European Society of Veterinary Oncology

Proceedings

26-28 May 2022

Annual Congress
Siracusa, Italy

European Society of
Veterinary Oncology

SCIENTIFIC COMMITTEE

Iain Grant (ESVONC)
Irina Gramer (ESVONC)

We would like to extend our thanks to the esteemed colleagues who have helped with the abstract review process:

Irina Gramer, Jane Dobson, David Killick, Joanna Morris, Erik Teske, Lorenzo Ressel, Stephen Baines, Owen Davies, Henrik Rönnberg, Ben Mielke, James Elliott, Rachel Pittaway, Jean Benoit Tanis, Kim Selting

ESVONC COMMITTEE

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Chiara Leo, Secretary
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Irina Gramer, Member-at-Large
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Congress support by the team of Pauwels Congress Organisers

Message from the President

Dear ESVONC members and ESVONC friends,

Welcome to Siracusa 2022. What a blessing to finally be together again. We have a lot to talk about, and a lot to share.

It has already been 3 years since our last *in-person* meeting in Hofheim. It seems like a decade! This period has brought stress and anxiety, pain and grief upon many of us. It has affected us personally and professionally in so many ways. The War is now striking our European brothers and sisters at a time when the World was just looking for peace, rest and recovery.

Yet, we must resist, stay together as a community, be supportive of each other, compassionate and live on. Our support naturally goes to the Ukrainian people and our colleagues who are suffering in their country or away from home *(see Stand with Ukraine page)*.

In retrospect, it is incredible to realise how much what we do, as veterinary oncologist, is more important than ever. One could wonder a couple years ago when the pandemic started, how much our field was a priority for the « World » around us, but our clients have never been closer to their pets and have never stopped demanding treatments during this time.

Our profession is essential!

Of course, your executive committee has continued working hard to get this Congress going (finally!). We wish to thank all our sponsors for their trust and renewed support in our community. The committee is also developing new initiatives with the focus to improve the benefits for our membership *(see EC page)*.

Finally, it is ESVONC 30th Anniversary this year. What an incredible adventure it has been since 1992 *(see ESVONC history page)*. The quality of our congresses is recognized internationally. I believe our diversity in Europe is the key to its success.
Let's celebrate and enjoy this Congress.

Yours,

Jérôme BENOIT,
President of ESVONC

On behalf of all our members and its executive committee, ESVONC stands in solidarity with Ukraine and supports our Ukrainian colleagues during the War. Our thoughts are with the people of Ukraine and their animals who are currently experiencing the most terrible situation.

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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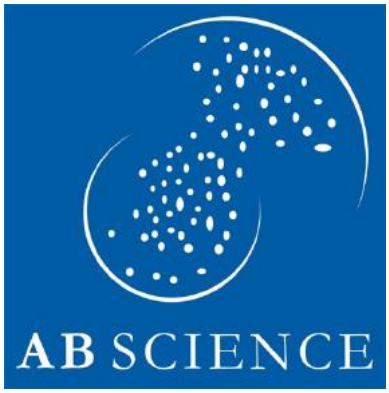

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SCIENTIFIC PROGRAMME

Times are CEST: UK is -1hr COLORADO TIME IS -8hrs and CALIFORNIA is -9hrs

RESIDENTS' WORKSHOP – Wednesday 25th May 2022

THE IMMUNE SYSTEM AND CANCER – ROOM LATOMIE		
14.00-15.00	Overview of the relationship between cancer and immune system	Dr. Federica Cavallo
14.45-16.00	Immunotherapy in veterinary oncology: past, present, and future	Dr. Phil Bergman
16.00 -16.30	*Coffee break*	
16.30-17.15	The Oncept melanoma vaccine experience	Dr. Phil Bergman
17.15-18.00	The Turin melanoma vaccine experience	Dr. Federica Cavallo
18.00-18.30	Interactive session	

MAIN PROGRAM – Thursday 26th May 2022

08.00-09.00	*Registration opens*	
	THEMED SESSION: SARCOMAS	
09.00-10.00	Clinical challenges and controversies in the management of soft tissue sarcomas	Dr. Bray
10.00-11.00	Pathologist contribution to understanding soft tissue sarcomas' behaviour	Dr. Avallone
11.00-11.30	*Coffee break*	
11.30-12.15	The proteomic landscape of soft tissue sarcomas	Dr. Huang
12.15-12-45	Panel discussion	
12.45-13.00	**Welcome ESVONC to Siracusa**	
13.00-14.15	**Lunch and poster session at the hotel – LAUDIEN ROOM**	
	ABSTRACTS	
14.15-14.30	Neoadjuvant Radiotherapy in the treatment of Feline Injection Site Sarcoma (FISS)	Dr. Hayes
14.30-14.45	Outcome and pattern of failure of a split protocol of RT and CCNU for canine non-visceral histiocytic sarcoma	Dr. Poirier
14.45-15.00	Percutaneous cementoplasty as a palliative treatment for dogs with osteosarcoma using a new self-setting bone substitute	Dr. Villamonte Chevalier
15.15-15.30	Pre-operative neoadjuvant vinblastine-prednisolone in canine mast cell tumours: a single-centre retrospective cohort study	Dr. Ossowska

15.30-16.00	**Coffee break**	
	ABSTRACTS	
16.00-16.15	**30 years of ESVONC**	ESVONC commitee
16.15-16.30	Retrospective evaluation of toxicity and response in 29 cancer-bearing cats treated with masitinib mesylate	Dr. Sayag
16.30-16.45	Monitoring treatment response and disease progression in Canine lymphoma using serial plasma nucleosome concentrations	Dr. Wilson Robles
16.45-17.00	Evaluation of Serum Amyloid A (SAA) and other clinical-pathological variables in cats with lymphoma	Dr. Schiavo
17.00-17.15	Safety and efficacy associated with 5 consecutive fractions of 4-5Gy in dogs with intracranial tumours	Dr. Del Portillo Miguel
18.30-21.00	**Welcome aperitivo Castello Maniace bar Sponsored by Anicura**	AniCura

MAIN PROGRAM – Friday 27th May 2022

08.00-09.00	FREE SPONSORED BREAKFAST BY VIRBAC STELFONTA – First hand perspectives and clinical insights Villa Politi Latomie park	Virbac Dr. Pamela D. Jones Dr. Charlotte Johnston Ms Inge Breathnach
	THEMED SESSION: GLIOMAS SESSION	
09.00-09.45	Matters of the Gray Matter: Radiotherapy Advances for Brain Tumors	Dr. Hansen
09.45-10.30	Barriers to Beat: Improving Drug Delivery to Brain Tumours	Dr. Tellingen
10.30-10.45	*Coffee break*	
10.45-11.30	What MR spectroscopy can tell us about intracranial neoplasia	Dr. Carrera
11.30-12.15	State of the art for gliomas: the human experience	Dr. Sponghini
12.15-13.00	Panel discussion	
13.00-14.00	*Lunch at the hotel – HOTEL PARK*	
14.00-15.00	**AG meeting**	
	ABSTRACTS	
15.15-15.30	Choroid plexus tumors treated with radiotherapy alone or combined with ventriculoperitoneal shunt in dogs and cats: a retrospective descriptive case series	Dr. Meier
15.30-15.45	Retrospective study of 30 canine presumed intracranial gliomas treated with external radiation therapy (RT) between 2007 and 2018	Dr. Ibish

15.45-16.00	Combined lomustine / temozolomide-irradiation proves efficacy even in resistant canine glioma cells	Dr. Fuchs
16.00-16.15	Validation of A Novel Non-Invasive Imaging System for Detection of Malignancy in Canine Subcutaneous and Cutaneous Masses Using Machine Learning	Dr. Dank
16.15-16.30	*Coffee break*	
	ABSTRACTS	
16.30-17.00	*Sponsored presentation – Volition*	Volition Veterinary Dr. heather Wilson-Robles
17.00-17.15	Epidemiological and clinical characteristics of frontal sinus carcinoma in 39 dogs (2001-2021)	Dr. Gedon
17.15-17.30	Long-term outcome of macroscopic anal sac adenocarcinoma in dogs treated with single-modality definitive-intent radiation therapy	Dr. Czichon
17.30-17.45	Clinical validation of a multi-cancer early detection blood-based “liquid biopsy” test in dogs using next-generation sequencing	Dr. Flory
17.45-18.00	A living biobank of canine mammary tumors organoids enables genetic modifications and drug testing to better understand disease heterogeneity	Dr. Inglebert
	Gala dinner Palazzo Beneventano, Ortigia Sponsored by Boehringer-Ingelheim	Boehringer Ingelheim

MAIN PROGRAM – Saturday 28th May 2022

	THEMED SESSION: SOTA	
09.00-09.45	Liquid biopsies: clinical application of cancer genetics in diagnostics	Dr. Arendt
09.45-10.30	Precision oncology in cancer care: state of the art	Dr. Cattrini
10.30-10.45	*Coffee break*	
10.45-11.30	Latest and greatest veterinary oncological immunotherapies	Dr. Bergman
11.30-12.15	Panel discussion	
12.15-13.45	*Lunch at the hotel’s park*	
	THEMED SESSION: SOTA	
13.45-14.30	The epidemiology and mechanisms of cancer pain: how should we individualize a patient's pain assessment and management?	Dr. Clark
14.30-15.15	From chronic inflammation to feline low grade intestinal lymphoma: a new model of lymphomagenesis	Dr. Freiche
15.15-15.45	*Sponsored presentation*	Virbac

	STELFONTA – Three continents and 12,000 cases – Wisdom from wins, learning from pitfalls	Dr. Pamela Jones
15.45-16.00	Coffee break	
16.00-16.15	**ALICANTE 2023**	Dr. Clemente
16.15-16.30	The mitotic regulator Polo-like kinase 1 as a potential therapeutic target for c-Myc-overexpressing canine osteosarcomas	Dr. Gola
16.30-16.45	Corticosteroids reduce interferon-gamma production in atezolizumab treated peripheral blood mononuclear cells of cancer bearing dogs	Dr. Zimmerman
16.45-17.00	Oncolytic virus therapy using genetically engineered Vaccinia virus encoding FCU1 protein in dogs diagnosed with malignant solid tumours	Dr. Beguin
17.00-17.15	HER-2 CAR-TILs as adjuvant treatment in dogs with spontaneous high grade and high stage malignancies	Dr. Ronnberg
	Farewell to next year in Alicante	
	Evening on your own	

NURSE PROGRAM – Friday 27th May 2022

08.00-09.00	*Registration opens*	
09.00-09.45	My patient has cancer - what are the options	Mrs. N. Read
09.45-10.30	What impact can nurses have on our patients' pain?	Dr. Clark
10.30-11.00	*Coffee break*	
11.00-11.45	Health Related Quality of Life in Oncology cases - advocating for your patients	Dr. Clark
11.45-12.30	Management of various drains (chest, abdomen, wound, bladder), management of feeding tubes and tracheostomy tubes, urethral stents, VAPs and SUBs	Dr. Bray
12.30-14.00	*Lunch and poster session at the hotel*	
14.00-14.45	Compounding chemotherapy drugs: advantages and pitfalls	Dr. Bergman
14.45-15.30	Side Effects of Oncology Therapeutics & How to Treat Them	Dr. Bergman
15.30-16.30	**Welcome ESVONC to Siracusa – 30 years of ESVONC** **Nurses board and future of ESVONC**	Esvonc board
	Free afternoon on your own	
	Gala dinner Palazzo Beneventano, Ortigia **Sponsored by Boehringer-Ingelheim**	Boehringer Ingelheim

NURSE PROGRAM– Saturday 28th May 2022

09.00-09.45	Owner support and education – what are your clients expecting from the oncology nurse	Ms. Breathnach
09.45-10.30	Role of social media in professional communication with clients, between professionals and setting up support networks for nurses and how to use this platform safely and appropriately	Ms. Breathnach
10.30-11.00	Coffee break	
11.00-11.45	The process of metastasis, predicting the pattern of neoplastic behaviour	Ms. Read
11.45- 12.30	The importance of nursing in RT - understanding what we do to best deal with side effects	Dr. Benoit
12.30-13.00	Specialising as an oncology nurse, what does that mean today?	Ms. Read
13.00-14.15	Lunch	
14.15-15.00	Diarrhoea management	Dr. Freiche
15.00-15.45	Vomiting management	Dr. Freiche
	Evening on your own	

The early years of ESVONC

(based on the archives of the ESVIM/ECVIM newsletters, and various testimonies from Erik Teske, Martin Kessler, Malcolm Brearley, Janos Butinar, and Henrik Rönnberg)

Written by *Erik Teske, former ESVIM newsletter editor and early member of ESVONC, and Gerard Rutteman, first membership secretary of ESVONC, and edited by Jérôme Benoit, current President of ESVONC.*

In the late 70's, scientists, active in the field of Veterinary and Comparative Oncology, formed a working group, to set up a TNM Classification System of tumors in domestic animals. The participants were: Dr R.S. Brodey, Philadelphia, USA; Dr E.L. Gillette, Colorado, USA; Dr V.N. Milouchine, Geneva, Switzerland; Dr W. Misdorp, Amsterdam, The Netherlands; Dr L.N. Owen, Cambridge, United Kingdom; Dr A.-L. Parodi, Alfort, France; Dr A.B. Syrkin, Moscow, USSR; Dr G.H. Theilen, Davis, California, USA. The work was edited by LN Owen and published in 1980.

Inspired by this previous initiatives, and by VCS already in existence in the USA, a discussion by fax, led to the initiative by scientists working in veterinary oncology to create the European Society of Veterinary Oncology. In the ESVIM Newsletter Vol 2, No 2, 1992, p24 an announcement was made that a Steering Committee had been formed. The objective of the Society were presented.

It was further planed that there would be scientific meetings once every two years and a newsletter.

Volume 2, No. 2, 1992
Official Bulletin of the European Society of Veterinary Internal Medicine

THE EUROPEAN SOCIETY OF VETERINARY ONCOLOGY (ESVONC)

A steering committee has been formed out of scientists active in the field of veterinary and comparative oncology in Europe. This in order to prepare the foundation of the European Society on Veterinary Oncology.
Members of the steering committee are:
Prof. Dr. N.T. Gorman (Glasgow), Dr. E. Hellmén (Uppsala), Prof. Dr. W. Misdorp (Utrecht), Prof. Dr. A.L. Parodi (Maisons-Alfort), Dr W. Ponomarkov (Moscow), Dr. L. Rossi (Genua), Dr. G.R. Rutteman (Utrecht).

It is the intention of the Steering Committee that the objective of the Society shall be to further scientific progress in veterinary and comparative oncology.
The Society shall endeavour to achieve this objective by:

(1) encourageing and facilitating coordination of research and other contributions to the knowledge related to pathogenesis, diagnosis, therapy, prevention and control of animal tumour-diseases.
(2) providing an organization for recognized specialists in veterinary oncology and for individuals who take interest in research, teaching or the practice of veterinary and comparative oncology.
(3) furthering education in veterinary oncology.
(4) facilitating the exchange of information on comparative oncology through interaction with other organizations for cancer research.

It is planned to organize scientic meetings once every two years and to publish a newsletter.

The Steering Committee shall formulate a constitution.
A vote on the proposed constitution will be organized at the Inaugural Meeting that is planned for Thursday September 24 1992, parallel to the WSAVA-congress held in Rome from 24-27 September. The Inaugural Meeting will be held in the Cavalierie Hilton Hotel, Hall Red, 16.00 - 17.00 hours.

Scientists interested to receive further information (including an application form for membership) are kindly requested to write to:

Dr. G.R. Rutteman,
ESVONC
Dept. Clin. Sci. Comp. Anim.
POB 80.154, 3508 TD Utrecht
The Netherlands
FAX: (31)-30-518126

Erik Teske, Wim Misdorp and Gerard Rutteman

The Steering Committee prepared a Constitution and an Inaugural Meeting was organized during 2nd ESVIM Meeting (parallel to WSAVA World congress) at the Cavalierie Hilton Hotel, in Rome on September 24, 1992. During that meeting the people present voted for the Constitution and Misdorp (President), Gorman (Vice-president), Rutteman (Secretary), Hellmén (Treasurer), Rossi (Meeting secretary), Ponomarkow (member), and Parodi (Member) were elected as the first officers of ESVONC. Dr Malcolm Brearley became editor of the Newsletter. It was also decided that the International Conference on Spontaneous Animal Tumours, held in November 1993 in Genoa, would be co-organized by ESVONC. As an annual fee the sum of 30 ECU was determined. The formal (legal) registration (KVK of the society (at Notary in Utrecht, NL), with Statutes and By-laws was in 1993.

In the early years of ESVONC, Annual Meetings took place during the ESVIM congresses, which later became the ECVIM-CA congresses. Usually, a two-day program was made. Also joined sessions with other Societies were organized. The first one was in Cambridge together with the ESVNU. The ESVONC newsletter was at the time incorporated into the ESVIM newsletter.

The membership of ESVONC was in those days not open for everyone. Full membership was open to those who were interested in veterinary oncology and who possessed an academic degree AND have worked actively in cancer research for at least one year. The General Meeting ruled on the admittance of full members.

On December 31, 1996 ESVONC had 54 registered members. In 1999 the first joint meeting of ESVONC and VCS was organized in Woods Hole, Massachusetts, November 13-16. However, only a very few European ESVONC members were present (including the colleagues Gerard Rutteman, Miriam Kleiter, Daniela Simon and Erik Teske) and it was absolutely not felt by our American colleagues as a joint meeting. Also, during the WSAVA congress in Amsterdam in April 2000, a joined precongress day on Oncology was organized by both VCS and ESVONC.

In January 2001 a new Executive Committee was elected by postal vote. David Argyle became the new President, Barabara Kaser-Hotz the Vice-President, and J Martin de Las Mulas as Member. One year later, January 2002, the first Interim ESVONC meeting was organized in Zurich, after which Interim Spring meetings became routine. In that very year Honorary

Membership was bestowed on Wim Misdorp and André Parodi for their contributions to comparative and veterinary oncology.
During the 11th Annual Meeting of ESVONC it was decided to have the new Veterinary Comparative Oncology Journal as the official journal of ESVONC. Barbara Kaser-Hotz became the new President and Martin Kessler the new Vice-President. In 2004, Eva Hellmén became the next President and Johan de Vos became treasurer and membership secretary. In addition, the present logo was elected from a few alternatives (see below).

That year, the first ESVONC meeting, independent from the ECVIM-CA, was hosted by Martin Kessler, in Hofheim, Germany, in a seminar room, at the basement of Martin Kessler's previous hospital. A certain cultural shift (or « *revolution* ») started to take place at the time, with a more diverse group of clinicians and non-clinicians, academics and non-academics, sharing a profound interest for oncology. This meeting is considered by many to be the foundation meeting of our Society as we know it today.

« the happy and beautiful crowd » at the Hofheim ESVONC Meeting, 7th-8th February 2004

In 2005 the number of ESVONC members approached the 100. Between 28th February – 1st March 2008, the second “First” joined meeting of ESVONC and VCS was held in Copenhagen. More than 200 participants and exhibitors from 24 countries registered to the meeting. During that year Malcolm Brearley became the next ESVONC president. In the mean time the spring ESVONC meeting grew and grew. In 2009 some 160 veterinarians attended the congress in Visegrad, Hungary. In that very same year also some sad news was received. The Honorary member Wim Misdorp had passed away at the age of 80. To honour is Memory and his contribution to science it was decided to name the ESVONC Junior Research Award the Wim Misdorp Award.

