excised SCMCT treated with adjunctive radiotherapy.

Materials and methods

Records of canine patients that underwent incomplete or narrow resection of SCMCT at four referral hospitals (2008-2016), who completed hypofractionated (30-40Gy in 1-2 weekly fractions) or fractionated (>40 Gy daily or M/W/F) protocols were reviewed.

Results

Fifty-five dogs were included with median time between surgery and radiotherapy 34 days (Range: 8-152). High grade histopathological features of SCMCT were observed in 7/55 patients, low grade in 46/55 and 2/55 were unknown. None had recurrent local disease with median follow up of 987 days (Range: 21-2444). Median survival time was 987 days. 4/55 patients had MCT related death, 38/55 were alive and disease free at the end of the study and 3/55 were lost to follow up. Eleven patients received fractionated radiation with median DFS 387 days (Range: 42-1471) and 44/55 hypofractionated with median DFS 1059 days (Range: 21-2444). Patients with low grade SCMCT had longer MST (1022 days) than high grade features patients MST (598 days). Eight dogs received concurrent chemotherapy; 3/8 were high grade, 4/8 low grade and 1/8 unknown.

Conclusions

Adjunctive radiation for SCMCT results in excellent long term local tumour control. Lower total doses and hypofractionated regimens may be adequate for the control of SCMCT

Radiotherapy Outcomes in Dogs with Solitary Plasma Cell Tumors: 29 Cases

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Introduction

Plasma cell tumors are common skin, oral, and vertebral neoplasms in dogs. To date, little information has been published on outcomes following treatment with radiation therapy. This retrospective study reports results from a single institution from 2008-2017.

Materials and methods

Hospital records were reviewed to identify patients with solitary plasma cell tumors not including multiple myeloma. Dogs with a minimum follow up of one year were included. 29 patients were identified with 31 tumors, receiving a total of 34 treatments (including retreatment of relapses and treatment of new tumors). Sites included 15 cutaneous, 7 oral, 4 vertebral, and 5 other locations. Outcomes were assessed for the following: gross vs microscopic, oral vs cutaneous vs vertebral, tumor volume at diagnosis, tumor response, time to first event (TFE), median survival (MST), radiation protocol, bone involvement, and mitotic index. Death due to tumor vs other causes was also evaluated.

Results

No statistically significant differences were identified among patients. 24% were alive at report time and were censored. Median TFE and MST were both 822 days. 78% of gross tumors had complete response. Local relapse occurred in 13% of patients. 82% of deceased patients died of other causes. Median survival was not reached for the remaining patients.

Conclusions

Radiotherapy is an effective treatment for plasma cell tumor regardless of location or size. It induces durable remissions in gross and microscopic disease and local relapse is uncommon. Even palliative protocols can induce durable remissions. Radiotherapy should be considered as a primary treatment for this tumor.

Outcome of canine high-risk cutaneous mast cell tumours treated with adjuvant radiation therapy alone or combined with chemotherapy

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Introduction

Data presented are part of a large multi-institutional retrospective study collecting information of dogs with cutaneous mast cell tumour (MCT) treated with adjuvant radiotherapy.

Materials and methods

The aim of the sub-analysis is to evaluate outcome and prognostic factors of patients with high-risk MCTs treated with adjuvant radiotherapy. MCTs were considered high-risk if they presented one of the following: high Patnaik/Kiupel grade, mitotic index or Ki67, or stage II.

Results

A total of 77 dogs met these criteria. At the time of data collection 44(57%) dogs were alive, 11(14%) died from unrelated causes, 20(26%) for systemic and 2(3%) for local disease. Median follow-up for dogs alive was 912 days (27-3399). The radiation protocol was definitive (daily/MWF; total dose>40Gy) in 38% and palliative (weekly; total dose

Conclusions

These results suggest that many dogs with high-risk MCTs benefit from radiotherapy with better than historically reported outcomes. Treatment with definitive protocols appears advantageous.

Malignant melanoma of the nasal planum in cats - A report of 6 cases

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Introduction

Malignant melanomas (MMel) are rare in cats with ocular, oral and digital as the most common primary sites. Although single cases of feline nasal planum MMel have been reported, the literature lacks detailed clinical information. To the authors knowledge this is the first case series of feline MMel of the nasal planum.

Materials and methods

Retrospective review of 6 cats with histologically confirmed MMel of the nasal planum presented to the oncology service of Hofheim Small Animal Hospital (Germany).

Results

Breed distribution was 4 Domestic shorthair, 1 Persian, 1 Ragdoll; mean age was 12.3 years (range, 7 to 17 years); there were 5 male and one female cat. All cats had a darkly pigmented nasal planum. Masses were present for 2 weeks to 33 months prior to diagnosis; 3/6 cats had lesions present for longer than 1 year, which had previously been diagnosed as benign melanomas. The tumors presented as 0.7-2 cm pigmented masses and were ulcerated in 2 cases. Two cats had confirmed lymphnode metastasis at time of diagnosis, one had also radiographic signs of pulmonary metastasis. Four of the 6 cats were treated with hypofractionated radiation therapy (4 x 9 Gy, once weekly) and had partial (n=2) or complete responses (n=2). Recurrences occurred after 30 and 92 days (PR), and 160 and 230 days (CR), mean ST of treated patients was 280.5 days (range, 180-334 days).

Conclusions

Elderly cats with pigmented nasal planum may be predisposed to MMel of the nasal planum. Hypofractionated radiation therapy can lead to short remissions.

Discovery of chromosomal amplifications in canine oral melanoma as a new prognostic factor

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Introduction

Canine oral melanoma is characterized by a local invasiveness and a high metastatic propensity. A better knowledge of the genetic bases is expected to improve management of this tumour. Copy number alterations are known characteristics of mucosal melanomas in dogs and humans. The goal of this study is to explore the prognostic value of specific relevant chromosomal amplifications in canine oral melanoma.

Materials and methods

The cohort includes 75 dogs with oral melanoma confirmed by histology, removed surgically without other adjuvant therapy and with a minimal follow-up of 10 days. Clinical information were obtained from referring veterinarians and histological data were reviewed by a board-certified pathologist. Quantitative PCR and Fluorescence In Situ Hybridization were performed on FFPE samples to identify these specific amplifications.

Results

The 75 dogs included in the study had a median survival time of 203 days. The studied chromosomal amplifications were recurrent (50% of dogs) and one of those was linked to poor prognosis (Log Rank test, p=5.7e-04). Other negative prognostic factors included gingiva location (p=5.2e-03), enlarged lymph nodes and tumor ulceration (p=0.024 and p=0.015), high mitotic index -MI- (p=1.2e-03) and achromic tumor (p=0.037). In bivariate analyses using Cox proportional hazards regression, this chromosomal amplification was associated with survival time (Hazard Ratio (HR)=1.97; p=0.020) independently of MI (HR=2.21; p=0.025 for dogs with MI>6), and tumor location (HR=2.02; p=0.013 for dogs with gingival melanomas and HR=2.14; p=9.6e-04 for dogs with chromosomal amplification).

Conclusions

This chromosomal amplification constitutes a new prognostic factor and may be an interesting therapeutic target in canine oral melanoma.

Controlled, Randomized Study of Intratumoral Tigilanol Tiglate (EBC-46) for Treatment of Canine Mast Cell Tumors

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Introduction

Tigilanol tiglate, isolated from the Australian rainforest plant Fontainea picrosperma, possesses antitumor activity and enhanced wound healing stimulation at the treatment site via activation of protein kinase C. Tigilanol tiglate may be effective when injected intratumorally as treatment for canine mast cell tumor (MCT).

Materials and methods

Dogs with cutaneous or lower limb subcutaneous MCT were enrolled and randomized 2:1 to treatment with a single intratumoral injection of tigilanol tiglate or sham treatment (untreated controls) in an investigator- and owner-masked multicenter study. Primary efficacy outcome was complete response (CR; disappearance of target lesion) on Day 28. Treated dogs with less than CR could receive a second intratumoral injection on Day 30 and untreated dogs could be crossed over to treatment.