As more and more practitioners who were interested in veterinary oncology became member of ESVONC, and under the influence of Johan de Vos, Malcolm Brearley and Martin Kessler, it was felt that the Constitution needed to be updated. Thus, the constitution was revised and the prerequisite that a member should have had at least one year experience in cancer research was deleted in 2009.

That year, a memorandum was signed between VCS (with Barbara Kitchell as President) and ESVONC, which paved the way for the current 4-yearly cycles. On 2012, the second WVCC was organised by ESVONC, in Paris and appeared to be a huge success.

Since that time, ESVONC has continued growing, becoming more inclusive and attracting members from all around Europe and more globally. We, ESVONC members, shall be grateful and thankful for the passion and hard work our *founders* have used to create ESVONC and make ESVONC the community it has become today.

Happy 30th Anniversary ESVONC!

Executive Committee's Report

President - Jérôme Benoit
Vice-President - Iain Grant
Secretary - Chiara Leo
Treasurer - Neil Palmer
Members at Large - Irina Gramer and Arno Roos

Recent achievements :

- Publication in VCO of our Congress Abstracts - gives better visibility to our presenters - to be renewed yearly
- Constitution revision (Chair Martin Kessler) - edits to be presented at the 2022 AGM + vote
- World Oncology Connections (WOC) - quarterly webinars co-organised by VCS, ARBROVET, AMONCOVET, JVCS and ESVONC

Ongoing and future projects :

- New Constitution 2023
- New relations with WSAVA, VSSO - future initiatives and projects
- Oncology Internship (standards and recommendations)
- Digitalization and communication (website, app, social media, registry and forum, podcasts)
- Welcome oncology nurses to our group

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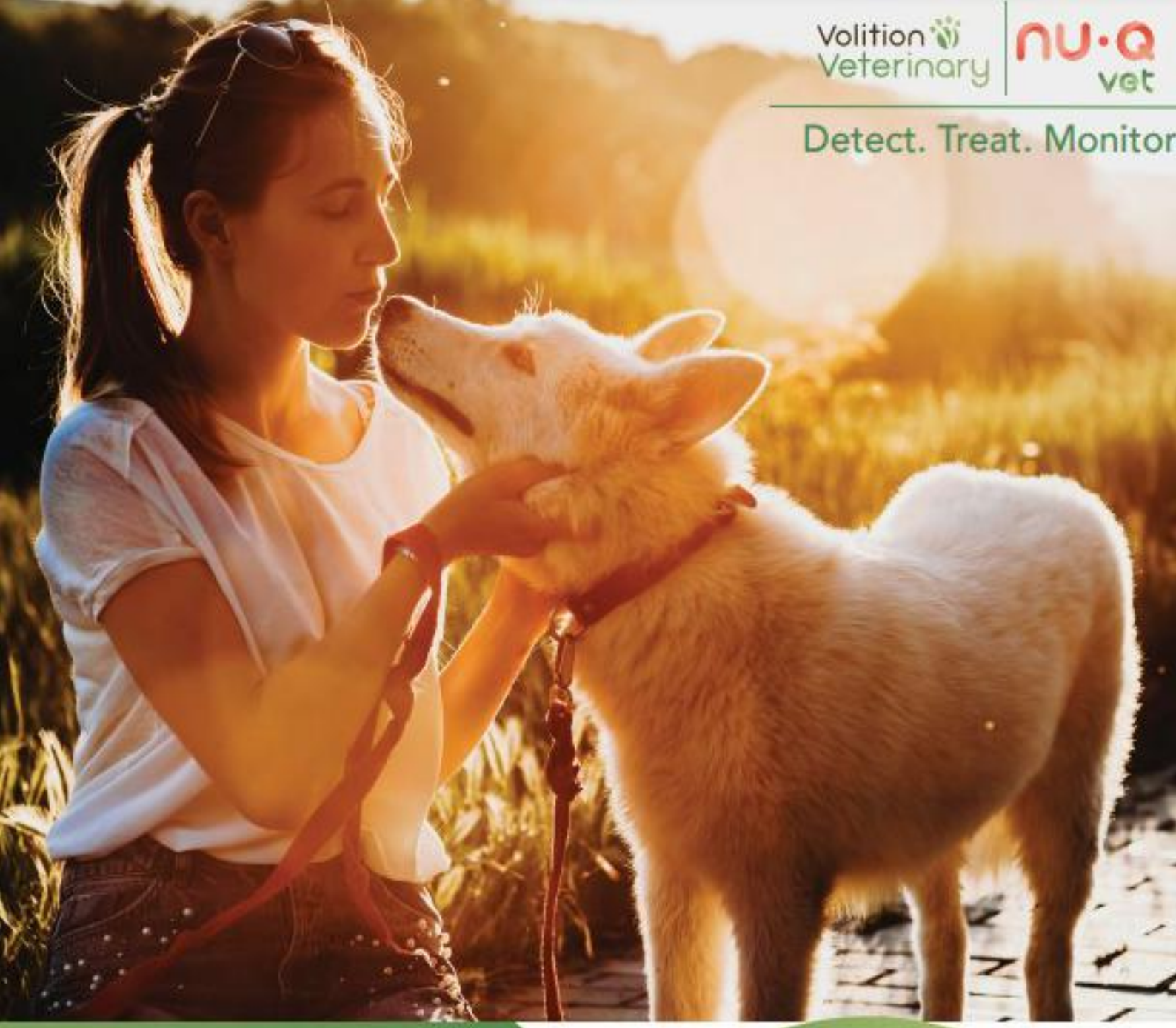

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TABLE OF CONTENT

ESVONC 2022 - Keynote Speakers

Dr. Federica Cavallo is Full Professor of Immunology (General Pathology) at the University of Turin, Medical School and carries out her research activities at the Department of Molecular Biotechnology and Health Sciences, situated in the Molecular Biotechnology Center "Guido Tarone", Via Nizza 52, Turin.
Graduating in Biological Sciences from Turin University in July 1989, she concluded the Ph. D. program (Tumor Immunology) in 1995. From 1996 to 1998 she was a post-doctoral fellow of the University of Turin and from 1998 to 2000 a researcher at the Center of Immunogenetics and Experimental Oncology CIOS, National Research Council, Turin, Italy. She became tenured researcher of the University of Turin in 2001, Associate Professor in 2006 and Full Professor in 2016.
She is a well-known, internationally reputed scientist, with a very good track record in the fields of onco-immunology, translational immunotherapy, and comparative oncology. Her research is mainly focused on active immunization against tumor antigens of breast cancer, melanoma, and osteosarcoma. As of April 2022, she is author/co-author of 198 peer-reviewed publications with an H Index of 46 and 8723 citations (data from Scopus). She is a member of the prestigious Academy of Sciences of Turin (Class of Physical, Mathematical and Natural Sciences) and of numerous other national and international scientific associations, among which: the Italian Society of Immunology, Clinical Immunology And Allergology (SIICA); the Italian Network for the Biotherapy of Tumors (NIBIT); the European Society for Cancer Research (EACR); the American Association for the Advancement of Science (AAAS); the American Association for Cancer Research (AACR); the American Association of Immunologists (AAI), and the Society for Immunotherapy of Cancer (SITC).

Dr. Phil Bergman is the Director of Clinical Studies for VCA. He is an adjunct faculty member of the Memorial Sloan-Kettering Cancer Center and the principal veterinary investigator for the canine melanoma vaccine (Oncept®), which was fully licensed in 2009. Prior to Dr. Bergman joining VCA, he served as the Chief Medical Officer for BrightHeart Veterinary Centers from 2007 to 2011; from 1999 – 2007 he was the head of the Donaldson-Atwood Cancer Center at AMC. After finishing veterinary school from Colorado State in 1990, he was an intern at Kansas State ('90-'91) and returned to CSU for his medical oncology residency ('91-'94) and then completed a PhD Fellowship in human cancer biology from the M.D. Anderson Cancer Center in Houston ('94-'99). He was previously Chair of the ACVIM Board of Regents and President of the Veterinary Cancer Society.

Dr. Jonathan Bray graduated from Massey University, New Zealand in 1988 and completed specialist training in Soft Tissue Surgery at Cambridge University, UK, becoming a Diplomate of the European College of Veterinary Surgeons in 1997. He gained a Masters degree in Veterinary Science in 1990, a Masters degree in Clinical Oncology in 2013 and a PhD in soft tissue sarcoma in 2020. Jonathan has spent periods of time in both academia and private referral practice since completing his residency, including positions as Head of oncology and soft tissue surgery at Davies Veterinary Specialists, Hertfordshire (2004-2010), and Head of the Companion Animal Group at Massey University (2012-2017). He is now a Senior Surgeon at Fitzpatrick Referrals Oncology and Soft Tissue in Guildford, UK.

Prof. Giancarlo Avallone graduated in veterinary medicine in 2004 at the University of Milan, where he attended a combined PhD/Residency program. In 2009 he passed the ECVP certification exam. From 2007 to 2011 he worked as a post-doc fellow at the University of Milan and then, with the same position, at the University of Bologna until 2015. From 2015 and 2018 he worked as senior lecturer and since 2018 as Associate Professor at the same institution where he is currently chief of the pathology service and director of the ECVP residency program. From 2015 to 2020 he was component and the last year chair of the ECVP examination committee and from 2020 component of the ECVP council. His major line of research is oncological pathology of domestic animals with special focus on tumors of soft tissues. He is author of 64 papers in indexed journals and of the most recent classifications of tumors soft tissue and tumors of bone.

Dr. Paul Huang is Head of the Molecular and Systems Oncology Laboratory at The Institute of Cancer Research (ICR) in London, UK. He received his PhD in Biological Engineering from Massachusetts Institute of Technology in 2008. He is currently Reader in Molecular Oncology within the Division of Molecular Pathology. His laboratory focuses on understanding aberrant signaling networks and drug resistance in sarcomas, with the goal of developing biomarkers and new therapies for these rare diseases. Paul is the Deputy Director of the Joint Royal Marsden-ICR Sarcoma Research Centre, one of the largest sarcoma research centres in Europe. He co-leads the multi-institutional Sarcoma Accelerator Consortium which seeks to develop a digital hub of clinical and molecular data to facilitate interdisciplinary research in patients with high-risk sarcoma. He also serves as Vice Chair of the Pathology & Translational Research Committee of the EORTC Soft Tissue and Bone Sarcoma Group. He was elected a Fellow of the Royal Society of Biology in 2020.

Katherine Hansen, DVM, DACVR (Radiation Oncology)
Katherine Hansen is a board-certified radiation oncologist who serves as an associate professor of clinical radiation oncology at the University of California-Davis. She received her DVM from UC Davis in 2013 and then completed a rotating internship at the University of Pennsylvania. After internship, she spent two years as a post-doctoral associate in the radiation oncology labs at Duke University, where she worked on a variety of projects involving murine modeling, hypoxia, and nanoparticle development. She trained as a resident in radiation oncology from 2011-2013, and then signed on as a faculty at UC Davis upon completion of her residency. Her appointment is primarily clinical in nature, and she spends most of her time training residents and treating patients in the clinic. Her research includes collaborations with the UCD-Medical Center physicists on quality assurance and software studies that benefit both human and veterinary radiation oncology. She also publishes on advanced veterinary radiation treatment options, especially in stereotactic radiotherapy.

Olaf van Tellingen
Group Leader, Division of Pharmacology, Netherlands Cancer Institute, Amsterdam

In 1993 I received my PhD on my thesis investigating the pharmacology of investigational Vinca Alkaloids in mouse models while working at the Netherlands Cancer Institute (NKI). I have remained working as a pharmacologist at the NKI. First as an associate researcher and then further as group leader. Between 1996 and 1997, I have been a visiting scientist in the lab of Prof. Josh Fidler (MD Anderson Cancer Center), where I became experienced in using orthotopic cancer models, including brain metastases models. Since then, my main research topic has been on glioblastoma (GBM), a devastating malignant brain tumor for which there are yet no curative therapies. Next to that, I am also involved as a pharmacologist in the support of in vivo intervention studies that are conducted in our mouse cancer clinic core facility.

Dr. Ines Carrera graduated in 2001 from the University of Santiago de Compostela (Spain). After a few years working in small animal private practice in Spain, she moved to Scotland to undertake a Master degree in Radiology at the University of Glasgow. She felt very passionate about Diagnostic Imaging, and after finishing her Masters she started a residency in Diagnostic Imaging at the University of Glasgow. She passed the European Diploma in Veterinary Diagnostic Imaging in 2010.

She has worked in several Universities (Illinois (USA), Sidney (Australia) and Zurich (Switzerland) for a total of seven years. During this period in Academia she has been fortunate enough to earn large teaching experience (with undergraduates and postgraduate students), has done extensive research (mainly focussed on Neuroimaging) and published numerous articles in peer-reviewed journals. She undertook a PhD degree in MR spectroscopy of the brain in dogs from the University of Bern-Zurich.

She has worked for the last 5 years in private practice in the UK (SCVS and Willows) and for VetOracle in the Neuroimaging service with Laurent Garosi and Simon Platt.

Dr. Andrea Pietro Sponghini
I graduated in Turin University (Italy) in 2004 in Oncology and in UPO (University of Piedmont Oriental) in 2014 in Palliative care.
My interest in particular is on Head & Neck cancer, Neuro-oncology, Melanoma and supportive care.
I work in a university hospital in Novara and I am in charge of the oncology department.
I collaborate with different scientific journals and I participate in international clinical trials.
I participate as a speaker at various congresses related to my pathologies.
I am member of some scientific societies such as IMI, AIOM, ESMO, AIOCC, NICSO.
In my free time I love being with my children, reading and traveling.

Maja Louise Arendt DVM, PhD, DipECVIM-CA(onc)

I am currently working as an associate professor in Veterinary and Comparative Oncology at the University of Copenhagen, faculty of Veterinary Clinical Sciences. I am also an affiliated researcher with the Comparative and Functional Genetics research group at Uppsala University.

I obtained the ECVIM diploma in medical oncology in 2019, after training as a resident at the Queens Veterinary School Hospital in Cambridge. I obtained my PhD at the University of Glasgow in 2009 on telomerase targeted gene therapy in cancer and did a Post Doc at Uppsala University from 2010-2012 focusing on comparative genetics. My main research focus is canine cancer genetics investigating both germline genetic risk factors and somatic cancer mutations. I try to combine my clinical work and research to assure that the research questions asked are relevant and of value to both human and animal cancer patients.

Dr. Carlo Cattrini is a 34-year-old human medical oncologist. He works as physician scientist and medical consultant at the University Hospital "Maggiore della Carità", Novara, Italy.
He graduated with honors from the University of Pavia in 2012. In 2013, he was awarded with a basic research fellowship, and he worked on preclinical studies in pediatric oncohematological malignances at the "Mario Negri" Institute for Pharmacological Research in Milan. He subsequently trained as Medical Oncologist at the University of Genoa (IRCCS Policlinico San Martino) from 2014 to 2019. During his training in Genoa, he worked on several preclinical and clinical studies in genitourinary tumors, and he was enrolled in the PhD Program in Translational Oncology. In 2019, he was awarded with the prestigious European Society for Medical Oncology (ESMO) Clinical Research Fellowship. Thanks to this grant, he spent 2 years at the Prostate Cancer Clinical Research Unit of the Spanish National Cancer Research Center (CNIO) in Madrid, Spain. Under the precious mentorship of David Olmos and Elena Castro, he has collaborated

on many projects in patients with advanced prostate cancer, including BRCA2men, Procure, BioChip and Capture. Now, he is currently involved in many academic and sponsored studies on prostate, renal and bladder cancer.
During this career, he has received several awards for his researches (Conquer Cancer Foundation of ASCO Merit Award at ASCO-GU 2020, Best Poster on Prostate Cancer at EMUC 2017, “Costa Foundation” Prize). He is a full member of ESMO, American Society of Clinical Oncology (ASCO), Associazione Italiana di Oncologia Medica (AIOM), Italian Network for Research in Urologic-Oncology (MeetURO) and MCCR Alumni Club.

Dr. Louise Clark is a European and Royal College of Veterinary Surgeons recognised Specialist in Veterinary Anaesthesia and Analgesia. She was awarded her Diploma in Veterinary Anaesthesia and Analgesia in 2003 following a residency at the University of Edinburgh R(D)SVS. She was then part of a large Anaesthesia/critical care team at the Animal Health Trust in Newmarket before moving to Davies Veterinary Specialists in Hertfordshire in 2007. In 2014 Louise was awarded an MSc (Distinction) in the Clinical Management of Pain from Edinburgh University and became a Fellow of the RCVS in 2020. She is currently Past President of the Pain Medicine Section Council at the Royal Society of Medicine and has been an invited examiner on the European Diploma examination and a Treasurer of the Association of Veterinary Anaesthetists. Her current interests revolve around raising the awareness of chronic pain in domestic species and improving the evidence base for treatment.

Dr. Valérie Freiche graduated from Ecole Nationale Vétérinaire d’Alfort, near Paris, France. After her graduation, she completed an internship and a French internal medicine specialisation at the Department of Internal Medicine in the same Vet School. She defended her PhD project about the Comparative Oncogenesis of Indolent T-cell Lymphoproliferative Intestinal Disorders in cats and humans. This study involved both human and vet researchers and this collaboration is still ongoing.
Nowadays Valerie is a member of the Internal Medicine Department of Alfort Vet School, and she is involved in clinical teaching and research.
She is currently the vice-president of the ECVIM-European Society of Comparative Gastroenterology (ESCG).
Her main interest is gastroenterology, particularly digestive oncology and interventional endoscopy. Her publication list consists of journal articles, research abstracts and book chapters. Valérie is strongly involved in veterinary continuing education. She created 4 years ago, with her PhD supervisor, Pr Olivier Hermine (Hôpital Necker, Paris-France), the first French Congress in comparative oncology to promote comparative research between human and vet res earchers according to the “One-Heath and One Medicine” concept.

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Inge studied for her veterinary nurse qualification in University College Dublin, graduating in 2007. She spent 8 years working in small animal practices in Ireland and Australia, before moving to a large soft tissue and oncology practice in Surrey in 2015. Here, she developed a passion for oncology nursing. In her role as Senior nurse clinician, she worked with an experienced and busy team, caring for both surgical and medical oncology patients. She gained her diploma in Advanced Veterinary Nursing from Harper Adams University in 2020, and is currently studying for a post-graduate certificate in Oncology from the same university. In 2020 she moved to a position as Senior Oncology Nurse at The Ralph Veterinary Referral hospital, a large multi-disciplinary referral practice, assisting them in setting up their Oncology referral service, which is now thriving.
Inge is passionate about helping enhance the quality of life for patients with cancer, while providing support and advice for their careers in what is a difficult and emotional time. She is also a firm advocate for veterinary nurse development and education, and enjoys sharing her oncology knowledge with others via her Instagram page @oncologyRVN. She also volunteers for Streetvet, and shares her home with a small opinionated chihuahua cross called Erik.

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Overview of the relationship between cancer and the immune system

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The elucidation of the many complex interactions between a tumor and the host immune system has contributed to the development of immunotherapy as a new cancer treatment. Indeed, cancer immunotherapy now represents the fifth pillar of cancer care, is in continuous and rapid evolution and is clearly endowed with the potential to revolutionize the way we treat cancer, for good (*1*).

The premise of cancer immunotherapy consists of granting a cancer patient's immune system the ability to recognize cancer cells as foreign, as they do with a virus or bacterium, and mount a response to kill them. The prospect of using anti-cancer vaccines has existed for decades, from the middle of the twentieth century when the race for tumor antigen identification began, and soon led to proof that tumor cells actually display antigens (*2*). Today, it is clear that these antigens can either be tumor-associated or tumor-specific. In the first case, they are native self-antigens that are abnormally expressed by multiple tumors of different histotypes, while, in the second, they are not expressed by normal cells and are tumor and often patient specific (*3*).

Over recent decades, a great deal of genome-sequencing-based research has demonstrated that tumors (in humans and animals) acquire from tens to thousands of somatic mutations during the processes of cancer initiation and progression, and that these are a result of DNA replication errors, and of exposure to endogenous factors (such as free radicals) and environmental (exogenous) carcinogens, such as various forms of radiation and mutagenic chemicals in food and air (*4, 5*). Thus, a tumor can acquire small genomic alterations, including single nucleotide variants (SNVs), as well as small insertions and deletions (collectively known as "indels") resulting in altered amino acid translation and altered protein products called "neoantigens"(*4, 6*). On a much larger scale, a tumor can undergo structural alterations that typically involve thousands to millions of nucleotides. Copy number variants (CNVs) are a common type of structural alteration that involve gains or losses of large stretches of DNA. Translocations are another type of structural alteration whereby two distant, otherwise unrelated, genomic regions are joined together, creating "gene fusions" that can drive tumor growth (*4*). These structural alterations may either result in the formation of neoantigens or in the deregulation of native self-antigen expression. Of the acquired neoantigens, some confer an intrinsic growth advantage to cancer (i.e., driver neoantigens), and are frequently shared by a variety of tumor types, while others (i.e., passenger neoantigens) do not have an impact on cancer growth and are mostly private to a given tumor, or cell clones in a given tumor (*7*).

Both neoantigens and oncoviral antigens are absent from normal human tissues and are only present on tumor cells, meaning that they can be spontaneously recognized by the host immune system (*6*). Tumor-associated antigens, thanks to their aberrant expression, can also induce an adaptive immune response to a certain extent (*5*). The adaptive immune response is based on the activation of T cells and the generation of memory cells. T-cell activation acts both by killing tumor cells and activating B cells to release high-affinity antibodies directed against the tumor antigens (*8*). These antibodies can, in turn, act either directly against tumor cells, by inhibiting growth factor receptors and/or inducing apoptosis, or indirectly via the engagement of complement, natural killer (NK) cells and macrophages, leading to cancer-cell elimination (*9*)

The central event in the adaptive immune response to tumors is therefore T-cell activation, which is a complex, highly demanding reaction orchestrated by cells of myeloid origin, called dendritic cells (DCs). These are professional antigen-presenting cells that are uniquely able to induce naïve T-cell activation and effector differentiation as they are able to capture the

antigens, process them inside of the cell and then expose them on the cell surface in the form of peptides bound to major histocompatibility molecules (MHC) (*10*). The DC's ability to perform cross-presentation, i.e., the presentation (in the context of class I MHC molecules (MHC-I)) of antigens captured from the extracellular milieu, allows them to trigger responses against antigens from other cell types, including tumors. This ability provides the means for the activation of the two main types of T cells; cytotoxic T lymphocytes (CTLs), which will kill tumor cells, and helper T cells that will aid B cells in their differentiation into antibody-secreting cells (*10*). Nevertheless, it is worth noting that CTLs are not the only cytotoxic cells involved in tumor cell killing. NK cells, whether of the T-cell lineage (NKT cells) or otherwise, can kill tumor cells in an antigen-independent way thanks to the expression of activating receptors, such as NKGD2, that recognize the expression of stress-induced ligands on tumor cells (*11*).

To summarize, tumors are antigenic and spontaneously recognized by the immune system, but they can develop and become clinically evident in an immune-competent organism. The explanation for this apparent contradiction can be found in the seminal experiments conducted by the research groups of Bob Schreiber and Lloyd Old at the beginning of the twenty-first century (*12, 13*). These experiments demonstrate that the immune system in an immunocompetent host is able to shape the immunogenicity of a tumor to the point at which it is no longer recognized by the immune system (*14*). This is the so-called "cancer immunoediting" process. It is an extrinsic tumor-suppressor mechanism that only engages after cellular transformation has occurred and intrinsic tumor-suppressor mechanisms have failed. It develops in three distinct phases, called "the three **E**s of cancer immunoediting" (*13, 15*). In the Elimination phase, innate and adaptive immunity work together to destroy developing tumors long before they become clinically apparent. If this phase proceeds to completion, the host remains free of cancer, and elimination thus represents the full extent of the process. If, however, a rare cancer cell variant is not destroyed in the elimination phase, it may then enter the Equilibrium phase, in which its outgrowth is prevented by immunologic mechanisms. T cells, IL-12 and IFN-γ are required to maintain tumor cells in a state of functional dormancy, whereas NK cells and molecules that participate in the recognition or effector function of cells of innate immunity are not required, which indicates that equilibrium is a function of adaptive immunity only. Equilibrium may also represent the end-stage of a cancer-immunoediting process and may restrain the outgrowth of occult cancers for the lifetime of the host. During the equilibrium phase, the editing of tumor immunogenicity occurs as a consequence of the constant immune selection pressure placed on genetically unstable tumor cells that are no longer recognized by adaptive immunity and become insensitive to immune effector mechanisms and/or induce an immunosuppressive state within the tumor microenvironment. These tumor cells may then enter the Escape phase, in which their outgrowth is no longer blocked by immunity, and they emerge to cause clinically apparent disease (*15*). As a consequence, an established tumor shows the so-called "immune hallmarks of cancer"; the ability to thrive in a chronically inflamed microenvironment, evade immune recognition and suppress immune reactivity by co-opting the mechanisms that normally regulate the immune system to avoid the development of autoimmune diseases (*16*). In other words, cancer is the price we pay for protection from autoimmune diseases (*17*).

Indeed, several mechanisms are responsible for immune resistance. Tumors modulate their expression of immunogenic ligands (e.g., NKG2DLs) and MHC-I molecules to escape detection by NK cells and CTLs. They can also co-opt survival pathways and anti-apoptotic proteins to resist immunogenic-cell death and immune-cell targeting. They can express immune checkpoint ligands, e.g., the ligands of (PD-L1 and PD-L2) of the Programmed Death 1 (PD-1), on their plasma membranes, effectively decommissioning anti-tumor T cells (*18*). Moreover, the dynamic tissue microenvironment consists of immunosuppressive immune cells and factors that suppress anti-tumor effector-cell functions and exclude them from acting on the tumor. Finally, T-regulatory cells (Tregs) and tumor cells can foster the activation-induced cell death of CTLs inside the tumor (*18*). Unfortunately, in many circumstances, these considerations have not been taken into account, causing the failure of numerous human and

veterinary clinical trials, which is why there is such a low number of approved anti-cancer vaccines.

Some therapies have been developed and proven to be effective at reversing the alterations that are responsible for tumor resistance. These include anti-apoptotic inhibitors, monoclonal antibodies that are targeted to immunosuppressive cell markers to deplete these cells, angiogenesis inhibitors that recondition the TME and thus enhance CTL activation and infiltration into the tumor, and molecules that can downregulate long noncoding RNA (lncRNA) and histone deacetylase expression, thus halting the transmission of the death signals in T cells and potentiating the formation of immune memory (*18*). Of particular importance are the therapies based on the use of chimeric antigen receptor (CAR)-T/NK cells and immune checkpoint inhibitors (ICIs).

CAR-T cells are engineered T cells that bear an extracellular single-chain fragment of an antibody variable region coupled to signaling domains, and that evoke enhanced anti-tumor activity by acting independently of MHC-I molecule expression on tumor-cell plasma membranes (*8*). Several of these drugs have been approved and effectively used in the clinic against different types of human liquid tumors. They induce powerful immune effector responses that are frequently associated with significant toxicities (*19*). CAR-NK cell therapies are also under evaluation (but not yet approved) as novel therapeutic options with potentially less side-effects (*20*).

As far as the use of ICIs is concerned, the pioneering work on the cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and PD1 immune checkpoints, led by the teams of James P. Allison and Tasuku Honjo, respectively, revealed that these molecules act as so-called "brakes" on the immune system, and showed that the inhibition of these checkpoint pathways allows T cells to eradicate cancer cells more effectively (*21*). These discoveries earned Allison and Honjo the Nobel Prize in Physiology or Medicine in 2018 and laid the foundation for the clinical development of ICIs, which have dramatically improved outcomes for many people with cancer and have been shown to induce robust and durable responses in cohorts of patients with a variety of solid tumors (*22*). ICIs, or checkpoint blockers, are monoclonal antibodies that block the immunosuppressive interaction between the inhibitory receptors expressed by activated T lymphocytes, such as CTLA4 and PD-1, and their ligands, such as PD-L1. By blocking the immune checkpoints, the antibodies interrupt these immunosuppressive interactions and restore the ability of T cells to eliminate antigen-expressing cancer cells. Ipilimumab is the first and only FDA-approved CTLA-4 inhibitor. Since its FDA approval, in 2011, more ICIs have been approved for human cancer therapy: four PD-1 inhibitors (nivolumab, pembrolizumab, cemiplimab, and dostarlimab); and three PD-L1 inhibitors (atezolizumab, avelumab and durvalumab). Very recently (March 2022) the FDA approved relatlimab, the first ICI targeting a third brake of T cells, LAG-3, to be given in combination with nivolumab, for people aged 12 and older with untreated unresectable or metastatic melanoma (*23*).

ICI and adoptive T-cell therapy with CAR T cells have changed the way cancer is treated, for good, leading to promising clinical outcomes, at least in some subsets of patients, with some durable responses. Nevertheless, these therapies can induce severe toxicities, which mainly include inflammatory autoimmune reactions (*19, 22*). Moreover, primary resistance to ICIs has been found in a substantial proportion of patients, while dramatic tumor-growth acceleration, so-called "hyper-progressive disease", has been described in some patients at the onset of anti-PD-(L)1 monotherapy (*24*). In addition, frequent tumor relapse has been observed in patients after initially successful CAR-T cell therapy, as has the induction of adaptive immune resistance to therapy. We are therefore still far from having found a cure for cancer and there is room for improvement in cancer immunotherapy.

A great opportunity, however, has been uncovered by the observation that ICI efficacy is higher in tumors with a high mutational load, such as melanoma (*25*), which, in turn, results in the formation of neo-antigens (*26*). This observation has spurred the search for neoantigens in tumors in order to generate personalized vaccines to be used in combination with ICIs. The

idea behind this approach derives from another important observation; response to ICIs varies according to the infiltration status of a tumor (*27*). High-CTL-infiltrated tumors ("hot" tumors) are generally highly responsive, whereas, low-CTL-infiltrated tumors ("cold" tumors) are poorly responsive. Vaccines against neoantigens would be effective in inducing a T-cell response in a host, that can transform a "cold" into a "hot" tumor, which, under treatment by ICIs, can be killed by the vaccine-induced CTLs. The problem here is that the pipeline to produce neoantigen-based vaccines is quite cumbersome and costly. It starts from the DNA and RNA sequencing of a tumor biopsy and its comparison with a sample of non-malignant tissue, normally the peripheral blood. It is necessary to both identify the somatic mutation and verify the expression of the mutated proteins by the tumor. The characterization of the patient's MHC allotype and the prediction of the expression of peptides from the neoantigens on the MHC molecules are also required to select candidate neoantigens for use as vaccines. Moreover, an optimistic prediction is that the needle-to-needle time is of 6 to 18 weeks, and the rate of success so far has been disappointing (*5, 28*). A possible explanation for this can be found in the different natures of the various neoantigens used for vaccines. If they are derived from passenger mutations, they can easily undergo immunoediting as a result of the immune pressure induced by the vaccine. Only neoantigens derived from driver mutations hardly undergo immunoediting. However, determining whether the neoantigen selected for vaccination has a causal role in maintaining the cell's neoplastic phenotype would require considerable time. Taken together, these factors render vaccination against neoantigens barely feasible, and attention is returning to tumor-associated antigens (*29*). Indeed, a role in promoting tumor growth and survival has been clearly demonstrated for many overexpressed and differentiation antigens, and the availability of ICIs can help to overcome central tolerance, leading to effective CTL activation in patients.

In conclusion, we now have several ways to counter the growth of tumors in patients, but much remains to be discovered, including the way in which the patient’s microbiota impacts on cancer progression, immune recognition, and response to therapy (*30, 31*). As the relationship between cancer and the immune system is so complex, the future of cancer-patient treatment must surely include the use of combined immunotherapeutic approaches.

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Canine Malignant Melanoma Update and Controversies

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Canine malignant melanoma (CMM) of the oral cavity, nail bed, foot pad and mucocutaneous junction is a spontaneously occurring, highly aggressive and frequently metastatic neoplasm. CMM is a relatively common diagnosis representing ~ 4% of all canine tumors and it is the most common oral tumor in the dog. CMM and advanced human melanoma (HM) are diseases that are initially treated with aggressive local therapies including surgery and/or fractionated radiation therapy; however, systemic metastatic disease is a common sequela. Based on

these similarities, CMM appears to be a good clinical model for evaluating new treatments for advanced HM.

Canine patients with advanced disease (WHO stage II, III or IV) have a reported median survival time of 1-5 months with standardized therapies. A combination of hypo-fractionated radiation therapy and chemotherapy have a reported median survival time of one year in stage I oral CMM. Human patients with deep AJCC stage II or stage III disease (locally advanced or regional lymph node involvement) have at least a 50% chance of recurrence after surgical resection; patients with stage IV melanoma (distant metastases) have a median survival of less than ten months and most of these patients eventually die of melanoma. Standard systemic therapy is dacarbazine chemotherapy in HM, and carboplatin chemotherapy in CMM. Unfortunately, response rates to chemotherapy in humans or dogs with advanced melanoma range from 8-28% with little evidence that treatment improves survival. It is easily evident that new approaches to this disease are desperately needed and multiple methodologies have been reported to date.

Active immunotherapy in the form of vaccines represents one potential therapeutic strategy for melanoma. The advent of DNA vaccination circumvents some of the previously encountered hurdles in vaccine development. DNA is relatively inexpensive and simple to purify in large quantity. The antigen of interest is cloned into a bacterial expression plasmid with a constitutively active promoter. The plasmid is introduced into the skin or muscle with an intradermal or intramuscular injection. Once in the skin or muscle, professional antigen presenting cells, particularly dendritic cells, are able to present the transcribed and translated antigen in the proper context of major histocompatibility complex and costimulatory molecules. The bacterial and plasmid DNA itself contains immunostimulatory sequences that may act as a potent immunological adjuvant in the immune response. In clinical trials for infectious disease, DNA immunization has been shown to be safe and effective in inducing immune responses to malaria and human immunodeficiency virus. Although DNA vaccines have induced immune responses to viral proteins, vaccinating against tissue specific self-proteins on cancer cells is clearly a more difficult problem. One way to induce immunity against a tissue specific differentiation antigen on cancer cells is to vaccinate with xenogeneic antigen or DNA that is homologous to the cancer antigen. It has been shown that vaccination of mice with DNA encoding cancer differentiation antigens is ineffective when self-DNA is used, but tumor immunity can be induced by orthologous DNA from another species.

We have chosen to target defined melanoma differentiation antigens of the tyrosinase family. Tyrosinase is a melanosomal glycoprotein, essential in melanin synthesis. The full length human tyrosinase gene was shown to consist of five exons and was localized to chromosome 11q14-q21. Immunization with xenogeneic human DNA encoding tyrosinase family proteins induced antibodies and cytotoxic T cells against syngeneic B16 melanoma cells in C57BL/6 mice, but immunization with mouse tyrosinase-related DNA did not induce detectable immunity. In particular, xenogeneic DNA vaccination induced tumor protection from syngeneic melanoma challenge and autoimmune hypopigmentation. Thus, xenogeneic DNA vaccination could break tolerance against a self tumor differentiation antigen, inducing antibody, T-cell and anti-tumor responses.

The signalment of dogs have been similar to those in previously reported CMM studies. No toxicity was seen in any dogs receiving the aforementioned vaccines with the exception of minimal to mild pain responses at vaccination, one muGP75 dog experienced mild aural depigmentation, and one muTyr dog has experienced moderate foot pad vitiligo. Dogs with stage II-III loco-regionally controlled CMM across the xenogeneic vaccine studies have a Kaplan-Meier (KM) median survival time (MST) of > 2 years (median not yet reached). The KM MST for all stage II-IV dogs treated with huTyr, muGP75 and muTyr are 389, 153 and 224 days, respectively. The KM MST for stage II-IV dogs treated with 50mcg MuTyr, 100/400/800mcg HuGM-CSF or combination MuTyr/HuGM-CSF are 242, 148 and > 900 (median not reached, 6/9 dogs still alive) days, respectively. For dogs on the Phase Ib MuTyr/HuGM-CSF/Combination trial, significant differences in MST were noted across pre-vaccination stage (stage IV MST = 99 days, stage III = 553 days and stage II > 401 days, P <

.001). The results from dogs vaccinated with huTyr were published in 2003 (Bergman et al, Clin Cancer Res 2003).
We have also investigated the humoral responses of dogs receiving HuTyr as a potential explanation for the long-term survivals seen in some of the dogs on this study. Utilizing standard ELISA with mammalian expressed purified human tyrosinase protein as the target of interest (kind gift of C Andreoni & JC Audonnet, Merial, Inc.), we have found 3/9 dogs with 2-5 fold post-vaccinal humoral responses compared to pre-immune sera. We have confirmed these findings utilizing a flow-cytometric-based assay of pre- and post-vaccinal sera in permeabilized human SK-MEL melanoma cells expressing endogenous human tyrosinase. Interestingly, the three dogs with post-vaccinal anti-HuTyr humoral responses are dogs with unexpected long-term tumor control (Liao et al, Cancer Immunity, 2006). Co-Investigators have also determined that normal dogs receiving the HuTyr-based melanoma vaccine develop Ag-specific IFN-γ T cells (Goubier et al, Vaccine, 2008).
The results of these trials demonstrate that xenogeneic DNA vaccination in CMM is: 1) safe, 2) develops specific anti-tyrosinase humoral and cell-mediated immune responses, 3) potentially therapeutic with particularly exciting results in stage II/III local-regional controlled disease and dogs receiving MuTyr/HuGM-CSF combination, and 4) an attractive candidate for further evaluation in an adjuvant, minimal residual disease Phase II setting for CMM. A safety and efficacy USDA licensure multi-institutional trial investigating HuTyr in dogs with locally controlled stage II/III oral melanoma was initiated in April, 2006. Human trials of xenogeneic tyrosinase DNA vaccination have recently initiated. In March 2007 and December 2009, we received conditional followed by full licensure (respectively) from the USDA for the canine melanoma vaccine. This represents the first US-government approved vaccine for the treatment of cancer across species.
Since 2003, four publications have reported limited to no efficacy in dogs treated with Oncept®, but all of these studies are uncontrolled with limited local control information, retrospective and contain less than 20-25 vaccinates per study. In a recently reported VCS abstract, our objective was to assess the safety and clinical outcomes from a large group of dogs with malignant melanoma treated with the Oncept® melanoma vaccine across VCA Oncology Centers. Our hypotheses were: 1) Oncept® melanoma vaccination in dogs with malignant melanoma will continue to have an excellent safety profile similar to previous publications; 2) Oncept® melanoma vaccination in dogs with malignant melanoma increases PFI and survival similar to the outcomes reported in the prospective 5-site USDA registrational trial reported by Grosenbaugh et al; 3) Oncept® melanoma vaccination in a larger cohort of dogs with malignant melanoma have longer PFI and survival than the outcomes reported in much smaller retrospective studies reporting on outcomes with 20-25 or fewer Oncept® vaccinates.
A total of 320 dogs from 12 VCA oncology centers were entered into the Medrio EDC system. Sixty-six percent, 17% and 9% had oral, digit and cutaneous tumor locations, respectively with 55%, 28%, 14% and 3% WHO stage I, II, III and IV dogs. Thirty-nine percent had adequate local tumor control and 11% had lymph node metastasis prior to starting Oncept®. Stage was highly prognostic for PFI and MST (Log-Rank $P < 0.01$). The median PFI was 3261, 805, 508, and 293 days for stage I, II, III, and IV dogs, respectively. The MST was > 3261 (median not yet reached), 2140, 2052, and 302 days for stage I, II, III, IV dogs, respectively. The long-term median outcomes noted in this study are similar to those reported in the USDA 5-site prospective licensure trial and compare favorably to outcomes reported with standardized therapies without Oncept®. The recent 2013-2016 retrospective studies reporting 20-25 (or fewer) Oncept® vaccinates do not mirror the results seen in larger dog cohort studies.
In summary, CMM is a more clinically faithful therapeutic model for HM when compared to more traditional mouse systems as both human and canine diseases are chemoresistant, radioresistant, share similar metastatic phenotypes/site selectivity, and occur spontaneously in an outbred, immuno-competent scenario. In addition, this work also shows that veterinary cancer centers and human cancer centers can work productively together to benefit veterinary and human patients afflicted with cancer.