Results

Enrolled dogs numbered 123 with 118 evaluable. Sixty of 80 dogs (75%) randomized to tigilanol tiglate treatment achieved CR after initial injection compared with 2/38 untreated dogs (5.3%) by Day 28 ($P < 0.0001$). Eighteen of 20 treated dogs not achieving CR received a second injection. Eighty-seven percent (68/78 evaluable) of treated dogs achieved CR within the two-dose treatment strategy. Ninety-six percent (55/57 evaluable) dogs achieving CR after first injection remained tumor-free at day 84. The most frequent adverse events were transient reactions at the treatment site, anticipated pathology associated with drug mechanism of action. Wounds developed in 92.5% (74/80) of treated dogs and healed rapidly from Day 7.

Conclusions

Tigilanol tiglate was highly effective for treatment of cutaneous and lower limb subcutaneous MCT in dogs and was well tolerated with manageable side effects.

Clinicopathologic characteristics, postoperative outcome and prognostic factors in 61 dogs with primary pulmonary carcinomas

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Introduction

Canine primary pulmonary carcinoma (PPC) is treated surgically. The role of chemotherapy remains unclear. The goal of this study was to broaden the veterinary database on postsurgical median survival times (mST), prognostic factors and Vinorelbine chemotherapy.

Materials and methods

Retrospective analysis of 61 dogs with PCC treated surgically between 2007 and 2017. Survival analyses were performed using the Kaplan-Meier and Logrank methods. Potentially significant variables were evaluated.

Results

Of the 61 tumours, 33 (54 %) were located peripherally in the lung, 22 (36 %) close to the hilus, 6 (10 %) affected the entire lobe. Tracheobronchial lymph nodes (TBLN) were histologically positive for metastasis (N1) in 9 cases, negative (N0) in 42 patients, no TBLN histology was performed in 10 cases. Long-term follow-up information was available for 47 dogs. Variables with prognostic impact were presence of TBLN metastasis (mST: N1 24 days, N0 570 days; p=0.0001), location of the tumour (mST: hilar 240 days, peripheral 650 days; p=0.00047) and lung metastases (mST: M1 157 days, M0 570 days; p=0.0042). Tumour size or carcinoma subtype was not prognostic. In 22 patients with negative prognostic factors (LN1 and/or M1 and/or hilar location) there was a trend towards better ST in 5 dogs treated with Vinorelbine (5-6 x 15mg/m²) compared to 17 patients that were left without adjuvant chemotherapy (mST with chemotherapy 515 days, no chemotherapy 240 days).

Conclusions

TBLN status, M1 and tumour location were confirmed as prognostic factors in canine PPC. Dogs with negative prognostic factors may profit from Vinorelbine chemotherapy.

Lymphoma risk by clade and age of diagnosis as potential markers of genetic risk in dogs.

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Introduction

Lymphoma is a common malignancy in dogs and is used as a model for human disease. Canine clades as described by Heidi Parker (Cell Reports, 2017) shows genetic linkage between breeds. Early onset of disease also might reflect genetic risk. This study looked at the lymphoma risk in the 23 clades and the age of onset within breeds.

Materials and methods

Data on 6205 Australian dogs with lymphoma was used. Dogs were assigned to a clade which was assessed for risk using OR. Individual breeds were compared to the overall clade risk. When the age of diagnosis was known, this was compared to control dogs of that breed and the lymphoma population.

Results

Six clades were found to be at statistically significant increased risk of lymphoma and five at decreased risk. There were five breeds that differed significantly from their clade. In all cases they were at decreased risk of lymphoma, whereas the clade was at increased risk. When analysed for age of onset, six breeds had a distinct early onset, four had a smaller peak early overall median older than the controls.

Conclusions

Patterns of lymphoma risk persist across clades, supporting an underlying genetic risk factor. The age of onset varies from younger than the median of the control population in some breeds, while in others it is older, suggesting that there are differing risk factors between breeds. The breeds that are contrary to the clade risk offer potentially unique opportunities to assess genetic risk factors.

Opportunities and challenges of active immunotherapy in dogs with B-cell lymphoma: a 5-year experience in two veterinary oncology centers

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Introduction

CHOP-based chemotherapy represents the treatment cornerstone for canine B-cell lymphoma (BCL); however, cure is rare. We have been treating dogs with BCL with chemo-immunotherapy since 2011.

Materials and methods

To better characterize safety and efficacy of immunotherapy, and to find the best candidates, we compared dogs treated with chemo-immunotherapy with dogs treated with CHOP-based chemotherapy. All dogs were completely staged and followed-up. Primary endpoints were time to progression, lymphoma-specific survival (LSS), and 1-, 2-, and 3-year survival rates.

Results

Three hundred dogs were enrolled: 148 received chemotherapy and 152 chemo-immunotherapy. Among dogs with diffuse large B-cell lymphoma, the benefit of chemo-immunotherapy was particularly relevant in dogs with a high serum LDH, stage V, substage b, and in those not previously treated with steroids (median LSS, 480 vs 85 days; P

Conclusions

Overall, adding immunotherapy to a CHOP-based protocol improved outcome in dogs with BCL, regardless of histotype and evaluated prognostic factors. Moreover, the identikit of the best candidate for immune-therapy was delineated.

Canine splenic masses: a systematic review of the literature for possible pre-surgical determinants of malignancy and creation of a new grading system

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Introduction

The difference in prognosis between benign versus malignant splenic masses is substantial and data more than 30 years old report a 1/3 benign versus 2/3 malignant distribution. Objectives of the study was; to determine, by means of systematic review of the literature, whether there are any pre-surgical parameters, either alone or in combination, that may aid predicting splenic mass malignancy.

Materials and methods

An electronic database search was performed. The data were collected and collated into the Preferred Reporting Items for Systematic Review and Meta-analyses (PRISMA) flow diagram. Inclusion criteria were: published within the last 30 years, canine patients, original data, presence of a splenic mass, final histology and pre-surgical data.

Results

A systematic search of the literature yielded a total of 17 papers that met inclusion criteria. Assessment lead to evaluation of several pre-surgical parameters: signalment, presenting signs, haemoabdomen, laboratory findings and diagnostic imaging. Overall, the review reported 1972 canine splenic masses of which 46.3% were benign and 53.7% were malignant. Other interesting finding is that German Shepherds were overrepresented in both benign and malignant group. Haemoabdomen was found in 60% of malignant versus 33% of benign splenic masses. More than 50% of splenic haematoma presented with an haemoabdomen. Based on the systematic review, a novel five-tier points grading system was formulated and will need to be evaluated prospectively.

Conclusions

Incidence of malignant splenic mass might not be as high as previously reported especially if incidental. No pre-surgical data appears to be predictive on its own but a new five-tier points grading system was developed.

USP7 inhibitor as a novel drug candidate for canine lymphoma/leukemia treatment

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Introduction

The ubiquitin specific peptidase 7 (USP7/HAUSP) is a cysteine protease which regulates the activities of numerous proteins like tumor suppressors, DNA repair proteins or immune responders. Aberrant USP7 activity has been linked to various type of cancers in humans, which provoked research into the use of USP7 inhibitors (USP7i) as anticancer drugs. Until now nothing is known about the activity of USP7i in canine cancers.

Materials and methods

Canine lymphoma/leukemia cells were used in the study. Expression of USP7 was examined by western blot. Metabolic activity of the cells was measured using MTT test. Induction of apoptosis was determined by Annexin V/PI staining. Cell cycle progression was measured by flow cytometry.

Results

USP7 is overexpressed in canine lymphoma. USP7i (P5091) clearly decrease cell metabolic activity as soon as after 24h of incubation (IC50 values were 8.04±0.53 for CLBL-1, 10.19±3.11 for CLB70, 8.21±1.63 for CNK89 and 12.77±3.08 μM for GL-1 cell lines). Cell cycle analysis revealed accumulation of the cells in G2/M phase. Annexin V/PI staining showed that P5091 treated cells undergo apoptosis. The proportion of apoptotic cells after 24h incubation with 8 μM of P5091 differed between the cell lines and accounted for 75.4±8.2 percent in CLBL-1, 64.4±11.3 in CNK89 and only 27.6±2.7 and 13.2±6.8 in CLB70 and GL-1, respectively.