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The Turin Melanoma Vaccine Experience

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The University of Turin's experience in canine melanoma vaccines stems from a collaborative effort, which began in 2010, between the Oncoimmunology group, headed by Federica Cavallo and Federica Riccardo at the Department of Molecular Biotechnology and Health Sciences, and the Clinical Veterinary Surgery group led by Paolo Buracco and Emanuela Morello at the Department of Veterinary Sciences.

Malignant melanoma (MM) is quite common in dogs and can affect several different anatomical sites, including the eyes, lips, skin, digit/footpad, and mucosae (*1, 2*). However, the most frequently affected location is the oral cavity, which constitutes 56% of all MM cases (*3*). Oral MM is locally aggressive, invading the bone in 57% of cases, and possesses high metastatic propensity, which ranges from 30% to 74% at the level of the regional lymph nodes, and from 14% to 92% to the lungs and other distant organs (*4-6*). Conventional therapies include surgical resection of the primary tumor and the addition of radiotherapy when local invasion is already present at the time of surgery, with correct surgical excision playing a fundamental role in disease outcome (*6, 7*). Prognosis is very poor in advanced-stage disease, with a 1-year

survival rate that does not exceed 30% even when surgery and/or radiotherapy are applied (*8, 9*). Chemotherapy is proposed as an adjuvant treatment, in these cases, in order to control the systemic dissemination of tumor cells, although it has been reported to be ineffective in increasing overall survival (*9*). This strong resistance to the standard therapies makes oral MM a disease that is still fatal, and for which innovative and long-lasting curative treatments are urgently required. The clinical benefits of the immunotherapeutic treatment of human melanoma patients and the similarities between canine and human melanoma both mean that advances in tumor immunology research in the veterinary field have been intensely sought after over recent decades (*8, 10, 11*). As a result, immunotherapy has also emerged as the fourth pillar in the anti-cancer arsenal, standing with surgery, radiotherapy, and chemotherapy, for veterinary oncology as well.

We have chosen to focus our attention on anti-cancer vaccination, of the current immunotherapeutic approaches available, because of its safety, specificity and long-lasting responses (*12, 13*). These features, and the consequent success of an anti-tumor vaccine, rely on the characteristics of the antigen that is targeted. A low level of expression in healthy tissues and a high level of expression in tumors, in addition to a “driving” role in the promotion of cancer development, render a molecule an “ideal” immunotherapeutic target (*14, 15*). In this context, chondroitin sulfate proteoglycan 4 (CSPG4) has arisen as one of the most appealing melanoma-associated antigens for DNA vaccination. It was initially discovered in a high percentage of human melanomas (*16*), and we later demonstrated its overexpression in over 60% of canine oral melanomas (*17*), as well as on derived cancer stem cells (CSC) (*2*). Whereas CSPG4 is not expressed in normal tissues, in tumor cells it performs a key oncogenic role, regulating several cancer-related processes, including proliferation, migration, invasion and drug-resistance (*15*). As for human melanoma patients, CSPG4 overexpression in canine melanoma is clinically relevant, as CSPG4-positive oral MM-affected dogs have worse prognosis than those whose tumors do not express the antigen (*2, 18*). Based on this evidence, CSPG4 targeting has the potential to become a triple-edged weapon in the fight against cancer; it can strike the primary lesion, its metastatic spread, and the CSC compartment, which is considered responsible for recurrences, metastasis, and treatment resistance. Therefore, we have decided to test the safety and clinical efficacy of anti-CSPG4 immunotherapy, by means of DNA vaccination, in combination with *in-vivo* electroporation (electrovaccination) in companion dogs affected by CSPG4-positive oral MM (*2, 6, 7, 18-20*).

However, it must be noted that CSPG4 is a non-mutated self-antigen, and as such, the development of an effective immune response is challenging. Immunization with DNA vaccines coding for xenogeneic antigens that share significant homology with the self-antigen have been shown to trigger better immune responses than self-homologous ones because they are able to circumvent self-immune tolerance. Since the amino-acid sequence of CSPG4 is highly evolutionarily conserved, with 88% similarity being displayed in the canine and human proteins, we used a plasmid coding for the human (Hu)-CSPG4 sequence as a vaccine, assuming that this approach would be effective in breaking host self-tolerance and consequently inducing an immune response that can cross-react against the canine antigen. Indeed, a humoral immune response against both the human and canine CSPG4 antigen was detected in Hu-CSPG4 electrovaccinated dogs (*2, 18, 19*). However, a low frequency of circulating canine CSPG4 reactive T cells was detected following anti-Hu-CSPG4 electrovaccination (*18*). This xenogeneic vaccination has resulted in a significant increase in the survival of vaccinated dogs compared to the control population, which was treated with surgery alone (*2, 18, 19*). Nevertheless, the clinical efficacy of the vaccine diminished when the primary tumor expressed low levels of the CSPG4 antigen, and dogs still died because of recurrences and metastasis. This could probably be linked to the presence of CSPG4-negative tumor clones within the lesion, but also to the low-affinity antibody production induced by xenogeneic vaccines (*20-22*). Improvements in this strategy are therefore paramount if more effective clinical achievements are to be reached and disease progression further impaired.

The use of hybrid DNA plasmids that are designed to code for chimeric molecules is an appealing approach thanks to the dual presence of a xenogeneic domain, which is instrumental in circumventing immune tolerance, and a homologous portion, which ensures the specificity of the immune response (*22*). We have therefore developed a second-generation anti-CSPG4 DNA vaccine, named HuDo-CSPG4 (MeraVax; WO2017115292A1), that codes for a chimeric human/dog protein and have tested its safety, immunogenicity and anti-tumor potential in the adjuvant setting in a prospective veterinary clinical trial that enrolled 80 client-owned dogs affected by CSPG4-positive, stage II-IV, oral MM after treatment with conventional therapies (i.e., surgery with or without radiotherapy). HuDo-CSPG4 electrovaccination was used in 52 out of 80 dogs and found to be well tolerated and immunogenic. The slight differences in amino acid sequence and the tertiary structure of the chimeric CSPG4 protein, which is encoded by the HuDo-CSPG4 plasmid, may result in the exposition of subdominant and/or new conformational epitopes, triggering an even more efficient humoral and cellular immune response than induced by the fully xenogeneic and homologous vaccines. Indeed, a higher affinity and more functional antibody response was elicited against the canine CSPG4 protein by the chimeric HuDo-CSPG4 than by the fully Hu-CSPG4 vaccine (*20*). These anti-CSPG4 antibodies are likely to eliminate CSPG4-positive cancer cells by multiple direct and indirect mechanisms. Importantly, the anti-CSPG4 immune response that was elicited appears to be clinically relevant in the immunized population, since a significant correlation was observed between the vaccine-induced antibody levels of the responder dogs and overall survival. Moreover, the detection of anti-CSPG4 IgA in the serum of a high percentage of vaccinated dogs suggests that mucosal immunity had been induced, and this may be partially protective against local recurrence. Interestingly, an increased percentage of B and $CD4^+$ T cells was observed, as was a decrease in myeloid-derived suppressor cells, in the peripheral blood of vaccinated dogs. It is also worth noting that an effective cellular response was elicited by HuDo-CSPG4 electrovaccination and that this was also correlated with better overall survival (*20*), whereas the neutrophil-to-lymphocyte ratio (NLR) and the lymphocyte-to-monocyte ratio (LMR) were neither prognostic nor predictive of response to vaccination (*23*).

Clinically, HuDo-CSPG4 electrovaccination is potentially beneficial in the treatment of CSPG4-positive oral MM, as it prolongs the survival of vaccinated dogs compared to controls that are treated with conventional therapies alone, regardless of the level of CSPG4 expression in the primary tumor (*20*). The results from this veterinary trial suggest that anti-CSPG4 therapy may represent a new therapeutic possibility for the treatment of CSPG4-positive oral MM, which behaves more aggressively, has less favorable prognoses (*2, 18*), and is more frequent (*17*) than its CSPG4-negative counterpart.

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Clinical challenges and controversies in the management of soft tissue sarcomas

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Soft tissue sarcoma is a term used to describe a number of different types of tumors of mesenchymal origin with similar histological features and biological behavior.(Ehrhart 2005; Dennis et al. 2011; Dernell et al. 1998) They are classically described as pseudoencapsulated masses with poorly defined margins. Because malignant tumor cells can penetrate this pseudocapsule, a surgical resection that passes through this plane can leave microscopic disease in situ, resulting in tumor recurrence.(Dernell et al. 1998) The extent of this recurrence-prone margin is poorly defined.

The prognosis for the majority of dogs with STS is generally good if a complete resection can be achieved.(Kuntz et al. 1997; Dennis et al. 2011; McSporran 2009) However, local recurrence can develop in up to 75% of dogs.(Baker-Gabb, Hunt, and France 2003; Banks et al. 2004; Bostock and Dye 1980; Cavanaugh et al. 2007; Chase et al. 2009; Heller et al. 2005; Kuntz et al. 1997; Selting et al. 2005; Stefanello et al. 2008) Rates of metastasis are less well-defined but may develop in between 1.7% and 41% of cases.(Banks et al. 2004; Dennis et al. 2011; Kuntz et al. 1997) Overall, about 20 to 30% of dogs will ultimately die of their disease.(Bacon et al. 2007; Angelov et al. 1999; Cavanaugh et al. 2007; Chase et al. 2009; Heller et al. 2005; Kuntz et al. 1997; McSporran 2009; Selting et al. 2005) Continued efforts to improve management options and to recognise those dogs at risk of recurrence and death remains important.

In reality, the owner of a dog with a STS doesn’t want to hear that the prognosis for their dog is “generally good”, or that there is a 20-30% chance that their dog will die from the tumour despite treatment. They want to understand what the prognosis is for their own individual dog, and whether treatment will be successful in ensuring the tumour does not come back. The answers to these more specific questions are harder for a clinician to answer. Cancer is a heterogenous disease, and one individual tumour will not present with the same characteristics as another. Variations in tumour size, location and other patient factors will impact on the ability of a surgeon to remove the mass with an appropriate cushion of healthy tissue. The generalised prognostic figures quoted above are also derived from retrospective analyses of clinical cases performed at different institutions, with different surgeons working under different conditions in different geographic locations around the world. These differences introduce bias and limitations into the case selection and application of surgical strategies that may impact on the characteristics of the tumour population being operated on, and the consistency of clinical management between different studies. Being retrospective in nature, determination of patient outcomes and the rates of local recurrence, metastasis and survival have the potential to be imprecise as relevant data may not have been collected in the first instance, or is reliant on recall or secondary knowledge. Finally, almost all of the studies have involved sample sizes of less than 100 animals, which restricts the statistical power necessary to correctly identify clinical features that may be influential in outcome.

In light of these limitations in the existing literature, it is understandable that controversy exists into whether certain characteristics of a STS may influence the outcome of patients more than others, or whether particular treatment strategies are more effective than others. In the next section, the evidence for some of the prognostic factors that may influence the outcome of STS in the dog will be examined. Comparative evidence from human STS will also be explored: this is because a similar debate on the factors that influence the management of STS has occurred in the human literature,(King, Hackbarth, and Kirkpatrick 2012; Kawaguchi et al. 2004; Rydholm and Rooser 1987; Enneking, Spanier, and Malawer 1981) and many of the treatment challenges posed by this tumour in humans are comparable to those that confront the veterinary surgeon. The individual prognostic factors that will be examined in the following section include: the histological type; histological grade and other known markers of proliferation; tumour size, location and palpable characteristics; the presence of metastasis; and finally the importance of surgical margins, including how resection margins are evaluated and the evidence to support what an appropriate width of resection margin is required. To conclude, the importance of STS structure and how the tumour microenvironment may influence whether an individual STS may recur after surgery will be reviewed.

Histologic type

As discussed by Avallone elsewhere in these notes, all subtypes of STS have traditionally been considered as a single group for prognostic purposes largely because differentiating individual tumour types by light microscopy can be unreliable.(Dennis et al. 2011) However, in human STS, there is increasing evidence that individual subtypes may exhibit differences in local invasiveness, metastatic potential and recurrence.(Tseng et al. 2012; Canter et al. 2010) In current studies on canine STS, any evidence for differences in outcome between various histologic subtypes is limited by small population sizes or a lack of rigour in histological diagnosis.(Dennis et al. 2011) There is a need to develop better tests that allow individual subtypes with variances in clinical behaviour to be identified with more confidence. This may require the increased use of immunohistochemical markers, or even molecular profiling.(Dennis et al. 2011)

Histologic grade

Histological grade is considered the most important prognostic factor in human STS,(Stojadinovic, Leung, Allen, et al. 2002; Mandard et al. 1989; Costa et al. 1984) and is also one of the most validated criteria to predict outcome following surgery in canine patients.(McSporran 2009; Kuntz et al. 1997) In one study, the histologic grade of a STS was found to be a strong predictor of local recurrence after surgery with recurrence rates for low, intermediate and high-grade tumours varying from 7, 34 and 75 percent respectively.(McSporran 2009) The findings of this paper are important, as it demonstrates a correlation between tumour grade and different rates of local recurrence for a cohort of STS that had all been resected with narrow margins. Current surgical advice for STS has been derived from evidence generated from cases treated in referral or academic practice.(Kuntz et al. 1997) However, cases managed in referral practice are a selected population; they have been referred for treatment at a specialist centre either because their STS was showing a more aggressive clinical appearance (e.g. large size, recent rapid growth, or a fixed and immobile characteristic) or were located in locations that made surgery more challenging. Because of this selection bias, interpreting the prognosis for patients in response to certain treatments needs to take into the account the population pool from which the treatment cohort was derived. Outcomes are likely to be better in those studies with a higher proportion of low-grade tumours,(Stefanello et al. 2008; Chase et al. 2009; McSporran 2009) compared to studies with more high-grade tumours.(Kuntz et al. 1997; Bostock and Dye 1980; Heller et

al. 2005; Banks et al. 2004; Baker-Gabb, Hunt, and France 2003) It follows that treatment advice may need to be stratified according to the grade of the tumour.

It is also well-recognised that the grading of tumours is subjective and variation in interpretation between different pathologists has been reported for STS and other tumour types.(Northrup et al. 2005; Regan et al. 2015) In one study on canine STS, the assigned grade or diagnosis of a mesenchymal tumour was modified in 5 out of 15 cases (33%) following review of the slides by a second pathologist.(Regan et al. 2015) In two of these cases, this revision led to an increase in grade (from grade 2 to grade 3), while in another two cases, the interpretation changed from a malignant mesenchymal tumour to a benign disease. In the final case, the diagnosis was modified from an oral sarcoma to a melanoma. These changes have the potential to alter the potential prognosis for these patients. When the original histologic assessment under-estimated the aggression or metastatic potential of the tumour, these dogs may have been denied consideration for adjuvant therapy that could have prevented or slowed tumour recurrence. For the dogs diagnosed with a malignant neoplasm when their tumour was actually benign, their outcome would obviously be better than expected. However, these dogs may have been subjected to treatments in excess of that needed for their underlying disease. The impact of this high error rate for an important prognostic indicator like tumour grade has implications not only on the management for an individual dog, but also on the ability to interpret the treatment recommendations from existing literature. Development of more objective predictive markers that correlate reliably with tumour behaviour would be important to help support clinical decision making.

Mitotic index and other proliferative markers

As a measure of proliferative activity within the tumour, the MI can provide additional prognostic information about an individual tumour.(Dennis et al. 2011) An MI of more than 9 mitotic figures per 10 high power fields (hpf) has been associated with increased (and earlier) rates of tumour recurrence, higher rates of metastasis and reduced overall survival in several studies.(Dennis et al. 2011; Bostock and Dye 1980; McSporran 2009; Kuntz et al. 1997; Ettinger et al. 2006) With an MI ≥9, MST range from 150 – 343 days, compared to 826 – 1138 days with an MI <9.(Bostock and Dye 1980; Simon et al. 2007)

The histologic determination of MI is actually a single ‘snap-shot’ of the proliferative activity of cells frozen in time at the time of tumour fixation. The use of various proliferative markers, such as Argyrophilic Nucleolar Organizer Region (AgNOR) and the Ki-67 protein, can provide additional information about the mitotic activity of a tumour as they detect chemical signals that may persist within the cell across the whole mitotic cycle.(Ettinger et al. 2006) In canine STS, increased AgNOR and Ki-67 scores have both been associated with reduced survival time and correlated with tumour grade and MI.(Ettinger et al. 2006) However, the use of these markers has not been routinely adopted in the evaluation of canine STS.

Tumour size and growth rate

Several canine and human studies have suggested that tumours larger than 5cm (golf-ball sized) have shorter disease-free intervals or survival times.(Kuntz et al. 1997; Monteiro, Boston, and Monteith 2011; Gustafson et al. 2003; Sampo et al. 2012; Guillou et al. 1997; Mandard et al. 1989; Stojadinovic, Leung, Allen, et al. 2002) However, other authors have not found any association between tumour size and outcome.(Bacon et al. 2007; Bostock and Dye 1980; Chase et al. 2009) A STS with a history of sudden or rapid growth, or the presence of tumour necrosis and ulceration, has also been suggested to imply a more aggressive growth characteristic,(Liptak and Forrest 2013) but this has not been validated in clinical trials. There may be confounders between tumour size and other prognostic factors that may influence outcome. Larger tumours may be more difficult to remove and may be more likely to impinge upon vital anatomical structures, which thus limits the ability to

maintain an appropriate resection margin about the entire tumour. Soft tissue sarcoma resected in first opinion practice also tend to be smaller than those managed in referral practice,(Chase et al. 2009; Kuntz et al. 1997) so the source of the tumour population also needs to be considered.

Palpable characteristics of the tumour

Most STS are readily palpable and may appear to be quite discrete and encapsulated. Other tumours may be multi-lobulated, soft and have very indistinct borders. Although the superficial aspects of the mass may appear quite mobile, the base can be indistinct and potentially attached to underlying bone or fascia.(Liptak and Forrest 2013) This difference in mobility between different tumours may be significant in terms of prognosis; tumours that feel more 'fixed' to underlying tissues have significantly decreased disease free intervals ($P<0.0001$) and survival times ($P=0.007$).(Chase et al. 2009) It is hypothesised that more adherent tumours may have a different tumour microenvironment that causes them to be more infiltrative or enables greater migration of tumour cells into the periphery.(Barker, Cox, and Erler 2012) However, interpretation of tumour mobility is a highly subjective feature and the prognostic significance of this finding has been inconsistently reported by other authors.(Dennis et al. 2011) This clinical finding needs to be validated in a prospective setting to see if it can help consistently predict prognosis.

Presence of metastases

The metastatic rate for dogs with soft tissue sarcoma has been reported to be between 1.7 and 41%.(Banks et al. 2004; Dennis et al. 2011; Kuntz et al. 1997) The published metastatic rate for grade 1 and 2 tumours is usually low, with most studies reporting incidences of less than 15%.(Kuntz et al. 1997; McKnight et al. 2000; Baker-Gabb, Hunt, and France 2003; Milovancev et al. 2020) For high grade tumours, the quoted figure is consistently higher and may be as much as 44%.(Ettinger et al. 2006; Kuntz et al. 1997; Selting et al. 2005) Other authors have reported intermediate levels of metastasis for grade 2 tumours, with rates between 27% and 33%.(Simon et al. 2007; Ettinger et al. 2006) Metastasis is five times more likely when tumours have a mitotic rate of 20 or more.(Kuntz et al. 1997) Other factors that have been associated with an increased risk of metastatic disease include the percentage of tumour necrosis and local tumour recurrence,(Liptak and Forrest 2013) although this latter characteristic is inconsistently reported. The accuracy of all of the data relating to STS metastasis is uncertain. Determination of metastasis is largely reported from retrospective studies, so there will be considerable bias and variation in the intensity of investigation for the presence of metastatic disease. Metastasis may not develop until many weeks or months after surgery so the period of follow-up of patients since surgery will affect the reported incidence; in one study, the median interval from surgery to detection of a metastatic lesion was 365 days.(Stefanello et al. 2011) In many studies, no histological confirmation of metastatic disease was performed, and a diagnosis of metastasis was reliant on imaging findings only.(Dennis et al. 2011) This raises the possibility that a newly discovered metastatic lesion may not necessarily be due to the previously resected STS; the majority of dogs with STS are elderly, so it is possible that some of these dogs could develop a new primary malignancy that may be occult to examination.

Resection margins

Wide resection of STS has long been considered an important requirement if adequate local control is to be achieved. In the first veterinary paper to describe outcomes for dogs STS, local recurrence developed in 25 of 103 (34%) of patients with MSTs of less than 2 years.(Bostock and Dye 1980) This paper does not specifically state what resection margins were used about the tumour, other than stating the "the mass was resected with as much surrounding normal tissue as permitted by the site". This paper was published at a time when

veterinary oncologic surgery in the UK was in its infancy, so it would seem unlikely that extensive resection margins of more than 1cm will have been attempted at that time. The next paper on STS was not published until 17 years later and came from a respected oncologic centre in the USA. By that time, the importance of oncologic principles were becoming realised, using comparative evidence from human oncology.(Withrow 2001) In this paper, the STS were managed with wide resection margins that included 3cm lateral to the tumour and a deep fascial plane; these margins were based on the resection margins being described for human musculoskeletal tumours.(Enneking, Spanier, and Goodman 1980; Enneking, Spanier, and Malawer 1981) Local recurrence was observed in 11 of 75 (15%) dogs,(Kuntz et al. 1997) with a median survival was almost 4 years. Subsequent studies where wide resection margins were used appeared to validate this finding, with local recurrence rates of 0 of 19 (0%),(Milovancev et al. 2020) 4 of 54 (7.5%),(Baker-Gabb, Hunt, and France 2003) and 10 of 50 (20%)(Avallone et al. 2014) dogs. Some studies showed that wide surgical margins were more likely to achieve complete tumour removal than marginal or narrow resection,(Baker-Gabb, Hunt, and France 2003; Banks et al. 2004) or that recurrence was more common with a narrow or marginal excision.(Chase et al. 2009) However, statistically significant correlations between resection margins and local recurrence have not been determined.(Chase et al. 2009; Baker-Gabb, Hunt, and France 2003) Radical excision has not been shown to improve survival times when compared to patients with other resection margins.(Kuntz et al. 1997)

More recently, there have been several studies that have challenged the necessity of wide resection margins to minimise the chances of STS recurrence. Local recurrence rates of just 10.8% (follow-up 210-2202 days) were reported in 35 dogs with low-grade spindle cell tumours of the extremities treated by marginal excision only.(Stefanello et al. 2008) In another prospective clinical study, 100% local disease control and 93% one-year disease free interval was achieved in 14 dogs with 1cm lateral resection margins and a single fascial plane beneath the tumour.(Banks et al. 2004) However, in that paper, patient follow-up times were only 12 months, which is inadequate for soft tissue sarcoma. In another paper examining outcomes for dogs with STS treated exclusively in first opinion practice, local recurrence rates of 20.8% were reported, despite marginal or narrow resections being performed in 77% of cases.(Chase et al. 2009) The median follow-up in that paper was 785 days.

Overall, it must be concluded that a relationship between resection margins and overall survival or local tumour recurrence has not been demonstrated in the existing literature. Moreover, the quality of any such evidence, even when it is present, must be considered poor due to the effects of numerous confounders, including tumour size, location and grade. Although the size of the resection margin is a metric that may be of greatest immediate relevance to the surgeon, it is probable there are too many variables that influence the relevance of such a macroscopic measurement, and other prognostic markers may be of more relevance.

Margin evaluation

Irrespective of the actual width of resection margin performed, demonstration of a histological margin that is clear of tumour cells – described as a “histological tumour free margin (HTFM)” is considered the best predictor for improved local tumour control of a STS.(Dennis et al. 2011; Milovancev et al. 2019) When neoplastic cells are seen immediately adjacent to the resection margins when examined using histology, tumour recurrence is more than ten times (95% CI 1.33-82.42) more likely to occur.(Kuntz et al. 1997)

Because of the association with increased local recurrence, obtaining a resection margin that is free of tumour cells on histological assessment is considered by many surgeons to be the

ultimate goal of oncologic surgery.(Eward et al. 2013). However, the histological assessment of surgical margins as an indicator of complete tumour removal in all planes can be highly flawed, either as a consequence of processing methodology or the practical realities of a commercial laboratory service.(Kamstock et al. 2011; Dennis et al. 2011) An important limitation of margin evaluation is that only a small fraction of the overall tumour circumference can be examined microscopically; most commercial veterinary laboratories evaluate neoplasm specimens using between three and six tissue sections. Pathologists therefore need to be strategic in assessing which sections of a large tumour bulk to evaluate.(Kamstock et al. 2011) Recommendations have been published to improve consistency in histologic processing and reporting.(Kamstock et al. 2011)

Aside from the practical limitations that prevent evaluation of the entire tumour circumference, there are other technical factors that can influence the accuracy of margin evaluation. Due to the effects of tissue elasticity and the deformation that occurs from the effects of fixation in formaldehyde and subsequent processing steps required to get a section of the tumour onto a microscope slides,(Dennis et al. 2011; Kamstock et al. 2011) the final measured histologic margin of tissue surrounding the visible tumour boundary can be 35% to 42% smaller than the original measured surgical margin.(Risselada, Mathews, and Griffith 2015) The extent of distortion has been found to differ for different tissues (e.g. skin, muscle, fat), based on their lipid content.(Upchurch et al. 2014) Contraction of tissues will also differ between tumours, probably due to variances in stromal characteristics and microscopic infiltration about the tumour boundary.(Milovancev et al. 2018; Risselada, Mathews, and Griffith 2015) There is a compounding effect on specimens composed of a tumour and multiple tissue types (e.g. skin, subcutaneous, or musculoskeletal tumours) that causes different tissue layers to distort and twist.(Kamstock et al. 2011) Further distortion of the tissue will occur during histologic processing; this is due to the effects of alcohol and xylene washing that prepares the tissue to be infiltrated and embedded in paraffin wax, and the fragmentation that can occur during microtomy and mounting on a slide.(Milovancev and Russell 2017) Due to the combined effects of these influences on tissue dimensions from excision to final interpretation on a microscope slide, the final measured histologic margin of tissue surrounding the visible tumour boundary was found to vary between 43% and 176% of the original measured surgical margin.(Upchurch et al. 2014; Risselada, Mathews, and Griffith 2015) This work suggests that the measured HTFM may actually bear little relevance to the actual surgical margin obtained; the histologic measurement of a tumour margin can under- or overestimate the actual extent of the tissue barrier that was maintained about the tumour during excision. Due to this variability in tissue shrinkage and deformation between patients, tissue and tumour types, extrapolating an optimal surgical resection margin from a desired HTFM will not be possible.

Another limitation to the accuracy of margin assessment is the ability of the histologist to reliably identify fascial tissues as a distinctive structure. As discussed previously, a defined fascial boundary is widely acknowledged as a crucial aspect of the deep resection margin.(Dernell et al. 1998; Kuntz et al. 1997) While a fascial layer may appear distinct to the surgeon, the same structure is often difficult to identify microscopically. From an oncologic perspective, if the pathologist cannot confidently recognise the fascial tissue that the surgeon utilised as a distinctive barrier during resection, the histological appearance of the deep margin may be interpreted as just a few cell layers of tissue, which raises concerns for an incomplete resection. Ultimately, the surgeon needs to interpret the histological findings in conjunction with the knowledge of their surgical plan. The surgeon knows best whether a thick or thin fascial barrier was utilised at any part of the dissection, which sections of the tissue appeared concerning at the time of surgery, or where margins were compromised due to proximity with vital anatomical structures. Coloured inks can be used to paint lateral and

deep margins of the excised tissue.(Kamstock et al. 2011) Inking can help overcome the difficulties in margin evaluation that occurs when different tissue layers become distorted during fixing, as the pathologist is able to observe the inked margin on the microscopic specimen. If tumour cells are seen to abut the section of tissue inked by the surgeon, there can be more confidence that this resection margin may be incomplete.(Kamstock et al. 2011)

The precise width of HTFM necessary to completely eliminate recurrence has not been investigated in the dog. Different studies use different criterion to define a HTFM width that equates to either a “complete margin” or “incomplete margin”, or often fail to describe one at all. When it is described, the widths of normal appearing tissue about the pseudocapsule may range from 1mm to 10mm.(Selting et al. 2005; McSporran 2009; Stefanello et al. 2008; Banks et al. 2004; Milovancev et al. 2020) These inconsistencies in the definition of what extent of HTFM is required to ensure complete excision of the STS makes it challenging to compare the outcomes from different clinical studies based on different sizes of gross resection margin.(Dennis et al. 2011) In one study, no recurrences were observed when a HTFM of >3mm was found between the tumour and surgical margins on histological review,(Stefanello D Fau - Morello et al.) but this paper was limited to dogs with low grade spindle cell tumours only. Another study showed no local recurrences in 30 dogs with a HTFM of ≥1mm;(McSporran 2009) while no influence of tumour grade was detected, this study was performed on cases submitted from first opinion practice so high-grade tumours were uncommon, representing only 7% of the study population. Only one paper has so far demonstrated a statistically significant correlation between HTFM (>2mm, in this case), local recurrence ($p<0.001$) and disease free interval ($p = 0.004$);(Scarpa et al. 2012) this was a study of 20 dogs with STS with 30% grade 1 and 70% grade 2 and 3 tumours. In all other studies where a HTFM was reported, interpretation of significance was affected by either inadequate case numbers,(Banks et al. 2004) inadequate follow-up times,(Milovancev et al. 2020; Kuntz et al. 1997) or the inclusion of dogs undergoing re-excision of a recurrent STS or surgical scar.(Bacon et al. 2007; Heller et al. 2005) In two studies, no correlation between HTFM and local recurrence was evident.(Baker-Gabb, Hunt, and France 2003; McSporran 2009) Once again, the current literature provides inadequate or insufficient evidence with which to draw definitive conclusions about what extent of HTFM is required to prevent local recurrence.

There is also confounding evidence that suggests tumour recurrence is not inevitable even when tumour cells are visible at the resection margins on histology. In studies where data on recurrence for canine STS with a positive HTFM has been reported, recurrence rates have ranged from as low as 17% and up to 100%;(Banks et al. 2004; Stefanello et al. 2008; Avallone et al. 2014; Bacon et al. 2007; McSporran 2009; Milovancev et al. 2020; Scarpa et al. 2012) across all studies, the mean rate of recurrence for an incompletely resected STS was 33% (38 of 114). Data from these same studies reveals that STS can also recur even when the histological margins indicate complete resection has been achieved. Rates of recurrence in this instance can range from 0% up to 50%; across all studies, the mean rate of recurrence for a STS that was considered to have been completely resected on histologic examination was 10% (16 of 164).(Banks et al. 2004; Stefanello et al. 2008; Avallone et al. 2014; Bacon et al. 2007; McSporran 2009; Milovancev et al. 2020; Scarpa et al. 2012; Prpich et al. 2013) A recent meta-analysis determined that having a HTFM of >0mm reduced the risk of recurrence by approximately 60%.(Milovancev et al. 2019)

The inconsistencies between margin analysis and recurrence risk are not limited to canine STS; they have also been reported in human STS as well as many other neoplastic conditions.(Beirne and Beirne 1959; Emmadi and Wiley 2012; Hayashi et al. 2014; Nurkin and Kane Iii 2012; Pilewskie and Morrow 2018; Wolf 2012; Wood 2013; Stojadinovic, Leung,

Hoos, et al. 2002) Reasons for this inconsistency could be due to the inherent limitations of histological analysis that were described above. However, there are several tumour-related reasons that could explain why established histologic methods are unable to distinguish the STS that may have a higher risk of recurrence, irrespective of margin status. These reasons include the profile of the pseudocapsule, the presence of satellite nodules and the influence of the tumour microenvironment.

Effect of the pseudocaspule and microscopic invasion

One of the defining features of a STS is the pseudocapsule that surrounds the gross boundary of the tumour and creates a discernible circumscription to the tumour.(Enneking, Spanier, and Malawer 1981) The pseudocapsule is formed initially by the compression and atrophy of the surrounding tissue as the tumour expands centrifugally. With continued expansion of the tumour, a reaction can develop between the capsule and normal tissue, which includes mesenchymal cell proliferation, an influx of inflammatory cells, haemorrhage, tissue oedema, and angiogenesis.(Liu et al. 2008; Enneking, Spanier, and Malawer 1981) This area is termed the reactive zone and may sometimes be visible grossly as a discoloured area that surrounds the tumour.

Historically, the pseudocapsule has not been considered to be a barrier to tumour invasion and dissection through this cleavage plane – equivalent to a marginal resection – would lead to high rates of local recurrence.(Enneking, Spanier, and Malawer 1981; Liptak and Forrest 2013) However, as discussed above, it is now recognised that some STS can be successfully managed with marginal resection margins.(McSporran 2009) Other STS – particularly those of higher grade – may require a wider HTFM. The extent of HTFM required to achieve adequate local control is therefore not binary and may vary according to individual characteristics of the tumour contour, and the microscopic invasion of cells beyond the gross boundary of the STS.

The peripheral contour of human STS has been described as either “pushing” (if no evidence of infiltrative growth was seen) or “infiltrative” (if the tumour contour was poorly defined, or satellite nodules were present).(Engellau et al. 2007) A pushing growth pattern was more commonly observed with low-grade tumours, but a proportion (18%) of high-grade tumours can also display this characteristic. None of the tumours with a pushing growth pattern recurred after resection regardless of histologic margin, whereas local recurrence developed in 6 of 26 (23%) people after marginal excision and 13 of 56 (23%) people after wide excision in STS with an infiltrative growth pattern. In a similar study, an almost 7-fold increase (HR = 6.7, p=0.005; 95% CI 1.82-26.13) in local recurrence was seen in STS that had an infiltrative contour.(Lintz et al. 2012)

There are four retrospective studies of canine STS that make an attempt to describe the contour of the STS.(Scarpa et al. 2012; Baker-Gabb, Hunt, and France 2003; Milovancev et al. 2020; Avallone et al. 2014) Each paper used a different criteria to describe whether the STS had a contour that was considered more or less invasive or infiltrative, so they are not directly comparable. One study showed that tumours with an infiltrative pattern were almost three times more likely to relapse (HR 2.45, 95% CI 0.61-9.89), but this finding was not significant. However, a significant relationship between a shorter recurrence free interval and dogs with an infiltrative tumour contour was demonstrated in another study.(Scarpa et al. 2012) In a further study, no recurrence was seen in 19 of 19 dogs with STS that were considered to have a less invasive/pushing growth pattern.(Milovancev et al. 2020)

The histological descriptions of human STS have also revealed discrete microscopic clusters of neoplastic cells – satellites or skip metastases - that extend some distance from the pseudocapsular boundary. These microscopic clusters, or even individual cells, are

separated from the pseudocapsule by microscopically normal tissue. In one study of human STS, microscopic tumour nodules have been identified between 1cm and 4cm from the main mass in 30% of cases.(White et al. 2005) Satellite nodules are more commonly observed with high grade than low grade lesions. When low grade STS do develop satellite nodules, they tend to be clustered close to the periphery of the pseudocapsule.(Azzarelli 1993; Enneking, Spanier, and Malawer 1981) The microscopic diffusion of tumour cells about the circumference for both mast cell tumour and STS has been described in the dog in only two studies.(Russell et al. 2017; Avallone et al. 2014) In one study, satellite lesions were described if there was at least 1mm of microscopically non-neoplastic tissue interposed between the satellite lesion and the neoplastic cells that remained in contact with the main tumour bulk. In this study of 19 STS, satellite lesions were observed in 6% of tumours with a mean distance of 3.8mm (range 2.9 – 17mm).(Russell et al. 2017) Almost 70% of the tumours in this study were low grade, and no high grade tumours were included. This may explain the relatively low incidence and distribution of the satellite lesions in this study, compared to what has been described in human STS.(White et al. 2005) In another study, satellite lesions were reported in 11 of 56 STS; these dogs had more than a 3.5 increased risk of relapse (HR 3.68 95% CI 0.81 – 16.69) when compared to 28 of 56 STS with an expansile profile, but this difference was not significant (p=0.09).(Avallone et al. 2014) This study did not correlate the tumour profile with the grade of STS.