Conclusions

USP7 protein may be an interesting target in canine lymphoma. Detailed research on the mechanism of action of USP7i in canine cancers are needed for the future selection of patients who may benefits from such anticancer therapy.

A novel aggressive T-cell lymphoma/leukemia in young dogs

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Introduction

During routine clinical immunophenotyping, we identified a unique CD4-CD8- T-cell lymphoma/leukemia expressing low levels of CD25 and class II MHC in young dogs, with a strong breed predilection for English Bulldogs. This study describes the clinical characteristics and outcome for this neoplastic entity.

Materials and methods

We retrospectively examined flow cytometric features, clinical presentation and survival data from English Bulldogs with a lymphocytosis of CD5+CD3+CD4-CD8-CD25loMHCIIlo T-cells. We then searched our clinical database to assess the frequency and clinical characteristics of this neoplasm in other dog breeds.

Results

We identified 56 English Bulldog cases with this CD4-CD8- T-cell lymphocytosis over a six-year period. The median age at presentation was 3 (range 1-9 years) and males comprised 78% of the cases. The median peripheral lymphocyte count was 42,293 cells/?L. The cells were commonly described as small and mature-appearing with scant cytoplasm, consistent with the small-size scatter properties identified by flow cytometry. Frequent clinical characteristics included elevated liver enzymes (87%), splenomegaly (68%), thrombocytopenia (68%) and hepatomegaly (56%). Median overall survival time (OST) from diagnosis was 22 days (range 1-490 days). Forty-nine additional cases of small cell T-cell lymphocytosis of this phenotype in breeds other than English Bulldogs were identified during the same time period. Clinical characteristics, cellular morphology, age (median 3.5, range 1-14 years), male predominance (71%), and OST (median 32, range 1-208 days) were similar to those described in the English Bulldogs.

Conclusions

CD5+CD3+CD4-CD8-CD25loMHCIIlo T-cell leukemia/lymphoma is an aggressive disease of small lymphocytes with a predilection for young, male, English Bulldogs.

Canine multicentric lymphoma (CML): towards a European consensus for diagnostic imaging ?

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Introduction

Imaging techniques are used for staging and follow-up of CML. However, there is currently no consensus on the choice of the technique. Proofs are lacking concerning the benefit of advanced imaging techniques in this indication, unlike in its human counterpart. Thus, we aimed to map out the current practices of diagnostic imaging for CML in European referral centers.

Materials and methods

An online survey was carried out among 143 veterinary specialists with the help of the European Canine Lymphoma Network and ESVONC. The survey was focused on their current practices such as choice, frequency of use of existing imaging techniques for CML diagnosis, staging and follow-up, and the possible use of FDG-PET in the future.

Results

33 responses were recovered, from 14 European countries. 75% recommended first chest X-ray (CXR) and abdominal ultrasonography (AUS) for staging. Whole body CT was the second choice (51%). The same techniques were recommended for patient follow-up, during (70%) and after (75%) treatment. Follow-up frequency varied widely. Despite cost limitations (45%), FDG-PET was considered necessary for better staging and recurrence detection (69%) and therapeutic response assessment (63%).

Conclusions

This preliminary study suggests that current practices in CML imaging in European referral centers are in line with literature recommendations but the use of CT imaging is limited due to anaesthesia, cost and lack of benefit evidence. This supports the project of a European multicentric study evaluating the value of advanced imaging (CT, PET) over chest X-ray and abdominal ultrasonography for staging and follow-up of CML.

Refining the double two-thirds rule: the importance of breed and clinical presentation to predict the diagnosis of canine splenic mass lesions in 288 dogs

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Introduction

Prediction of the diagnosis of canine splenic masses can determine appropriate intervention. This study explores the predictive value of breed and clinical presentation on the diagnosis of a malignant splenic mass lesion.

Materials and methods

Records from the Royal Veterinary College (2007–2016) were reviewed. Dogs with splenic masses were included if they had a histopathologic or cytologic diagnosis, or imaging findings consistent with disseminated metastatic disease. Signalment, physical examination, haematology, imaging findings and pathology reports were recorded. Breeds were grouped according to phenotype and genome. Binary logistic regression was performed to identify predictors of malignancy and haemangiosarcoma.

Results

288 dogs were identified: 27% female and 63% male, 21% entire and 79% neutered; German Shepherd was the most common breed (11%). Six phenotype groups (PG) of 15–54 animals and 5 genotype groups (GG) of 21–65 animals were formed. Median age was 10 years and median bodyweight 25 kg. Thirty-nine percent of dogs presented with haemoabdomen; a splenic mass was found incidentally in 28%. Sixty-eight percent had malignant tumour of which haemangiosarcoma comprised 66%.

On multivariate analysis, GG (p=0.002), haemoabdomen (p=0.000) and neutrophil count (p=0.017) predicted malignancy and GG (p=0.025), haemoabdomen (p=0.000) and non-incidentally-found masses (p=0.000) predicted haemangiosarcoma. One hundred percent of the German Shepherd/Retriever group with haemoabdomen and neutrophil count >12x10e9/l had a malignant tumour whereas 12% of the Poodle/Spaniel group without haemoabdomen and neutrophil count

Conclusions

Signalment and clinical presentation may have predictive value to diagnose a malignant splenic mass and haemangiosarcoma.

POSTERS

Metallo-balance analysis of serum trace elements for prediction and differentiation of hepatic masses in dogs

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Introduction

The purpose of this study was to evaluate the prediction and differentiation of hepatic masses by metallo-balance analysis of serum 17 trace elements in dogs.

Materials and methods

One hundred thirty dogs with hepatic masses (HM group) and 78 clinically healthy dogs (Control group) were included. Serum samples were collected from all dogs. In HM group, surgery was performed, followed by the histopathological diagnosis. In HM group, 72 dogs underwent the serum collection from 10 to 20 days after the operation. In each sample, the serum concentration of 17 trace elements (Li, Na, Mg, P, S, K, Ca, Fe, Co, Cu, Zn, As, Se, Rb, Sr, Mo and Cs) was measured by inductively coupled plasma-mass spectrometry. The serum 17 trace elements were evaluated for the classification between HM and Control groups by discriminant analysis, and discriminant score (metallo-balance index) was calculated. Metallo-balance index was evaluated to differentiate hepatic masses and compared between before and after the operation.

Results

Serum S, Fe, Cu, Se and Cs were significantly different between HM and Control groups. The accuracy of classification between HM and Control groups was 86.2%. The metallo-balance index in HM group was significantly higher that in Control group. In HM group, the metallo-balance index had no differences among hepatocellular carcinoma (HCC), hepatocellular adenoma, focal nodular hyperplasia and cholangiocarcinoma. In the dogs with HCC, the metallo-balance index significantly decreased postoperatively.

Conclusions

Metallo-balance analysis of serum 17 trace elements might serve the prediction of hepatic masses as a screening test in dogs.

Comparison of diagnostic accuracy of different fine-needle cytology techniques in canine thyroid neoplasia.

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Introduction

Cytology is the first step in thyroid nodules diagnosis in human medicine. The diagnostic accuracy of this technique has not been specifically evaluated in dogs, considered low in some publications due to frequent blood contamination. The objective of this retrospective study was to compare the diagnostic accuracy of cytology obtained by fine needle biopsy, as well as to evaluate the quality of the samples obtained with different techniques in dogs with thyroid neoplasia.

Materials and methods

Patients with a histopathological diagnosis or a presumptive imaging diagnosis (CT, ultrasound) of a thyroid tumour in which cytology samples had been obtained with 23G or 25G needles, by fine needle aspiration (FNAC), fine needle with no aspiration (FNNAC) occluding or without occluding the needle hub were included. The quality of the samples was classified into three categories (I non-diagnostic, II diagnostic, III superior diagnosis) based on blood contamination, cellular quantity and cellular features.

Results

The diagnostic accuracy in all cases (n=41), regardless of the technique used or the needle gauge, was 79%. The highest diagnostic accuracy (94%) was achieved in the FNNAC occluding the needle hub group (n=26), with 54% of the samples being classified as type III quality. In the other groups FNNAC without occluding the needle hub (n=10) and FNAC (n=5) the diagnostic accuracy was lower, 42% and 40% respectively.

Conclusions

These results suggest that cytology is a useful technique in the initial approach to the diagnosis of a thyroid mass, and specifically the FNNAC, results that should be evaluated in prospective studies.

Retrospective evaluation of the combination of zoledronic acid, toceranib phosphate (Palladia ®) and meloxicam in cats with oral squamous cell carcinoma.

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Introduction

Feline oral squamous cell carcinoma (FOSCC) is the most common form of feline oral cancer frequently invading the mandibular and maxillary bone contributing to morbidity and mortality. Bisphosphonates and in particular zoledronic acid have been shown to be safe and efficacious in reducing bone loss and tumor growth used alone or in combination with meloxicam in different models of FOSCC. Toceranib phosphate has also shown some biological efficacy treating FOSCC. The objective of the study was to determine the clinical benefit and adverse event profile of the combination of zoledronic acid, meloxicam and toceranib in the treatment of FOSCC.

Materials and methods

Medical records of cats with OSCC were searched between 2012 and 2018 treated with toceranib (2.4-3.25mg/kg M/W/F) zoledronic acid (0.2mg/kg in 25 ml SSF 0.9% during 15 minutes) and meloxicam (0,05 mg/kg PO SID). Clinical variables (staging) at diagnosis, response (RECIST criteria), progression free survival (PFS), and toxicity (VCOG criteria) were assessed.

Results

Twelve cats with a histopathological diagnosis were included. The overall biological response rate was 66%. There was one CR, two PR, and five SD The median progression-free survival (PFS) was 82 days, and median overall survival (OS) was 196 days. Treatment was well tolerated, with the most common side effect being mild gastrointestinal toxicity.

Conclusions

The combination was well tolerated and showed biological efficacy, with similar PFS and OS times reported previously with other treatment options. Prospective evaluation of this combination is warranted to assess response in an effort to achieve a more durable response in the treatment of FOSCC.

High dose rate (HDR) interstitial 192-Ir brachytherapy for the treatment of a recurrent dermal vascular hamartoma in a horse: a case report

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Introduction

Hamartomas are benign tumor-like malformations of tissues otherwise normally present within the organ of origin. A complete surgical excision may be curative. When margins are incomplete, cryo- and lasertherapy have been reported as unsuccessful to prevent recurrence in horses. Irradiation of hamartomas has not been evaluated in animals.

Materials and methods

A fourteen-month-old male horse was referred on June 2017 for recurrent and non-resectable (5*4*3 cm) dermal vascular hamartoma on the coronary band (hoof), three month after a marginal excision. The lesion had been present since birth. At the time of consultation, the lesion was exophytic, broad based and presented with frequent bleeding. A HDR needle-based interstitial brachytherapy protocol, of four once daily 7 Gy fractions (6 needles, TD 28 Gy) was performed under sedation. The implant was computerized and 3D dosimetry was performed (Oncentra, Elekta). PFI and tolerance to treatment (VRTOG) were evaluated.

Results

The treatment was tolerated without significant acute side effects (grade I). A complete response was observed within 3 months with a progressive regression, necrosis and a subsequent detachment of the mass. Complete scar healing was seen within 5 months and the remission remains complete after 31 months. Only a partial alopecia and dryness persist at the site (grade I).

Conclusions

This is the first report of an hamartoma treated with radiotherapy in veterinary medicine. HDR brachytherapy was safe and effective in this case and may be recommended in horses for the treatment of benign vascular malformations when surgery is not an option.

Presumed Primary Skeletal Muscle Lymphoma In A Cat - Case Report

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Introduction

Most feline lymphomas are represented by the extranodal forms, affecting especially the gastrointestinal tract, nasal cavity, kidneys and other miscellaneous locations. There is no description of a primary muscular lymphoma in cats, which is the focus of this report. Only 1 primary muscular lymphoma has been described in dogs.

Materials and methods

An 18-months-old Domestic long-haired cat presented for evaluation of a subcutaneous mass that involved the right pelvic and femoral region, showing lameness and lethargy. Blood analysis, abdominal ultrasound, X-ray, PCR for FIV/FeLV and fine needle aspiration were performed. For further information, multiple biopsies were submitted to the laboratory for histopathology and immunohistochemistry (CD3, CD45R, PAX-5, Vimentin). A multi-agent chemotherapy protocol (COP/COP + Doxorubicin) was initiated.

Results

Cytology, histopathology and immunohistochemistry revealed a large B-cell lymphoma which infiltrated the skeletal muscle and partially the subcutaneous adipose tissue. Blood analysis, abdominal ultrasound and x-ray were not contributory. PCR for FIV/FeLV was negative. The COP protocol was initiated, followed by a complete remission after 10 days. The recurrence appeared in the 28th day of treatment. The chemotherapy protocol was changed by adding Doxorubicin and Epirubicin. Despite of mild improvement in the performance status, the patient died 49 days later.

Conclusions

In comparison with other reported feline extranodal lymphomas, in this case, the survival time and the length of remission were shorter, suggesting a poor prognosis. Although the incidence of extranodal lymphoma in young cats is low, it should be considered as a differential diagnosis for choosing an efficient treatment and improve the patient's performance status.

Immunophenotypic profiles of canine mammary tumors

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Introduction

Mammary gland tumors are the most commonly occurring neoplasm in the female dog and represents a heterogeneous group in terms of morphology and biological behavior. The molecular-based classification system adopted for breast cancer is a valuable tool for assessing prognosis in canine mammary tumors. This study has the objective to report the immunophenotype profiles of canine mammary tumors and correlate with survival.

Materials and methods

This study included 170 malignant canine mammary tumors. Luminal tumors were subdivided into luminal A (RE e/ou RP+, HER2- and Ki6720%) and luminal B-HER2-positive (RE e/ou RP+, HER2+ and Ki67>20%). The HER2-overexpressing subtype as characterized by the absence of staining for hormone receptors (ER and PR) and positivity for HER2. The triple-negative cases were defined as the absence of positivity for ER, PR and HER2 and classified as basal cases when positive for CK5/6 and or EGFR.

Results

Four immunophenotypes were identified: 32% luminal A (55/170), 51% luminal B HER2- (86/170), 5% luminal B HER2+ (9/170) and 12% triple-negative (20/170). HER2-overexpressing phenotype was not identified. Bitches with luminal A tumors exhibited a longer survival (did not reach the median survival). Luminal B HER2- had a median survival of 435 days and the luminal B HER2+ median of 102 days, whereas for triple-negative subtype, the median survival time was 187 days.

Conclusions

Canine mammary tumors may present different immunophenotypes. Luminal B HER2+ subtypes predominated, and the best prognosis was associated with the luminal A subtype.

Evaluation of Kappa/Lambda ratio in canine B cell lymphomas by flow cytometry

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Introduction

Evaluation of immunoglobulin light chains (kappa or lambda) expression by flow cytometry is a key element in the diagnosis and monitoring of human B cell lymphomas. Normal and reactive B cell lymphocyte populations typically exhibit expression of both kappa and lambda light chains at an expected ratio, while neoplastic B cell proliferation exhibit a monotypic expression. However, there is no published information regarding kappa/lambda ratio (K/? ratio) in canine B cell lymphomas.

Materials and methods

Kappa and lambda expression were evaluated by flow cytometry in 27 dogs with B cell lymphomas (CD45+/CD21+/CD3-/CD5-). The K/? ratio was calculated following the human criteria for clonality, being a ratio that is outside of the range of 3:1 and 1:0,3 considered as monoclonal.

Results

Twenty-seven (26/27; 96,3%) of the B cell lymphomas showed clonal expression of light chains (K/? ratios >3 or

Conclusions

Most of the dogs with B cell lymphoma had a good correlation with the range of the K/? ratio previously published in humans for clonality evaluation. Further studies with more animals are needed to determine the utility of this ratio in the identification of subtypes of canine B cell lymphomas or any significance as prognostic marker.