Taken overall, this evidence suggests the pseudocapsule of the STS is actually a more complex and nebulous structure than originally presumed and likely plays an important role in influencing recurrence of a tumour after surgery. In some tumours, the fibrous pseudocapsule may actually provide an effective barrier against tumour growth and infiltration but this probably holds true for a proportion of (mostly) low-grade lesions only.(Enneking, Spanier, and Malawer 1981; Liu et al. 2008; Takahashi, Sato, and Miura 1993) In those instances, successful local control could indeed be achieved with excision of the mass including a narrow rim of normal tissue, as has been suggested by some authors.(Stefanello et al. 2008; Chase et al. 2009) However, in other tumours, the reactive zone that surrounds the pseudocapsule is an area of nascent and evolving neoplastic activity, with isolated clusters of neoplastic cells and a permissive stromal microenvironment that supports cancer initiation, neovascularization and tumour migration. In these cases, there is a higher likelihood for tumour recurrence if the plane of surgical excision passes through this area.(Kind, Stock, and Coindre 2009; White et al. 2005) The description of isolated tumour nodules located several centimeters from the tumour pseudocapsule may also provide an explanation for why local recurrence could still occur following complete removal of the gross tumour;(Voros et al. 1998; White et al. 2005; Enneking, Spanier, and Malawer 1981) there are likely complex mechanisms at play in the tumour microenvironment that impact on which tumours recur, and those that do not.(Beacham and Cukierman 2005; Bolouri 2015; Lu, Weaver, and Werb 2012)

The influence of these differing tumour contours and extent of microscopic invasion of tumour cells into the surrounding tissues will likely have a profound, but as yet unmeasured impact on tumour recurrence. Because the presence of these characteristics cannot be reliably predicted for each individual tumour, there is an argument that wide surgical margins should be the appropriate strategy, as this would ensure that if satellite nodules are diffusely present around a particular STS they will be contained within the resected block of tissue.(Bostock and Dye 1980; Kuntz et al. 1997; Ehrhart 2005; Liptak and Forrest 2013) However, if it was that simple, existing data should demonstrate improved outcomes with increasing resection margins; as outlined above, the current literature does not support this correlation. This may suggest there are more complex elements involved. In human STS, the issue of ‘how much to resect’ has been circumvented by the routine inclusion of radiotherapy

(adjuvant or neoadjuvant) into almost all treatment strategies.(Rosenberg et al. 1982; Karakousis et al. 1986; Pisters et al. 1996; Zagars, Ballo, Pisters, Pollock, Patel, and Benjamin 2003) Radiotherapy in combination with surgery has allowed shrinkage of the resection margins without compromise for patient outcome.(Rosenberg et al. 1982; Miller, Xu-Welliver, and Haglund 2015) In the canine patient, routine inclusion of adjuvant RT is unlikely to become the standard of care for the treatment of STS, due to the costs and logistical challenges of delivering this treatment. Therefore, efforts to develop novel prognostic markers or adjuvant treatments that are targeted to STS behaviour would assist efforts to manage this tumour.

Conclusion

STS is a complex disease and many uncertainties surround the biology of the tumour and the best options for clinical management. Historically, the tendency has been to recommend wide excision margins in all patients, but this is not fully supported by recent evidence. Nevertheless, it is accepted that inappropriately conservative treatment will affect the outcome for a patient with more aggressive disease.

The “biologic aggressiveness of a soft tissue sarcoma” was recognised in 1981 as the key factor in human STS to guide the selection of an appropriate surgical margin required to achieve local control.(Enneking, Spanier, and Malawer 1981) Despite this awareness, the veterinary profession continues to struggle with the management of canine STS almost half a century later. Because there are no diagnostic tests that can reliably predict the amount of surgical margin required for a particular tumour, there is a mismatch between treatment and disease: some dogs are overtreated for their disease, resulting in large wound reconstructions or amputation when smaller surgical margins would have been effective. Other dogs are undertreated and suffer tumour recurrence and premature death due to inadequate initial treatment. Current evidence suggests it is not the extent of resection that influences successful patient outcome, but the biological behaviour of the tumour.(Weitz, Antonescu, and Brennan 2003) However, considerable deficiencies exist in the literature to help reliably determine the prognosis for an individual patient. This highlights the need for more reliable and objective prognostic markers to be developed in canine and human STS.(Kilvaer et al. 2010; Dennis et al. 2011; Engellau et al. 2005; Gustafson et al. 2003; Kondo et al. 2013; Matsumine et al. 2007; Pisters et al. 1996; Sampo et al. 2012; Yudoh et al. 2001; Zagars, Ballo, Pisters, Pollock, Patel, Benjamin, et al. 2003) If prognostic markers for tumours with either favourable or aggressive behaviour could be identified and predicted with more confidence, more appropriate and targeted treatment could be provided.

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A full reference is available on request

Pathologist contribution to understanding soft tissue sarcomas' behavior

Giancarlo Avallone, DVM, PhD, Dipl ECVP. DIMEVET-University of Bologna

Soft tissue sarcoma (STS) is a group of tumors of mesenchymal origin encompassing different tumor types.13 STS have been studied more extensively in the dog, and this lecture will focus on this species. The main contribution of the pathologist is to provide a diagnosis and assessing histological features which are known to be associated with prognosis, the most important of which are the status of surgical margins and histological grade.

The term "sarcoma" should be limited to neoplasms with a malignant behavior, and the term "soft tissue" refers to the extraskeletal mesenchymal/connective tissues of the dermis, subcutis and fascia, striated and smooth muscle, vessels, serosal and synovial linings, and nerve sheaths.13 Thus, sarcomas arising in viscera and parenchymal organs should not be included in the group of STS.13

The veterinary literature unfortunately does not apply rigorously these definitions, and many studies include in the STS group different tumor types and frequently benign soft tissue tumors, such as benign nerve sheath tumors.2,6,10,15 Because of these inconsistencies, studies published in the literature are often difficult to compares, and for these reasons, a standardized classification should be applied.

The status of surgical margins is one of the most important prognostic criteria, being associated with local recurrence.1,8,10,14 This parameter is different than the surgical dose (aggressiveness of the surgery), and it is represented by the distance of neoplastic cells from the surgical margins assessed at the microscope by the pathologist. It can be reported as a quantitative parameter (in mm), or as a qualitative parameter. Debate exists among pathologist on how to report qualitatively the status of margins: a two-tier system (clean vs infiltrated margins), as used in human medicine, has been suggested, and the use of the intermediated category of "close margins" has been discouraged.3,5,7,9 In consideration of the type of sampling performed in veterinary medicine, which cannot be as extensive as in humans, an intermediate category seems to have some advantages, nevertheless it should be standardized before its use in routine diagnostic settings.

The second most important prognostic parameter for canine STS is the histologic grade. Tumor grading is defined as a method to quantify the putative clinical aggressiveness of a neoplasm based on specific histological features.12 And the purpose of a grading is to subdivide neoplasms of a given histologic type into categories that provide additional prognostic information for the clinician/oncologist. It should not be confused with the "biological behavior" of a tumor and represents a morphological category. The grading system used in veterinary medicine is the same used in human medicine and developed in the '80 by the Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) also known as the "French" system, which take into account the differentiation, the total amount of necrosis in the tumor, and the mitotic count.4 The assessment of these parameter, and especially of the differentiation, can be subjective, and consequently there can be discrepancies in the grade provided by different pathologist. In veterinary medicine one study reported that 1 pathologist out of 3 provide a different grade in 50% of the cases, and in human medicine a maximum of 60-70% of agreement between pathologist is reported.16 Furthermore, the assessment of the grade in pre-surgical biopsies is reported to provide a grade different that on excised tumor in up to 40% of cases, most of the differences being represented by underestimation of the grade. This occurs because the presurgical biopsies may not be representative of the whole sarcoma in respect of the parameters used to calculate the grade.11

In the dog the histological grade has been associated with the overall survival and, when the status of histological margins is not clean, with the local recurrence. It is important to point out the grade is not a predictor of local recurrence per se, but only when margins are not clean. In cases with clean margins, STS do not recur in most of the cases regardless of the grade.1,8,10

The grading system does not apply to some entities: histiocytic sarcoma, because it is not a STS, but it is a leukocytic tumor; hemangiosarcoma of soft tissues and malignant nerve sheath tumors of brachial plexus, which are by definition a STS, but have never been included in grading studies and thus no information are available; and fibrosarcoma of the oral cavity, because up to 50% of low grade fibrosarcomas are the so called high-low fibrosarcoma (sarcomas with histologically bland appearance but aggressive behavior).12 Information on the validity of the histological grade in visceral sarcoma are preliminary and needs further validation.

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The proteomic landscape of soft tissue sarcomas

Paul Huang

Soft tissue sarcomas are a group of rare tumours of mesenchymal original. Efforts such as The Cancer Genome Atlas Consortium have systematically characterised the genomic and transcriptomic features of a subset of soft tissue sarcomas. While these large-scale efforts have undoubtedly improved our understanding of the biology of these tumours including the identification of new diagnostic tools and drug targets, very few of these findings have been translated for use in the clinical management of patients with sarcoma. In contrast to genomic and transcriptomic studies, few proteomic analyses of sarcomas have been undertaken, in part due to the technical challenges associated with the profiling of formalin fixed paraffin embedded (FFPE) tissue. Proteins form the functional biological units in the cell and are therefore responsible for the vast majority of pathophysiological processes in tumours. Furthermore, most targeted drugs that are approved or in clinical development target proteins and not DNA or RNA. Hence the ability to comprehensively map proteins in clinical specimens will enable likely lead to the discovery of robust biomarkers and actionable targets for drug development. In this lecture, I will give a background of the application of proteomics to clinical specimens and the inherent advantages and challenges with such analyses. I will then provide several exemplars of how proteomics can be used in the field of soft tissue sarcoma research both in translational studies for histological subtype classification and biomarker discovery as well as basic science studies in the identification of new therapeutic targets and development of patient-derived preclinical models.

For more information on this topic, please see the following references:

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Brain Tumor Radiation: Supplemental Notes

Katherine S. Hansen, DVM, DACVR (Radiation Oncology)

Intracranial tumors:

In the veterinary literature, intracranial tumors are reported in various ways, including anatomic location and distribution (intraaxial vs. extraaxial), and distinction between primary tumors (glial, meningeal, ependymal, and choroid plexus), and secondary tumors (metastatic, or directly compressing/invading such as pituitary or nasal tumors) is common.

For radiotherapy, most intracranial tumors treated are primary brain tumors, with pituitary masses being the most notable secondary tumor. Most intracranial tumors presenting for radiotherapy are also imaging diagnosed, and have not received any surgical intervention for definitive diagnosis. Brain tumors are correctly identified as a tumor on MRI roughly 90% of the time; however, correct imaging diagnosis of tumor type may be more difficult, and in one study 70% of primary tumors were correctly identified on imaging. Additionally, as many as 47% of canine cerebrovascular accidents were misdiagnosed as gliomas, and up to 12% of gliomas were misclassified as strokes.

Tumors types:

Meningiomas are extraaxial primary tumors, frequently uniformly contrast-enhancing on MRI, and commonly accompanied by edema. While meningiomas are considered the most common primary brain tumors in the dog and represent roughly half of all primary brain tumors, glial tumors comprise nearly 1/3 of primary brain masses. In cats, more than ½ of primary brain tumors are meningiomas. Lymphoma and pituitary masses comprise the majority of secondary tumors in cats.

Gliomas are intraaxial tumors that are commonly T2 hyperintense, variably and often non-uniform in their contrast- enhancement, and frequent accompanied by edema. Brachycephalic dog breeds are overrepresented with glial tumors, and in contrast to humans, dogs have oligodendrogliomas or astrocytomas more commonly than glioblastoma.

Clinical Signs:

Clinical signs referable to primary brain tumors largely depend on their location. Masses that arise in the brainstem can result in reduced consciousness, cranial nerve deficits, and proprioceptive deficits. Masses of the cerebellar region can result in ataxia, dysmetria, intention tremors, and vestibular signs. Glial cell tumors frequently arise in the forebrain, and as such seizures are very common with gliomas, although other signs are seen as well (e.g., behavior changes, circling, head pressing, visual deficits, hemineglect, proprioceptive placing deficits, and neck pain can be seen with forebrain lesions).

Advances in radiotherapy for intracranial tumors:

Many recent advances in radiotherapy have improved our ability to deliver treatment for brain tumors.

- **Anesthesia:**

- Newer anesthesia drugs have greatly improved our ability to perform consecutively daily anesthesia and shortened the recovery period.

- **Technological advances:**
  - Several recent advances have improved our ability to minimize dose to normal tissues and to sharply focus dose onto the tumor target.
    - On-Board Imaging
      - kV imaging and cone-beam CT scanners are now widely available to improve certainty of radiotherapy positioning
    - Positioning Devices
      - A variety of veterinary-specific and adopted devices are used to improve accuracy and precision in positioning
    - Multi-leaf collimators (MLC)
      - Radiation can be conformed closely around the tumor shape with MLCs, reducing the normal tissue in the field
    - IMRT/VMAT
      - Intensity Modulated Radiotherapy and Volumetric Arc Radiotherapy are two techniques that move MLCs during treatment to create a dose cloud conforming to the tumor, and they both result in rapid dose fall-off in the adjacent normal tissues

- **Fractionation:**
  - Fractionation is breaking up the total radiation dose into smaller treatments to help limit normal tissue damage while still delivering a high total dose to the tumor target.
  - Until recently, small dose-per-fraction protocols (i.e., definitive) were considered the most desirable way to deliver brain radiation.
    - Definitive
      - Definitive radiotherapy allows for treating larger regions of the brain with many small, daily doses of radiation, which helps minimize long-term damage to normal brain and nerves
      - Classic definitive protocols are typically delivered in 16-22 fractions. There are also reports of more condensed protocols delivering 10 doses
    - Palliative
      - Coarse fractionation, wherein moderate to high radiation doses are delivered over a few (weekly) treatments, are also reported
    - Stereotactic

- Stereotactic radiotherapy takes advantage of advanced techniques, and it spares normal tissue by avoidance rather than fractionation
- Targets ideally include minimal normal tissue, so minimal fractionation is needed
- Generally, 1-5 consecutive daily treatments
- With some tumor types, the outcomes may rival definitive protocols, but with the convenience of limited hospital visits and anesthesias

Radiotherapy outcomes:

- Side effect profiles from brain radiotherapy, and specifically newer stereotactic protocols, have not been aggressively defined with follow-up or necropsy.
- *See outcomes tables below for summaries of palliative, definitive, and stereotactic radiotherapy outcomes.*

Canine Suspect Meningioma Radiation Outcomes:

Surgery + Definitive Radiotherapy: MST ~ 16.5-24+ months

Reference	Cases	MST (months)	RT Protocol (Gy X fractions) Total Dose (TD)
Theon, 2000	*20*	*2-year PFS 68%*	*4 Gy X 12 TD: 28 Gy*
Axlund, 2002	*31*	*16.5*	*TD 40-49.5 Gy*
Uriarte, 2015	*7*	*17*	*3 Gy X 12 TD: 36 Gy*
Keyerleber, 2013	*21 RT 10 Surgery + RT*	*19.0*	*2.5-3.0 Gy X 15-20 TD: 45-54 Gy*

Definitive Radiotherapy: MST ~ 8.3 – 26.7 months

Reference	Cases	MST (months)	RT Protocol (Gy X fractions) Total Dose (TD)
Spugnini, 2000	*29 (mostly meningioma)*	*8.2*	*3 Gy X 16 TD 48 Gy*
Keyerleber, 2015	*21 RT 10 Surgery + RT*	*19.0*	*2.5-3.0 Gy X 15-20 TD: 45-54 Gy*
Schwarz, 2018	*31*	*26.7*	*2.5 Gy X 20 or 4 Gy X 10 TD: 40-50 Gy*
Treggiari, 2017	*15*	*24.9*	*2-4 Gy X 9-20 TD: 37-48 Gy*
Magalhaes, 2021	*12 definitive 4 palliative*	*17.5*	*2.75-3.27 Gy X 12-16 TD: 44-48 Gy*

Stereotactic Radiotherapy: MST ~ 11-18.4 months

Reference	*Cases*	*MST (months)*	*RT Protocol (Gy X fractions) Total Dose (TD)*
Mariani, 2013	*38*	*13.1*	*15 Gy X 1 TD: 15 Gy*
Griffin, 2016	*30*	*18.4*	*6-8 Gy X 3-5 doses TD 24-30 Gy*
Kelsey, 2018	*31*	*16.8*	*16 Gy X 1 20-24 Gy boost*

Palliative Radiotherapy: MST ~ 11.5 – 17.5 months

Reference	Cases	MST (months)	RT Protocol (Gy X fractions) Total Dose (TD)
Magalhaes, 2021	*12 definitive 4 palliative*	*17.5*	*4.75-8 Gy X 4-8 TD: 31.5-38 Gy*
Brearley, 1999	*41*	*11.5*	*5-9 Gy X 5 TD: 38 Gy*

Canine Trigeminal Nerve Tumor Radiation Outcomes:

Stereotactic Radiotherapy: MST ~ 10.6-14.5+ months

Reference	*Cases*	*MST (months)*	*RT Protocol (Gy X fractions) Total Dose (TD)*
Hansen, 2016	*8*	*10.6 (24.5 mo disease specific)*	*8 GY X 3, 15 Gy X 1 TD: 15-24 Gy*
Swift, 2017	*15*	*14.5*	*8 Gy X 3 TD: 24 Gy*

Canine Mixed Tumor Type Outcomes:

Stereotactic or Definitive: MST ~ 10.7-23.9 months

Reference	*Cases*	*MST (months)*	*RT Protocol (Gy X fractions) Total Dose (TD)*
Zwingenberger, 2016	*34*	*10.7*	*8 Gy X 3, 15 Gy X 1 TD: 15-24 Gy*
Rancilio, 2017	*12*	*11.9*	*8 Gy X 3 TD: 24 Gy*
Van Asselt, 2020	*52*	*18.1*	*2.25-2.5 Gy X 18-20 TD: 45-50 Gy*
Rohrer Bley, 2005	*46*	*23.9*	*2.5-4 Gy X 10-17 TD: 35-52.5 Gy*

Canine Suspect Glioma Radiation

Definitive Radiotherapy: MST ~ 7.4-23 months			
Reference	***Cases***	***MST (months)***	***RT Protocol (Gy X fractions) Total Dose (TD)***
Schwarz, 2018	*12*	*7.4*	*2.5 Gy X 20 or 4 Gy X 10, TD: 40-50 Gy*
Debreuque, 2020	*38*	*23*	*3 Gy X 15 TD: 45 Gy*
Magalhaes, 2021	*10 definitive 6 palliative*	*16.8*	*2.75-3.27 Gy X 12-16 TD: 44-48 Gy*

Stereotactic Radiotherapy: MST ~ 8.5-12.6+ months			
Reference	***Cases***	***MST (months)***	***RT Protocol (Gy X fractions) Total Dose (TD)***
Dolera, 2017	*42*	*12.6 (RT alone) 13.8 (RT + TMZ)*	*4.2-7 Gy X 5-10 TD: 33-42 Gy*
Moirano, 2020	*22*	*8.5 (one course) 21.6(RT+chemo) 28.4 (mult-course)*	*8-10 Gy X 3 TD: 24-30 Gy*
Lester, 2001	*1*	*12 mo*	*15 Gy X 1 TD: 15 Gy*

Palliative Radiotherapy: MST ~ 9-16.8 months			
Reference	***Cases***	***MST (months)***	***RT Protocol (Gy X fractions) Total Dose (TD)***
Magalhaes, 2021	*10 definitive 6 palliative*	*16.8*	*4.75-8 Gy X 4-8 TD: 31.5-38 Gy*
Brearley, 1999	*34*	*9*	*5-9 Gy X 5 TD: 38 Gy*

Canine Pituitary Tumor Radiation

Definitive Radiotherapy: MST ~ 11.7-33 months			
Reference	***Cases***	***MST (months)***	***RT Protocol (Gy X fractions) Total Dose (TD)***
Theon, 1998	*24*	*11.7*	*4 Gy X 12 TD: 48 Gy*
Fornel, 2007	*12*	*17.7*	*3 Gy X 12 TD: 36 Gy*
Kent, 2007	*19*	*33*	*3 Gy X 16 TD: 48 Gy*
Marcinowska, 2015	*12*	*32*	*3.8 Gy X 10 TD: 38 Gy*

Stereotactic Radiotherapy: MST ~ 8-11.7 months			
Reference	***Cases***	***MST (months)***	***RT Protocol (Gy X fractions) Total Dose (TD)***
Hansen, 2018	*45*	*8 (Cushings) 20.6 (non-Cushings)*	*8 Gy X 3, 15 Gy X 1 TD: 15-24 Gy*
Gieger, 2020	*13*	*11.7*	*16 Gy X 1 TD: 16 Gy*

Palliative Radiotherapy: MST ~ 4.8-6 months			
Reference	***Cases***	***MST (months)***	***RT Protocol (Gy X fractions) Total Dose (TD)***
Brearley, 1999	*8*	*4.8*	*5-9 Gy X 5 TD: 38 Gy*
Marcinowska, 2015	*12*	*6*	*5-8.25 Gy X 5 TD: 38 Gy*

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Barriers to Beat: Improving Drug Delivery to Brain Tumours

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ABC-transport inhibitors as a platform to enhance brain delivery of drugs

The ABC-transporters ABCG2 and ABCB1 are expressed at the BBB and reduce the brain penetration of many drugs. Although valuable for normal brain functioning, they hamper the pharmacotherapy of many CNS-diseases. Even in brain tumors where this barrier is more leaky, we have shown that ABC transporters are expressed and can restrict drug efficacy. Inhibition of these transporter would be a platform technology to enhance drug delivery to the brain. We are repurposing elacridar, a drug that was previously developed to modulate ABCB1-mediated drug resistance in tumor cells, as a pharmaco-enhancer for drug delivery to the CNS.