Evaluation of a novel topical formulation of liposome-encapsulated bleomycin as a therapy for non-melanoma skin cancers in veterinary species

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Introduction

Bleomycin is an anti-tumour antibiotic that shows efficacy against several malignancies including non-melanoma skin cancers; however, its clinical use is restricted due to its severe systemic toxicity and limited penetration through the skin. The aim of this study was to investigate a novel, non-invasive topical formulation of the drug consisting of bleomycin encapsulated inside ultra-deformable liposomes: Bleosome.

Materials and methods

This study is three-tiered: (1) to investigate the molecular effects of Bleosome in vitro on a panel of cancer cell lines; (2) to assess the skin penetration of Bleosome ex-vivo, labelling the drug with a fluorophore and visualizing treated canine skin explants with multiphoton microscope; and (3) to evaluate the efficacy and side-effects of Bleosome in vivo, as treatment of equine sarcoids following CO2 laser excision.

Results

Here we show that Bleosome is effectively taken up by cancer cells more readily than free bleomycin and has comparable cytotoxic effects. Furthermore, liposomes improve the penetration of bleomycin through the skin. We have translated this research into the clinic and observed that 75% (6 out of 8) of equine patients, treated topically after surgical excision, showed no relapse or adverse reactions after an average of 3 months follow-up.

Conclusions

Bleomycin is an established drug but its utilisation has been limited by associated side-effects and limited penetration. Here we show that Bleosome can aid both uptake into cells and penetration through the skin, and maintain the cytotoxic profile of the drug. Further work is ongoing to establish the molecular mechanism of Bleosome and to substantiate its clinical relevance.

Evaluation of lomustine, L-asparaginase and prednisone as a first rescue protocol for resistant canine non-Hodgkin high-grade B-cell lymphomas following a 19 week University of Wisconsin (UW-19) induction protocol.

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Introduction

The combination of lomustine, L-asparaginase, and prednisone (LAP) has been previously evaluated as an effective rescue treatment for canine lymphoma (LSA). The aim of this study was to evaluate efficacy and toxicity of this protocol, in a Spanish cohort of resistant canine B-cell multicentric lymphomas previously treated with a UW-19 protocol.

Materials and methods

Medical records were searched retrospectively for dogs with resistant B-cell multicentric lymphomas treated initially with a UW-19 protocol that received LAP as a first rescue treatment from 2014-2018. Clinical variables (staging) at initial diagnosis and relapse, response rate, progression free survival (PFS), and toxicity (VCOG criteria) were assessed.
Twenty nine client-owned dogs met the inclusion criteria. Lomustine (70-90mg/m2) was administered orally at 3-week intervals for a total of 5 doses, concurrently with subcutaneous L-asparaginase (10000 IU/m2) administered the first two doses of the protocol. Prednisone was administered at a tapering dose for the duration of the protocol.

Results

The overall response rate (ORR) for dogs treated with this protocol was 55% (15), with a median progression free survival of 59 days. Twelve dogs achieved a complete response (44%), 3 a partial response (9%) and 1 stable disease (3%). The median progression free survival (PFS) was 59 days. The median PFS for the initial UW-19 was 200 days. Toxicoses were mild and self-limiting in 24 of 29 cases.

Conclusions

Despite a lower response rate compared to previous published data, the LAP protocol is a viable first rescue treatment option for dogs with B-cell multicentric LSA treated with a UW-19 week protocol.

Infrared Thermography in Cats with Feline Injection-Site Sarcomas

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Introduction

Cutaneous neoplasms are associated with increased angiogenesis and tissue perfusion, with a consequent rise in local temperature. The detection of temperature gradients through Infrared thermography could aid in diagnosis, tumor grading and monitoring of disease prognosis and progression. The present study evaluated the temperatures of the feline injection site sarcoma (FISS), correlating findings with tumour histological grade and the patient's long term survival after surgery.

Materials and methods

Cats of any breed, sex or age with diagnosed FISS were included on study. The temperature of the tumor area and the healthy area distant at least 3 cm from the tumour margin was measured using the thermographic camera FLIR T650sc (Flir Systems®).

Results

Twenty-five cats were evaluated, 17 (68%) females and 8 (32%) males, mean age of 9.1 ± 3.8 years, 4.8 kg ± 1.4. The temperature of the tumour area ranged from 32.1 to 38.4°C (mean 35.5°C ± 1.5); and in healthy areas from 31.9 to 37.8°C (mean 34.9°C ± 1.6). Most of the tumours (58%) were warmer than the surrounding tissue (mean difference 1.5oC ± 0.9), while 31% were cooler (mean difference 0.7oC ± 0.6). Three tumours had the same temperature as the surrounding tissue.

Conclusions

Although thermography was highly sensitive in detecting variations in tumour temperatures, there was no correlation between tumour temperature and histological grade ($p = 0.42$) and survival ($p = 0.78$) after surgery. Further investigation is necessary to determine the contribution of this technique to the diagnosis and prognosis of FISS.

A long-term follow-up study of Adenoviral (AdCD40L) Immunotherapy of Canine Malignant Melanoma

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Introduction

Malignant melanoma is a serious condition in both human and canine medicine, often carrying a poor prognosis and high metastatic potential. Canine malignant melanoma (CMM) represents up to 7% of all malignancies in dogs and is the most common oral malignancy. Radiation and surgery is successful in loco-regional control of CMM, whilst treating non-resectable disease with chemotherapy has been unrewarding. Consequently, multiple immunotherapeutic treatment strategies have been developed. When treated with surgery alone the median survival time for dogs with stage II melanoma is reported to be < 5 months, whilst for stage III-IV it is < 2-3 months when treated with surgery alone.

Materials and methods

The aim was to evaluate the efficacy of adenoviral vector (AdCD40L) immuno-therapy in dogs with spontaneous malignant melanoma, with overall survival (OS) as endpoint. Between May 2005 and May 2013, 32 dogs were given 1-7 intratumoral injections of AdCD40L every 7 days, combined with cytoreductive surgery in 20 cases and only immunotherapy used in 12. As follow-up was performed five years after last patient treated, all but one of the patients included were now deceased. Previously only 19 of these dogs had been included in statistical analyses and assessed for efficacy.

Results

The median survival for dogs with stage II-IV melanoma in this study was 168 days, or roughly 5.5 months.

Conclusions

This compares favorably with the generally reported survival time suggesting AdCD40L immunotherapy in CMM warrants further clinical trials. This especially as the adverse events mainly was transient fever and swelling at injection site.

Diagnostic significance of metallo-balance analysis with serum trace elements in canine prostate adenocarcinoma

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Introduction

Unlike human prostate cancer, any tumor markers such as prostate specific antigen have not yet been established for screening test and evaluation of therapeutic effect in canine prostate adenocarcinoma (PAC). The purpose of this study was to evaluate the diagnostic significance of PAC by metallo-balance analysis of serum 17 trace elements in dogs.

Materials and methods

Twenty-three male dogs with prostate adenocarcinoma (PAC group) and 46 clinically healthy male dogs (Control group) were included. Serum samples were collected from all dogs. In PAC group, prostatectomy or prostatocystectomy was performed, followed by the histopathological diagnosis. In PAC group, the serum samples were postoperatively collected again. In each sample, the serum concentration of 17 trace elements (Li, Na, Mg, P, S, K, Ca, Fe, Co, Cu, Zn, As, Se, Rb, Sr, Mo and Cs) was measured by inductively coupled plasma-mass spectrometry. The serum 17 trace elements were evaluated for the classification between PAC and Control groups by discriminant analysis, and discriminant score (metallo-balance index) was calculated. In addition, metallo-balance index was compared between before and after the operation.

Results

Serum Cu was significantly different between PAC and Control groups. The accuracy of classification between PAC and Control groups by the metallo-balance index was 91.3%. The metallo-balance index in PAC group significantly lower than that in Control group. In PAC group, the metallo-balance index was not significantly different between before and after the operation.

Conclusions

Metallo-balance analysis of serum 17 trace elements might be useful for the diagnosis of PAC in dogs.

Aggressive surgical treatment of an invasive anal sac SCC in a cat.

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Introduction

Squamous cell carcinoma (SCC) of the anal sac is an uncommon location for feline patients and may have a different behaviour from other common locations. Our case showed fast growth, extremely aggressive local behaviour and early metastatic potential. Surgical treatment of this condition has not been previously described.