What MR spectroscopy can tell us about intracranial neoplasia

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Imaging is an essential component of tumour diagnosis and assessment. Magnetic resonance imaging (MRI) provides accurate description of the tumor size, location and its relationship with its adjacent structures, and in addition, there are many MRI sequences that provide information on the biological properties of the tumour.

Magnetic resonance spectroscopy (MRS) of the brain is a non-invasive technique that allows the determination and quantification of brain metabolites. MRS gives metabolic information related to neural integrity, cell proliferation or degradation, energy metabolism and necrosis. This metabolic information may support clinical diagnoses and enhance the understanding of neurological disorders.

Field strengths of at least 1.5 T are necessary for proper differentiation of the chemical shifts of metabolites in 1H MRS, since the chemical shift of protons is very small (≈10 ppm). Spectral resolution, particularly for coupled spin systems (eg, glutamate, glutamine, and myoinositol), and signal-to-noise ratio are higher at 3.0 than 1.5 T. Therefore, the use of a 3.0 T field improves reliability of detection of these compounds.

The result of MRS can be visualized as a graph of signal intensity with respect to its frequency. Proton signals of different metabolites or even different protons of one molecule can occur at different positions (frequencies) within the MRS spectrum. The shift of peaks in their relationship to one another on the frequency axis is called chemical shift. The relative concentration of the metabolites can be measured by numerical integration (normally using metabolite ratios) or by sophisticated software such as LCModel.

Two general categories of spatial localization techniques are used in 1H MRS: single-voxel and multi-voxel (also called chemical shift imaging). Single-voxel MRS measures the MR signal of a single selected region of interest (ROI) whereas signal outside this area is suppressed. In multi- voxel several small voxels are obtained in a stack volume of the brain. Single-voxel provides a more stable spectrum, without lipid contamination and intra-voxel contamination; however, only a focal area of the brain can be examined.

Proton MRS can detect several metabolites, such as N-acetyl aspartate (NAA), choline, creatine, glutamate, glutamine, myoinositol, and glutathione, in the brain of clinically normal subjects. N-acetyl aspartate, which resonates at 2.01 ppm, is considered a neuronal marker and is present only in neurons, axons, and dendrites. Choline resonates at 3.2 ppm and is involved in membrane synthesis and degradation, whereas creatine resonates at 3.0 ppm and is involved in energy metabolism. Glutamate and glutamine metabolites resonate closely at 3.75 ppm and between 2.1 and 2.5 ppm, respectively. Glutamate is an excitatory neurotransmitter that plays a role in mitochondrial metabolism; glutamine is involved in detoxification and regulation of neurotransmitter activity. Myoinositol is a pentose sugar that resonates at 3.5 to 3.6 ppm and is part of the inositol triphosphate intracellular second messenger system. Glutathione resonates at 3.77 ppm; it is an antioxidant and is essential for maintaining RBC structure and maintaining hemoglobin in a ferrous state. In pathological conditions, these metabolites may be found in abnormal concentrations (absent, lower, or higher concentrations), and other metabolites (eg, lipids or lactate) that typically are not present in a healthy brain may be detected.

The metabolic profile of intracranial neoplasias share some features amongst different tumoral types, while there are some specific metabolites that may help to differentiate between different tumours, tumoral grade and even response to treatment.
NAA concentration is low in neoplasia as there is neuronal loss or death. In extra-axial tumours, it should not be any NAA as there is no neural tissue. Choline concentration is normally very increased in all tumoral types, since there is a high cell membrane breakdown. High grade neoplasia shows frequently increased lactate due to hypoxia and high concentration of lipids, due to necrosis. These features are not present in low-grade neoplasia. Some specific metabolites, such as glutathione, has been shown in people to correlate with the response of radiotherapy treatment of meningiomas in people. In people, there are studies also that correlate the concentration of some metabolites with survival (higher concentration of lipids and scyllo-inositol correlated with poorer prognosis).

MRS should be seen as an adjunct technique that may add information to narrow down the differential diagnosis in combination with the remainder MRI sequences.

THEMED SESSION - Sota

Liquid biopsies: Clinical application of cancer genetics in diagnostics

Maja Louise Arendt DVM, PhD, DipECVIM-CA(onc)

Cancer is a genetic disease in the sense that all cancer cells have changes in their genome. In the past 10-15 years we have started to gain more and more insight into which genetic changes are present in cancer cells, not just in humans but also in companion animals and in particular dogs. Elucidating genetic changes in particular cancer types can be useful for understanding the cancer cell behaviour and identifying therapeutic targets or reasons for treatment resistance. However, these genetic changes can also be used as diagnostic targets to detect the presence of disease.
Liquid biopsies refer to the use of body fluids or cells as a minimally invasive technique to gain information regarding diagnosis, stage or early relapse of disease. In particular human oncology, liquid biopsies have proven to be useful for early detection of disease relapse, by detecting small amounts of circulating cancer DNA, also known as cell free DNA (cfDNA), in the blood of cancer patients. Due to the turnover of cells within cancerous tissues, small amounts of cfDNA are continuously released into the bloodstream which means that tumor

specific mutations can be captured in a blood sample using very sensitive and specific PCR techniques.
We have some diagnostic challenges in veterinary oncology where either obtaining tissue biopsies from tumors is problematic or associated with morbidity for the patient or where inflammatory cells infiltrating tissue can make it difficult to make a conclusive histopathological or cytological diagnosis. Using minimally invasive biopsies looking for cancer specific genetic changes can be a practical and cost-efficient way to overcome some of the diagnostic challenges within veterinary oncology.

The era of precision oncology in cancer care

Carlo Cattrini

The panorama of precision oncology in human cancers has completely changed in recent years. The advent of molecular profiling has impacted clinical practice, being a guidance for diagnosis and therapy in many tumor types (Mateo et al, Nat Med 2022). A significant number of molecularly guided treatment options have been approved on the basis of genomic biomarkers for various tumors (Malone et al, Genome Med 2020). Some drugs proved activity in different cancers that share the same molecular alteration, and received broad or tumor-agnostic approvals. Examples of such alterations are NTRK fusion, microsatellite instability or DNA mismatch repair deficiency (Adashek et al, Trends Cancer 2021). Non-small-cell lung cancer (NSCLC) represents an example of success of biomarker-guided therapy, and tyrosine kinase inhibitors (TKIs) have provided an illustrative example of the achievements in targeting oncogene addiction (Yuan et al, Sig Transduct Target Ther 2019). EGFR and BRAF mutations, ALK, RET and ROS1 rearrangements are now routinely tested in patients with NSCLC to choose the most appropriate treatment strategy. Similarly, genetic aberrations in DNA repair pathways have become a cornerstone of precision oncology in several cancer types (Lozano et al, Br J Cancer 2021). The synthetic lethal effect of PARP inhibitors in patients with impaired BRCA function represents one of the major triumphs of translational medicine (Ashworth et al, Nat Rev Clin Oncol 2018). Indeed, germline and somatic alterations in BRCA genes are currently assessed in patients with breast, ovarian and prostate cancer to predict for response to PARP inhibitors. Genomic sequencing has driven most of the recent advances in terms of biomarker-guided interventions. However, transcriptomics, proteomics and immune profiling are also being studied to predict for patients' response and prognosis. An example is represented by the Programmed Death-Ligand 1 (PD-L1). Its expression, as measured by immunohistochemistry, is assessed to predict for response to immune-checkpoint inhibitors (ICIs). However, its clinical use is controversial and restricted to specific tumors, given that many trials have shown efficacy of ICIs in patients with PD-L1 negative cancers (Grossman, Oncogene 2021). Tumour mutational burden (TMB) is another emerging biomarker for predicting response to ICIs in various tumor types, but the optimal predictive cut-point vary widely by histology (Yarchoan et al, N Engl J Med 2017). The advent of new tailored drugs has also prompted multitargeting therapy and combinatorial approaches to combat drug resistance (Dupont et al, FEBS J 2021). An example is represented by the combination of TKIs and ICIs in melanoma, lung and renal cancer. TKIs-ICIs combos provided meaningful clinical benefit to patients, resulting in additive, and sometimes synergistic, effects. However, this new era of precision medicine brings significant challenges for healthcare to enable wide access to these drugs for patients. Costs related to diagnostic tests, technologies and drugs result in a significant gap between advances in anticancer drug development and administering these drugs to patients. In some cancers, the use of comprehensive genomic

profiling, instead of multiple single-biomarker tests, can improve quality of treatment and cost-effectiveness (Chawla et al, JCO Precis Oncol 2018).

Veterinary Oncology Immunotherapies – A Mini Review

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While the immune system is normally thought of as providing protection against infectious disease, the immune system's ability to recognize and eliminate cancer is the fundamental rationale for the immunotherapy of cancer. With the tools of molecular biology and a greater understanding of mechanisms to harness the immune system, effective tumor immunotherapy is now a reality. This new class of therapeutics offers a more targeted and therefore precise approach to the treatment of cancer. Cancer immunotherapy is now recognized as one of the pillars of treatment alongside surgery, radiation and chemotherapy; the various cancer immunotherapy modalities will be outlined in this short review.

IMMUNE SYSTEM

The immune system is generally divided into two primary components: the *innate immune response*, and the highly specific, but more slowly developing *adaptive or acquired immune response*. The innate and adaptive arms of immunity are not mutually exclusive; they are linked by: 1) the innate response's ability to stimulate and influence the nature of the adaptive response, and 2) the sharing of effector mechanisms between innate and adaptive immune responses. Immune responses can be further separated by whether they are induced by exposure to a foreign antigen (an "active" response) or if they are transferred through serum or lymphocytes from an immunized individual (a "passive" response).

ANTITUMOR IMMUNITY

There are significant barriers to the generation of effective antitumor immunity by the host. Many tumors evade surveillance mechanisms and grow in immunocompetent hosts as easily illustrated by the overwhelming numbers of people and animals succumbing to cancer. There are multiple ways in which tumors evade the immune response including, but not limited to, immunosuppressive cytokine production, impaired dendritic cell (DC) function via inactivation ("anergy") and/or poor DC maturation, induction of cells called Regulatory T cells (Treg), which were initially called suppressor T cells, etc.

BACTERIAL & BIOLOGIC RESPONSE MODIFIERS

Dr. William Coley, a New York surgeon in the early 1900's, noted that some cancer patients developing incidental bacterial infections survived longer than those without infection. Coley developed a bacterial "vaccine" ("Coley's toxins") to treat people with sarcomas which provided complete response rates of approximately 15%. (1) His seminal work laid the foundation for nonspecific modulation of the immune response in the treatment of cancer. There are numerous non-specific tumor immunotherapy approaches ranging from biological response modifiers (BRM) such as BCG (Bacillus Calmette-Guérin; interestingly, Guérin was a veterinarian),*Corynebacterium parvum*, *Salmonella typhimurium* (VNP20009), Mycobacterial cell wall DNA complexes, MTP-PE (muramyl tripeptide-phosphatidylethanolamine), oncolytic viruses and imiquimod (Aldara®), and recombinant cytokines, growth factors or hormones. The European Committee for Medicinal Products for Veterinary Use (CVMP) adopted a positive opinion in March, 2013 for the veterinary product Oncept IL-2 (feline Pox virus expressing recombinant feline IL-2). This product from Merial (now Boehringer Animal Health) also received conditional licensure from the USDA CVB (Center for Veterinary Biologics) in 2015. It is labelled for use in addition to surgery and

radiation in cats with stage I fibrosarcomas without metastasis or lymph node involvement, to reduce the risk of relapse and increase the time to relapse.
The ultimate goal for a tumor immunotherapy with a specific target is elicitation of an anti-tumor immune response which results in clinical regression of a tumor and/or its metastases. Responses to cancer vaccines and other cancer immunotherapies may take several months or more to appear due to the slower speed of induction of the adaptive arm of the immune system. The ideal cancer immunotherapy agent would be able to discriminate between cancer and normal cells (ie specificity), be potent enough to kill small or large numbers of tumor cells (ie sensitivity) and lastly be able to prevent recurrence of the tumor (ie durability). The immune system detects tumors through specific tumor-associated antigens (TAAs) and/or abnormal disease-associated antigens (DAAs). A variety of approaches have been taken to date to focus the immune system on the aforementioned targets, including whole cell, tumor cell lysate and/or subunit vaccines, DNA vaccines which immunize with syngeneic and/or xenogeneic (different species than recipient) plasmid DNA, viral and/or viral vector-based methodologies designed to deliver genes encoding TAAs and/or immunostimulatory cytokines, DC or CD40-activated B cell vaccines and adoptive cell transfer. Antibody (Ab) approaches for cancer immunotherapy include monoclonal antibodies (mAbs), anti-idiotype Abs, conjugated Abs and engineered Ab “variants (e.g. bispecific mAbs, single chain variable fragments, single chain Abs, etc.).

ANTITUMOR VACCINES
One particularly exciting vaccine approach is the use of a HER-2 targeting attenuated listeria therapeutic vaccine. (2) This approach was utilized by Mason et al in dogs with appendicular osteosarcoma after being treated with amputation and adjuvant carboplatin chemotherapy. It is currently unknown how much of the therapeutic efficacy is from the xenogeneic human HER-2 vs the listeria, but may be considered in the future with other HER-2 related histologies. This product received USDA-CVB conditional license in 2018.
This author has developed a xenogeneic DNA vaccine program for melanoma utilizing xenogeneic tyrosinase DNA vaccines in collaboration with human investigators from Memorial Sloan-Kettering Cancer Center.(3) Antigen-specific (huTyr) IFNγ T-cells were found along with 2- to 5-fold increases in circulating antibodies to huTyr which can cross react to canine Tyrosinase, suggesting the breaking of tolerance. The clinical results with prolongation in survival have been reported previously. Based on these studies a multi-institutional safety and efficacy trial for USDA licensure in dogs with locally controlled stage II/III oral melanoma was initiated in 2006 with granting of conditional licensure in 2007, which represented the first U.S. governmental regulatory agency approval of a vaccine to treat cancer across species. Results of this licensure trial documented a statistically significant improvement in survival for vaccinates vs controls and a full licensure for the HuTyr-based canine melanoma vaccine from USDA-CVB was received in December, 2009 (Oncept™, Merial, Inc.).

MOST RECENT ADVANCES
Tumor immunology and immunotherapy is one of the most exciting and rapidly expanding fields at present. Significant resources are focused on mechanisms to simultaneously maximally stimulate an antitumor immune response while minimizing the immunosuppressive aspects of the tumor microenvironment. The recent elucidation and blockade of immunosuppressive cytokines (e.g. TGF-β, IL-10 and IL-13) and/or the negative costimulatory molecule CTLA-4 and PD-1 (Programmed Cell Death 1 or CD279) along with the functional characterization of myeloid derived suppressor cells (MDSC) & T regulatory cells, have dramatically improved cell-mediated immunity to tumors by “taking the brake” off the immune system.
The aforementioned greatly expanded molecular understanding of the immune system has recently translated into human cancer immunotherapeutics which confer a survival benefit such as the use of the checkpoint inhibitor anti-CTLA-4 Ab, ipilimumab (Yervoy™, Bristol-Myers Squibb), Abs directed against another checkpoint inhibitor known as PD-1 receptor or

against human PDL-1 (L = ligand) such as Nivolumab (Opdivo®, Bristol-Myers Squibb) and Pembrolizumab (Keytruda®, Merck). Furthermore, pembrolizumab was recently given FDA approval for unresectable or metastatic, microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) solid tumors that have progressed following prior treatment. This is truly revolutionary as it represents the first cancer-agnostic FDA approval and is one of many recent FDA approvals based on single arm studies.

Another area of extreme promise in IO is a form of adoptive cell therapy called CAR-T (Chimeric Antigen Receptor-T cells). T cells are harvested from a patient and then genetically engineered to express a CAR on their cell surface with expansion in vitro before being reinfused back into the patient. (4) This CAR is specifically designed to recognize a specific TAA with a domain responsible for activating the T-cell when the CAR-T binds its TAA. The latest generations of CAR-T are engineered to contain important costimulatory domains that further enhance the immune response against the cancer cell containing the TAA. In 2017, an expert panel of the FDA unanimously (10-0) recommended approval of CTL019 (tisagenlecleucel), an investigational CAR-T therapy utilizing CD19 as its CAR of choice for patients with B-cell acute lymphoblastic leukemia (ALL). Many CAR-T studies have found > 80-90% objective tumor responses, but side effects, including death can occur. Furthermore, CAR-Ts are currently difficult to make and carry a high cost of goods to produce, making for a difficult, but not impossible commercial development path in veterinary medicine.

Checkpoints, checkpoint inhibitors and other adoptive cell transfer technologies like CAR-T and others are also starting to be better understood and pursued in veterinary diseases. Furthermore, the exciting race to develop commercial veterinary specific IO therapeutics like checkpoint inhibitors and CAR-T is currently ongoing with a handful of animal health companies. As these therapeutics reduce immune tolerance and more easily generate specific anti-tumor immune responses in patients, pathologic autoimmunity was predicted and is now being seen clinically in human patients as a side effect.