Materials and methods

A 12 years old neutered DSH was presented for anal mass and constipation. SCC was histologically confirmed. CT scan showed a 4.13 cm mass presumably originated from the right anal sac, obliterating anus and distal rectum, and enlarged inguinal superficial and internal iliac lymph nodes.
Complete anal and distal third of the rectum resection were performed. Enlarged lymph nodes were also removed. Mucocutaneous anastomosis of the remaining rectum to perineal skin was performed.

Results

Wide margins mass removal including distal third of the rectum was achieved without postsurgical complications, or fecal incontinence. Recovery was uneventful, patient was ambulatory and defecation was controlled by the patient, using its litter box since 2 days after surgery. Toceranib was prescribed as adjuvant therapy. Twelve weeks after surgery, multiple nodules were detected on legs and caudal abdomen and patient deteriorated rapidly. Euthanasia was performed two weeks later, but no local recurrence was detected in the surgical area.

Conclusions

Complete anal and caudal rectal resection achieved a good surgical outcome and an excellent quality of life. Remaining rectum showed capacity to hold fecal material and emptying in a conscious manner avoiding use of diapers. Toceranib was not useful on controlling tumor metastases.

In vitro and in vivo Evaluation of High-Dose Ascorbate in Dogs

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Introduction

Studies in humans have determined that supraphysiological concentrations of ascorbate (plasma concentrations 20 mM, i.e. pharmacological ascorbate (P-AscH-)) readily produce high fluxes of H2O2 via its oxidation; this H2O2 preferentially induces cancer cell death compared to normal cells. The vast majority of in vitro studies have focused on the effects of P-AscH- on human tumor cells. The effects of P-AscH- on canine osteosarcoma, and if high levels of ascorbate are achievable in dogs, are unknown.

The purpose of this study was twofold: (1) determine the pharmacokinetic (PK) profile of high-dose intravenous ascorbate in healthy Beagle dogs; and (2) determine the effects of P-AscH- on canine osteosarcoma cells.

Materials and methods

Eight Beagle dogs were administered two doses of ascorbate (250 mg/lb or 1000 mg/lb) via intravenous infusion over six hours, on separate days. Plasma ascorbate concentrations were measured at 12 time points during and after infusion for PK analysis. Clonogenic assays were performed on 2 canine osteosarcoma cell lines and 1 normal canine fibroblast cell line after exposure to high concentrations of ascorbate.

Results

Plasma ascorbate levels peaked at 9 mM following the higher dose and returned to baseline 6-8 hours after dosing. Minor adverse effects were seen in two dogs: mild nausea or vomiting.

Ascorbate significantly decreased survival of osteosarcoma cells in vitro, while sparing normal fibroblasts.

Conclusions

These data indicate: P-AscH- is preferentially cytotoxic to canine-derived cancer cells; and high levels of ascorbate can be safely administered to dogs. Further studies are needed to determine the effects of P-AscH- on canine cancer patients.

Treatment of feline fibrosarcoma with wide surgical excision and intraoperative orthovoltage irradiation: preliminary results

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Introduction

Long term control of feline fibrosarcomas with surgery and radiation remains a challenge. Orthovoltage, in comparison with megavoltage, offers advantages such as lower cost and limited shielding requirements which might lead to wider availability. We hypothesized that tumor bed intraoperative irradiation before surgical closure with orthovoltage would be well tolerated and provide disease free survival (DFS) comparable to reports with radical surgery and adjuvant megavoltage postoperative radiation.

Materials and methods

24 client owned cats, with naïve (n=14) or recurrent (n=10) fibrosarcomas, evaluated with CT scan, were treated with wide surgical excision (3 cm gross margins, one fascial plane deep margin) and tumor bed irradiation (8Gy 22 cats, 10Gy 2 cats) prior to surgical closure. 4 cats received additional external beam orthovoltage radiation (3.75GyX9 Monday Wednesday Friday).

Results

Complete margins (5mm or more) were obtained in only 9 cats, close margins (less than 5mm) in 11 cats, incomplete margins in 4 cats. Nine cats (37.5%) experienced delayed healing including 5 with close margins and 4 with complete margins. Six cats (25%) experienced recurrence including the 5 deceased cats. Among the 5 deceased cats, 2 had incomplete margins, 2 close margins, 3 had recurrent disease. Average follow up was 11.7 months (1 to 35 months). DFS was 11 months with 19 cats (79%) still alive.

Conclusions

Preliminary results are encouraging, with delayed but acceptable healing. Tumor control is comparable to some reports with traditional megavoltage irradiation. Wide surgical excision with intraoperative orthovoltage appears as a viable alternative for treatment of cats with fibrosarcomas.

Canine high grade – low grade orofacial fibrosarcomas – a retrospective analysis of 70 cases

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Introduction

Oral fibrosarcomas can be subgrouped based on histologic grade. A distinctive category called “biologically high-grade histologically low-grade fibrosarcomas” (HG-LG-FSA) has been described, characterized by highly differentiated cells without features of malignancy, yet a biologically malignant behaviour. The aim of this study was to describe clinical and computed tomographic (CT) findings in HG-LG-FSA in a large number of cases.

Materials and methods

Retrospective analysis of 70 dogs with histologically confirmed HG-LG-FSA.

Results

80% of the cases belonged to large breeds with a predisposition for Retrievers (all Retrievers 24%, Golden Retrievers 18%). No brachycephalic breeds were affected. There was a slight male predominance (40 males vs 30 females). Median age was 8 years (range, 3 to 13 years). The tumours were firm, indolent, broad-based masses, rostrolaterally on the maxilla near the canine tooth or premolars (68%). The caudal maxilla (16%), mandible (10%) or other parts of the cranium (6%) were rarely affected. There was no primary ulceration of the tumor.

CT studies were available for 50 patients and showed a large, broad–based soft tissue mass (median length x width 49 x 31.5 mm) with pronounced peripheral contrast uptake. A (pseudo-)capsule and soft tissue calcification were identified in 76% and 23% of the cases, respectively. A permeative type osteolysis of adjacent bone was typical. Based on thoracic CT, none of the dogs showed signs of metastasis at time of presentation.

Conclusions

This tumor presents with rather characteristic CT features which differ from “classical” oral fibrosarcomas. The previously reported breed predisposition for Retrievers was confirmed.

Outcome of first-rescue cyclophosphamide treatment for relapse small cell gastrointestinal lymphoma in cats

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Introduction

Lymphoma is the most common intestinal tumor in cats. The small-cell variant carries a good prognosis, usually treated with chlorambucil and steroid therapy. No standard treatment recommendation has been established for relapse small cell gastrointestinal (GI) lymphoma. The objective of this study was to investigate the outcome in cats with relapse small cell GI lymphoma treated with cyclophosphamide after failing chlorambucil therapy.

Materials and methods

Medical records from three institutes between 2002 and 2017 were retrospectively reviewed. Cats treated with cyclophosphamide for relapse lymphoma following first-line chlorambucil/steroid therapy were included. Median progression-free survival (PFS) and overall survival (OS) were analyzed using Kaplan-Meier method. Cox regression analysis was performed to assess the impact of signalment, clinical signs, concurrent disease, location of lymphoma, and response to treatment on outcome.

Results

Twenty cats met the inclusion criteria. Median dose of cyclophosphamide was 206.9 mg/m2 (161.3-281.8 mg/m2) every 14 days. The median PFS and OS were 215 days (95% CI 102-328) and 1065 days (95% CI 974-1156), respectively. Complete resolution of clinical signs was noted in 18 of 20 cats (90% complete response rate). Complete resolution of clinical signs while receiving cyclophosphamide had a significant impact on PFS (P=0.02, HR=0.14), while duration of clinical signs (P=0.03, HR=1.003) and response to prior chlorambucil therapy (P=0.01, HR=0.995) had a significant impact on OS.

Conclusions

Cyclophosphamide treatment for relapse small cell GI lymphoma in cats resulted in moderate response duration with a high complete response rate. A prospective randomized study is necessary to determine the superiority of cyclophosphamide over other rescue chemotherapeutics.

Automatic Detection of Canine Soft Tissue Sarcoma Using Transfer Learning Algorithms

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Introduction

A major challenge in the diagnosis of canine soft tissue sarcoma (STS) is the high inter-reader variability between pathologists. However, deep learning methods are capable of automatically learning representations of such data and so could improve the accuracy of tumour detection. In this study we aimed to evaluate the use of transfer learning to classify grade 1 (lowest grade) canine STS against normal tissue.

Materials and methods

Whole-tissue digitized images of histopathology slides (WSI) obtained from an oncology referral hospital were used for training/validation of the deep learning algorithm. The canine STS dataset was created from 10 WSIs that contained STS grade 1 and normal tissue. Sample patches of 256 x 256 pixels suitable were used. Approximately 4000 patches were used for training and 1000 patches for validation. Transfer Learning was applied via fine-tuning of the VGG19 convolutional neural network (CNN) using a publicly available dataset and then bottleneck feature extraction applied to the STS data set.

Results

Pilot results demonstrate improved performance using transfer learning compared to training on the dataset alone, with accuracy increasing from 48.50% to 69.20% and precision, recall and F1-scores all increasing from between 0.40-0.47 to 0.69. Aggregated transfer learning with ‘fine-tuning’ marginally improved performance further.

Conclusions

Transfer learning is a promising technique for digital pathology through improved classification performance. Aggregated Transfer Learning produced marginally better classification metrics compared to models pre-trained using the ImageNet dataset. Further studies will aim to improve the statistical significance of the results, the morphological features assessment and recognition for the grading of canine STS.

Combination therapy with APAVAC® immunotherapy and low dose cyclophosphamide as adjuvant treatment for feline aggressive mammary carcinomas – A Pilot study

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Introduction

Feline mammary carcinomas are a very aggressive type of disease with high rates of metastasis after radical surgery. Adjuvant chemotherapy has been suggested but reports are few and with heterogeneous results. The purpose of this preliminary evaluation is to assess the efficacy of a chemoimmunotherapy (tumor microenvironment-oriented therapy) combining APAVAC® (Heat-Shock-Protein96 based vaccine) and metronomic cyclophosphamide.

Materials and methods

Eleven female cats with mammary tumors were enrolled after full staging procedures. All cats were submitted to radical mastectomy and tumor classification was performed (WHO classification scheme). For vaccine preparation, HSP96 were isolated and purified from the tumor. Oral cyclophosphamide (15mg/m2 SID) and vaccine administration were used as adjuvant treatment. Disease-free interval (DFI), time to progression (TTP), Tumor specific survival (TTS) and treatment toxicity were assessed and compared with previous reported literature.

Results

Most of the cats (82%) were stage III. Five cats (45%) were diagnosed with mammary carcinoma for the first time, the remaining with recurrent disease. Side effects were observed in 82% of patients (grade 1 and 2 VCOG-CTOE). Only two cats died before completing the immunotherapy protocol. The median DFI and TTP were 297 and 377 days respectively. 64% of the cats are still alive.

Conclusions

To the authors knowledge this is the first description of the application of chemoimunotherapy in feline mammary carcinomas. This treatment may be a valid option for aggressive feline mammary carcinomas, with low toxicity and similar disease-free intervals compared with previous reports. Further studies with a large number of patients are needed to evaluate is true efficacy.

A pilot study of immune infiltrates in canine cutaneous mast cell tumours

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Introduction

The traditional view of cancer focussed on genetic changes affecting cancer cells, but recently there is increasing interest in the role of the tumour microenvironment (TME). Immune infiltrate - comprising tumour infiltrating lymphocytes (TILs), tumour associated macrophages (TAMs) and T-regulatory cells (Tregs) - forms a key TME component. The aim of this project was to describe the immune infiltrate in low-grade and high-grade canine cutaneous mast cell tumours (MCTs), and evaluate correlation with metastatic disease and clinical outcome.

Materials and methods

Twenty client owned dogs with naturally occurring MCTs were reviewed. Eleven were Kiupel low-grade and nine high-grade; Patnaik grading gave 5 grade I, 9 grade II and 6 grade III. Immunohistochemistry was performed for CD3, CD4 and MAC387 using standard validated protocols; immunoreactivity was quantified for each slide in a blinded, randomised manner using previously described methodology.

Results

High-grade tumours had higher median TAMs (53.3 V 16.7) and CD3+TILs (110.2 V 35.9) but lower Tregs (37.3 V 67.7). Higher CD3+TILs correlated with Patnaik grade III, metastatic disease, MCT-related death and survival time < 6 months. Tumours with metastases, regardless of grade, had higher CD3+TILs than those without (median 87.3 V 36.8) but lower Tregs (median 27.9 V 58.6). TAMs showed no particular correlation with outcome or metastatic disease.

Conclusions

This pilot study suggests that high CD3+TILs and low Tregs are associated with higher biological and pathological grade in canine cutaneous MCTs. This phenomenon is also described in cutaneous squamous cell carcinoma in people and suggests that T-cell infiltrates are involved in developing and maintaining a malignant phenotype.

Immunoexpression of Cox-2, C-kit and EGFR immunoexpression in dog and cat lung tumours

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Introduction

Lung tumours are a very type of neoplastic aggressive disease with scarce therapeutic options both in dog and cats. This study aimed to evaluate the imunoexpression of Cox-2, C-kit, EGFR and Proliferation index in order to evaluate their potential as therapeutic targets.

Materials and methods

Eighteen lung carcinomas (11 Papillary Adenocarcinomas; 5 Bronchioloalveolar carcinomas and 2 Acinar Adenocarcinomas) from 13 dogs and 5 cats were included and analyzed by immunohistochemistry for Cox-2 (Antibody Anti-Cox-2, Clone SP21, dilution 1:40, Termo Scientific®); C-kit (Polyclonal Rabbit Anti-Human CD117 Dako®, dilution 1:100) and EGFR (Antibody Anti-EGFR, 1:100, Invitrogen®).

Results

The medium age for dogs was 11.69±3.06 years (min-8, max-18) and for cats 12.8±2.38 years (min-10, max-16). Cox-2 overexpression was detected in 88.8% of the cases (16 out of 18 cases), being statistically associated with high mitotic index (p=0.024). C-kit expression was detected in 83.3% (15 out of 18) and was statistically associated with histological type (p=0.026) and nuclear pleomorphism (p=0.013). Overexpression of EGFR was detected in 94.4% (17 out of 18) of the samples, showing a statistically significant association with high nuclear atypia (p=0.039) and mitotic index (p=0.045).

Conclusions

Our results showed an overall high immunoexpression of the three biomarkers in lung tumours of dog and cat, independently of the histological type revealing its promising role as therapeutic targets in those species.

VEGF-A and VEGFR-2 expression in canine cutaneous histiocytoma

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Introduction

Canine cutaneous histiocytoma (CCH) is a Langerhans cells epidermotropic tumour, frequent in young dogs. The regression phenomena to which it is associated makes it an attractive system for analysis of tumoral behaviour. The aim of this study is to clarify the role of angiogenesis in CCH regression.

Materials and methods

The expression of Vascular Endothelial Growth Factor (VEGF-A, JH121, NeoMarkers®, 1:100) and its receptor (VEGFR-2, Flk-1, Santa Cruz Biotechnology®, 1:100) were analysed in 50 CCH, categorized in four histological groups according to the stage of the regressive process.

Results

Most of the tumours were negative for VEGF-A (n=37; 74%) or had focal (n=6; 12%) or diffuse positivity (n=7; 14%). VEGFR-2 immunoexpression was observed in the majority of cases (n=39; 78%); 7 tumors (17%) had focal positivity, 17 cases (43.5%) a multifocal labeling and 15 cases (38.5%) a diffuse labeling. The differences obtained between the histological groups were statistically significant both for VEGF-A (p=0,002) and VEGFR-2 (p

Conclusions

Although tumour cells expressed VEGF receptor in the early stages of tumour development, it seems that they do not produce VEGF. Our results suggest that the poor expression of VEGF-A in early stages of HCC development and the imbalance between VEGF-A and VEGFR-2 could compromise vascular growth in CCH and contribute to its regression.

Prevalence of p53 dysregulation in feline oral squamous cell carcinoma and non-neoplastic oral mucosa

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Introduction

Squamous cell carcinoma is the most common malignant oral tumor in cats. The late presentation is one of the factors contributing to the detrimental prognosis of this disease. The immunohistochemical expression of p53 tumor-suppressor protein has been reported in 24-65% of feline oral squamous cell carcinomas (FOSCC), but the presence of TP53 mutations has never been systematically evaluated. The aim of this study was to determine whether p53 immunohistochemistry accurately reflects the mutational status of the TP53 gene. Additionally, the prevalence of p53 dysregulation in FOSCC was compared with that of feline non-neoplastic oral mucosa, in order to investigate the relevance of these dysfunctions in cancer development.

Materials and methods

Twenty-six biopsies of FOSCC and 10 cases each of lingual eosinophilic granuloma, lymphoplasmacytic stomatitis and normal oral mucosa were retrospectively screened for p53 immunohistochemical expression and TP53 mutations in exons 5-8.

Results

Eighteen FOSCC (69%) expressed p53 and 18 had TP53 mutations. The agreement between immunohistochemistry and mutation analysis was 77%. None of non-neoplastic samples showed positive immunohistochemical staining; while one case each of eosinophilic granuloma and lymphoplasmacytic stomatitis harbored gene mutations. Unlike previously hypothesized, p53 dysregulation was not associated with exposure to environmental tobacco smoke.

Conclusions

These results suggest an important role of p53 in feline oral tumorigenesis. Moreover, the immunohistochemical expression of p53 appears to reflect the presence of p53 mutations in most cases. It remains to be determined if the screening for p53 dysregulation, alone or with other markers, can eventually contribute to the early detection of this devastating disease.

Agreement between immunohistochemistry and flow cytometry in the assessment of Ki-67 index in lymphoma bearing dogs.

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Introduction

The prognostic significance of the proliferation marker Ki-67 has been demonstrated in different tumor types, including canine lymphoma. Immunohistochemistry (IHC) on histological samples is the gold standard technique to determine Ki-67 index. Evaluation of Ki-67 by flow cytometry (FCM) in fine needle aspirates has been shown useful in canine diffuse large B-cell lymphoma. The aim of this study was to investigate the agreement between IHC and FCM in the assessment Ki-67 index, to evaluate whether FCM may serve as a non?invasive alternative method to discriminate between high? and low?grade canine lymphomas.

Materials and methods

Dogs with previously untreated nodal lymphoma undergoing diagnostic lymphadenectomy were prospectively enrolled. Ki-67 index was assessed by both FCM and IHC, and expressed as percentage of positive cells. 10x103 cells were categorized by FCM. By IHC, a manual count was performed in five 400x fields.

Results

Twenty-three dogs with 17 DLBCL, 1 nodal MZL, 2 TZL, 2 PTCL and 1 TLL matched the inclusion criteria. The median time elapsed between FCM and histology was 3.5 days. The median Ki-67 index was 49% (range, 1.7-99.5%) with FCM and 49.7% (range, 7.5-86.4%) with IHC. According to the Bland-Altman plot, the bias between the techniques was -4% and 95% limits of agreement were -49%-40% (Spearman's r=0.6; P=0.003).

Conclusions

Data indicate agreement between the two techniques. The observed bias with wide confidence intervals is caused by method-inherent differences in the number of categorized cells. Due to the higher number of cells evaluated by FCM, this method might prove more accurate than IHC.

A New prototype ELISA for determining TK1 protein levels in canine hematological malignancies and its clinical applications

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Introduction

Thymidine kinase 1 (TK1) is an ATP dependent enzyme involved in DNA-precursor synthesis. TK1 activity has been used as a valuable marker for staging and monitor therapy response with hematological malignancies in both humans and dogs. The purpose of this study was to evaluate performance of a new canine TK1 ELISA for diagnosis and monitoring therapy response in hematological malignancies

Materials and methods

Serum samples from dogs with hematologic malignancies i.e. malignant lymphoma (N=37), acute leukemia (n=4) and sera from healthy dogs (n=141), were analyzed for TK1 activity with a [3H]-deoxythymidine (dThd) phosphorylation assay and TK1 protein concentrations by a TK1 sandwich ELISA based on poly-clonal rabbit anti dog TK1 antibodies from Alertix AB3.

Results

Both TK1 assays showed significantly higher levels in hematological malignancies compared to healthy dogs (P

Conclusions

These results showed that the polyclonal canine TK1 ELISA is as precise and sensitive as the TK1 activity assay for detection and monitoring of therapy in canine hematological malignancies. The combination of TK1 ELISA with CRP may aid in the diagnostics and clinical monitoring of different canine malignancies and thus serve as a valuable tool in veterinary medicine

Clinical features and cytokine profiles of dogs with cytokine-producing lung adenocarcinoma and leukocytosis

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Introduction

This study aimed to report the clinical features of canine cytokine-producing lung adenocarcinoma and to demonstrate the association between the cytokine and leukocyte profiles.

Materials and methods

Two dogs (Cases #1 and #2) with a solitary lung adenocarcinoma and leukocytosis underwent surgery. The resected tumors were analyzed on the gene expressions of granulocyte colony-stimulating factor (G-CSF) and granulocyte macrophage colony-stimulating factor (GM-CSF), colony-stimulating factor 3 receptor (CSF3R) and Interleukin-6 (IL-6) by the quantitative real-time PCR compared with the normal lung tissues. The medical records of the patients were reviewed.

Results

Neither patients had any of clinical signs and fever. Preoperative white blood cell count (WBC) was 58,300 and 32,900 in Cases #1 and #2, respectively: especially, the segmented neutrophils accounted for the majority. Preoperative C-reactive protein (CRP) was 2.35 and 0.5 in Cases #1 and #2, respectively. Neither patients had any of metastasis and dirty surgical margins. The increased WBC and CRP postoperatively decreased to the normal ranges. The gene expression of G-CSF increased 6.2 and 19.7-fold in Cases #1 and #2, respectively whereas that of GM-CSF was little changed. The gene expression of CSF3R also increased 10 and 4.0-fold in Cases #1 and #2, respectively. The gene expression of IL-6 markedly increased 30-fold in Case #1 whereas it slightly increased 1.9-fold in Case #2. The postoperative survival time of Cases #1 and #2 was 347 and 118 days, respectively.

Conclusions

Canine cytokine-producing lung adenocarcinoma with high G-CSF and IL-6 caused leukocytosis and might be associated with poor prognosis.

Identification of Canine Mast Cell Tumour-associated miRNAs through NGS and qPCR techniques

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Introduction

Canine cutaneous Mast Cell Tumour (MCT) is one of the most frequent skin neoplasm in dogs (incidence 7%-21%). It derives from mast cells in cutaneous and subcutaneous tissue and its variable behavior makes characterization and diagnosis complex. MicroRNAs (miRNAs) are small non-coding RNAs regulating of gene expression. miRNA expression is dysregulated in cancer through various mechanisms. OMICS techniques may provide a holistic approach to identify new tumor-associated miRNAs.

Materials and methods

A retrospective study on Formalin Fixed and Paraffin Embedded (FFPE) MCT samples was performed. Nineteen tumors and intra-patient healthy controls were enrolled. Next Generation Sequencing (NGS) was asses to obtain a complete miRNomic profile, using NextSeq-500 platform (Illumina). qRT-PCR was used to validate differentially expressed miRNAs (DE-miRNAs) selected based on logFC (-2.4 >logFC>2.4) and false discovery rate (FDR). Gene Ontology (GO) and KEGG pathway enriched analysis was done to understand DE-miRNAs functions.

Results

Result showed that a total of 356 and 203 miRNAs has been identified in control and tumour samples, respectively. qPCR data confirm that, among previously selected 9 DE-miRNA, 2 were up- and 6 were down-regulated in tumor respect to control samples (p value

Conclusions

This results provide the evidence that miRNAs are involved in neoplasm progression and can discriminate tumor from healthy control.Further studies could lead to better understand microRNA role to obtain a more specific tumor profile important for diagnosis and therapy.