In summary, the future looks extremely bright for immunotherapy. This author ardently looks forward to the time when cancer immunotherapy plays the same significant role in the treatment and/or prevention of cancers in veterinary patients like it currently does in human cancers. (5)

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The epidemiology and mechanisms of cancer pain: How should we individualise a patient's pain assessment and management

Dr. Clark

Epidemiology of cancer pain - the human perspective

There are increasing numbers of people living with and beyond cancer, and this presents a clinical challenge for pain management. A large proportion of these patients experience pain secondary to their disease or its treatment, impeding rehabilitation and significantly impacting upon their quality of life. The successful management of this pain presents a considerable challenge. The World Cancer Report 2014 highlighted the increasing global prevalence of cancer. In 2003, 10 million people developed malignancies with 6.2 million dying from the disease. In 2015, there were 17.5 million new cancer cases and 8.7 million related deaths. Fewer cancer-related deaths have led to an increase in cancer survivors. In 2018, there were an estimated 43.8 million people living with cancer (within 5 years of diagnosis) worldwide. In epidemiologic data of cancer pain collected between 2006 and 2011, overall cancer pain prevalence data indicated the presence of pain at the time of cancer diagnoses at 35%, and a mean prevalence of moderate to severe pain throughout the disease course of 46%, varying widely (21%–84%) based on specific cancer and disease stage. Most studies report pain prevalence escalated in advanced cancer to approx. 75%, with similar end-of-life pain prevalence slightly higher in most studies. Pain prevalence variations were found specific to cancer type and site, but substantiated the presence of pain across neoplastic diagnoses, including haematologic cancers, such as lymphoma with pain prevalence reports of 20% to 87%. Pain has a marked impact on cancer patients; a pan-European survey of patients with all stages of cancer identified that 69% had activities of daily living impeded by pain, and, despite the availability of effective treatments, cancer-related pain may be inadequately controlled in up to 50% of patients. The psychosocial consequences of cancer pain (particularly anxiety and depression) are well documented. Despite the impact that pain has on quality of life, half of those undergoing cancer treatment report that their quality of life is not deemed a priority in the overall care that they receive. Considerable improvements in identification and treatment of malignancies have led to the increased survival rates, with unintended negative impact upon wellbeing and quality of life.

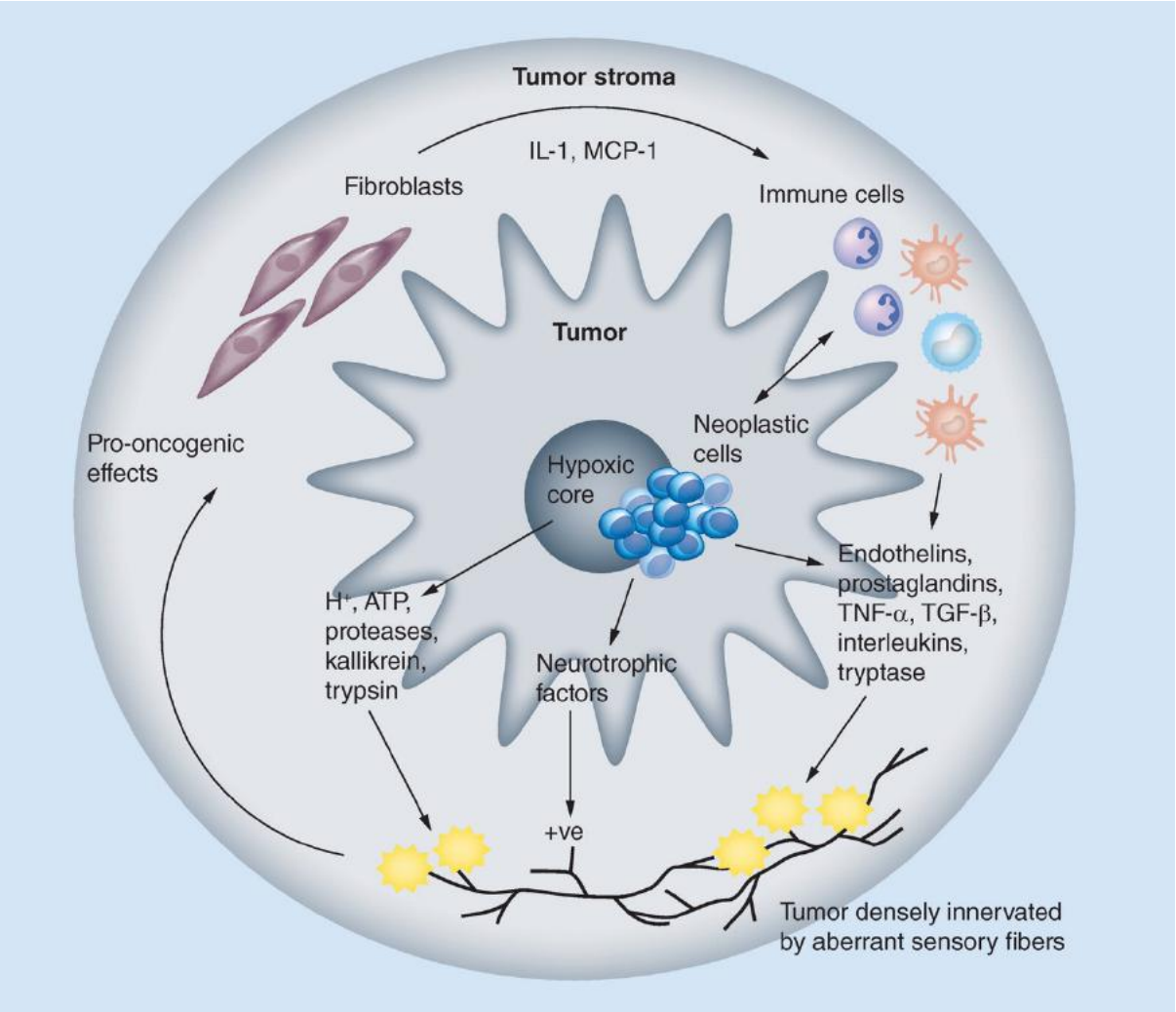
We have little comparative data upon which to base our clinical approach. It would be appropriate to be cognisant of the increased prevalence of pain as the disease advances, and that euthanasia is an ethical option throughout cancer management and is an ethical imperative where suffering cannot be alleviated. We must also investigate the use of quality of life assessment tools (see notes from nursing day specifically pertaining to this).

Mechanisms of cancer pain – where is the pain coming from?

We do fully not understand many of the biological mechanisms responsible for cancer pain. Depending on the type and anatomic location of cancer, different mechanisms may be contributors. For example, the cause and current understanding of metastatic bone cancer pain is different than the cause of pain from a squamous cell carcinoma in the tongue.

A complex relationship exists between a malignant lesion and its microenvironment, where the tumour has a dynamic relationship with host cells. Tumour and host cells both secrete numerous mediators that are implicated in pain, peripheral sensitization, and angiogenesis. Mediators implicated in cancer pain include protons, endothelin, adenosine triphosphate, neurotrophic factors, and cytokines. Neurotrophic factors include nerve growth factor (NGF), neuturin and BDNF.

Tumour and its microenvironment.



From: Magee D, Bachtold S et al. (2019)

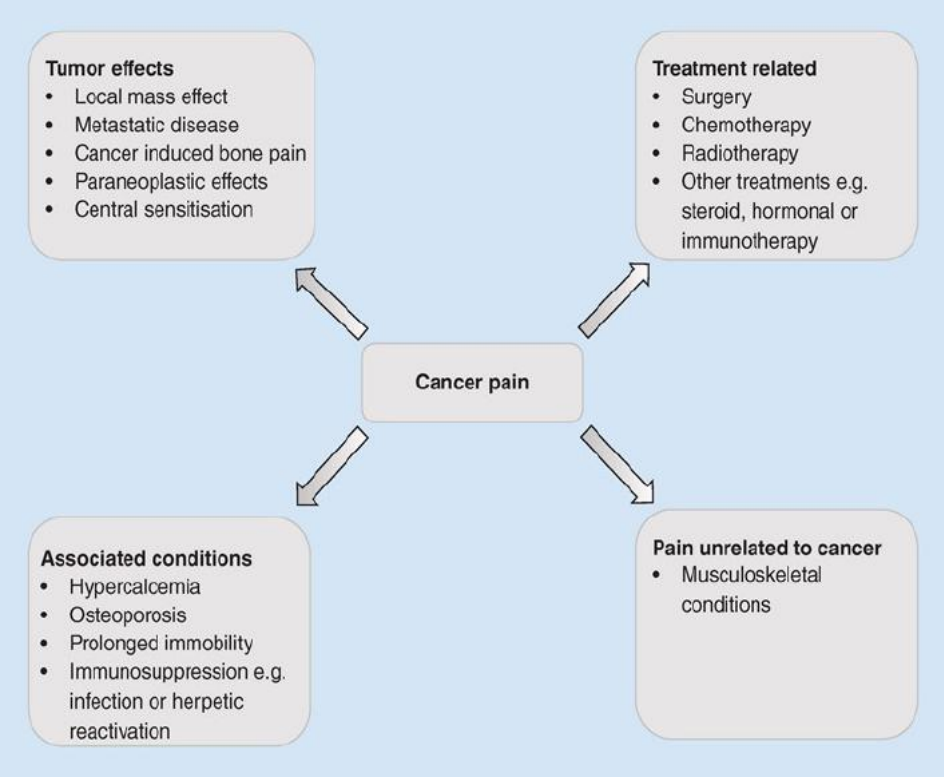
Nociceptive pain arises when stimuli, whether thermal, mechanical, or chemical are transduced into sensory neurone action potentials. The transduction of chemical mediators is best described and can involve both the transient receptor potential vanilloid (TRPV) and acid-sensing ion channel families (ASIC). Cationic (Na^+ and Ca^{2+}) flux triggers depolarization once threshold potentials are reached.
Peripheral sensitization results in transcriptional and translational modification to receptors and ion channels, producing a lower threshold for neuronal activation and increased magnitude of response observed. The processes involved in this are similar in cancer and non-cancer pain.
The mechanisms by which central sensitization in the dorsal horn of the spinal cord occurs, include increased membrane excitability and synaptic transmission, decreased descending inhibition and glial neuronal interactions. The specific mechanisms are complex, differ according to the pain state and are still being elucidated but the overall effect is to increase the “gain” of the system resulting in states of facilitation, potentiation, or amplification.

Neuropathic Pain – what are the differences?
Neuropathic pain arises as a direct consequence of a lesion or disease affecting the somatosensory system. It develops because of damage to, or dysfunction of, the nervous system. A cross-sectional study found that more than 40% of cancer patients with moderate to severe pain had neuropathic symptoms that interfered with their daily activities. In patients with cancer, neuropathic pain results from malignant infiltration of nerves, or nerve damage during surgical intervention. The most common cause of neuropathic pain is the direct

invasion of the cancer and the resulting nerve damage. This type of pain usually presents as a plexopathy, which is defined as “injury to nerve fibres in a specific distribution,” typically brachial, thoracic, and lumbosacral.
Several cancer treatments result in neuropathic pain as a side effect; for instance, surgery or radiation-induced neurological injury and chemotherapy-induced neuropathy. The major chemotherapy medications that result in neuropathy are cisplatin, vincristine, and procarbazine.

Cancer-induced bone pain
Tumour deposits within bone (primary or secondary) result in pain through disparate mechanisms. These are, structural disruption, increased local osteoclastic activity, inflammatory mediator release and changes in sensory innervation. These pain mechanisms are often co-existing and co-dependent. E.g., local inflammatory mediator release (IL-1, IL-6, TGF-β and RANKL) increases osteoclast activity and subsequent bone destruction. The resulting net bone resorption then allows room for expansion of the tumour. Disruption to periosteum, marrow or cortex can result in pain. Malignant bone pain has a neuropathic component. NGF is implicated in significant sprouting of aberrant sensory and sympathetic nerve fibres, in periosteum, mineralized bone and marrow.

Contributors to pain in the cancer patient



From: Magee D, Bachtold S et al. (2019).

Treatment-related pain
Cancer treatment can itself cause substantial painful morbidity.
The extent of this is possibly less in our patients than in human cancer patients but it should not be ignored, and pain should always be considered in any risk/benefit equation.
Persistent post-surgical pain
Surgery represents an integral component of the management of many cancers. Persistent post-surgical pain (PPSP) is prevalent throughout the human post-surgical population and is particularly pertinent to cancer patients. A formal definition of PPSP does not exist, but it has been proposed that *“it is pain that develops or increases intensity following a surgical procedure, either as a continuation of acute postoperative pain or developing after an*

asymptomatic period. It must be isolated to the surgical field, the territory of a nerve or dermatome associated with the surgical field."
The pain must have been present for at least 3–6 months, and significantly impact upon quality of life. Additionally, any other causes (e.g., infection or recurrence of malignancy) must have been excluded.
In people, PPSP is more frequently observed following certain procedures including thoracotomy, breast surgery and limb amputation, although it may occur after even limited surgery. A significant proportion of patients with PPSP have neuropathic features; although variable, these features have been identified in up to 68% of patients. The presence of neuropathic features has a greater impact upon quality of life. PPSP has been identified in veterinary patients and in animal disease models but has not been properly investigated or adequately considered. Despite peripheral nerve injury being a clear risk factor for the development of PPSP, the relationship is complex. PPSP is a significant problem after breast cancer surgery and contributes adversely to patients' quality of life.
There is a recent initiative from Cornell University to develop a scale to diagnose and assess post-amputation pain in dogs, potentially another step on our PPSP education pathway.

There are several risk factors for PPSP development in people:
Patient:

- Anxiety
- Depression
- Raised BMI
- Pre-existing pain
- Pain catastrophizing
- Genetic factors
- Young age†

Procedure:

- Longer duration of procedure
- Open procedure
- Retraction/destruction nerves
- Use of drains
- Surgery in low-volume centres
- Poorly controlled peri-operative and post-operative pain – single biggest factor!

Other:

- Chemotherapy
- Radiotherapy
- †Older age risk for phantom limb pain.

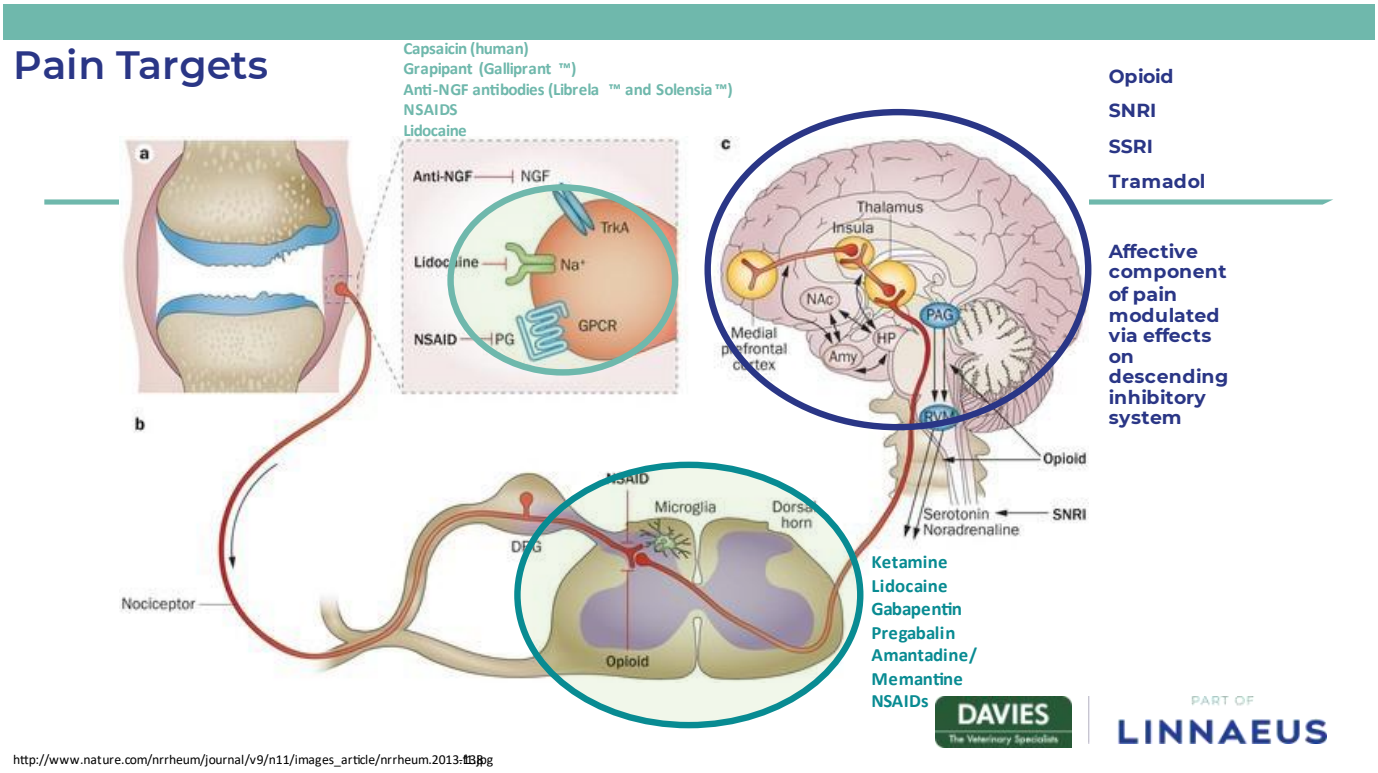
Although certain risk factors are more manageable than others, e.g., peri and post-operative acute pain control, no single risk factor has been identified that can be targeted by an intervention to prevent PPSP.

Chemotherapy-induced peripheral neuropathy

Chemotherapy-induced peripheral neuropathy (CIPN) occurs following chemotherapy, typically affecting longer, sensory nerve fibres. The risk of development and severity depend on several factors including drug type and cumulative dose of treatment. Overall, in human studies prevalence rates as high as two thirds have been reported during the first month of chemotherapy. Platinum compounds (cisplatin, carboplatin and oxaliplatin), vinca alkaloids (vincristine) and taxanes (paclitaxel) are commonly implicated. The pathophysiology of CIPN is not fully understood, although multiple contributory processes have been identified. Different agents may result in alternative pathophysiological mechanisms and therefore result in a constellation of signs and symptoms. The lower does used in veterinary oncology may spare patients the risk of CIPN but this appears yet to be investigated.

Radiotherapy
Radiotherapy utilizes the application of ionizing radiation to cause cellular DNA changes, resulting in cell death. The effects of radiotherapy depend on numerous factors, but ultimately, non-malignant tissues can also be vulnerable to injury. Toxicities including mucositis and nerve plexopathies are among the most encountered following radiotherapy in people. Radiotherapy-induced damage can result in either early or late toxicities, depending on the time taken for development of injury after exposure to ionizing radiation. Early manifesting toxicity involves cell loss from tissues possessing high cellular turnover, such as mucosa or epidermis-causing mucositis and epidermal desquamation, respectively. Conversely, late manifesting tissue toxicities occur secondary to injury to slower-renewing tissues such as nerve or muscle, manifesting as fibrosis, necrosis, and atrophy. Cytocidal effects of ionizing radiation result in direct cellular and tissue damage. Indirect effects describe the reactive effects that take place in other cells or tissues because of radiation-related injury, which includes bystander effects, a phenomenon in which nonirradiated cells, near those irradiated, display genetic damage themselves.
Nervous tissue is particularly susceptible to radiotherapy damage both through direct damage from ionizing radiation and the fibrosis that can result. Radiation-induced brachial plexopathy presents with variable onset and timing
Radiation-induced oral mucositis is an expected tissue injury that results from acute inflammation of oral mucosa, tongue, and pharynx. Radiation-induced oral mucositis can be a significant barrier to adequate nutrition and consequently may interrupt or limit desired cancer treatment, thus proving a major challenge in the treatment of head and neck cancers in people.

Managing cancer pain – pharmacological approaches.
These images provide the outline of some of the potential drug targets in chronic pain.
It`s beyond the scope of this lecture to provide detailed resources relating to each specific drug.
The appropriateness of each pharmacological agent can be assessed once the anatomic location and the type of pain (mechanistically) is determined. Examples will be discussed during the lecture.



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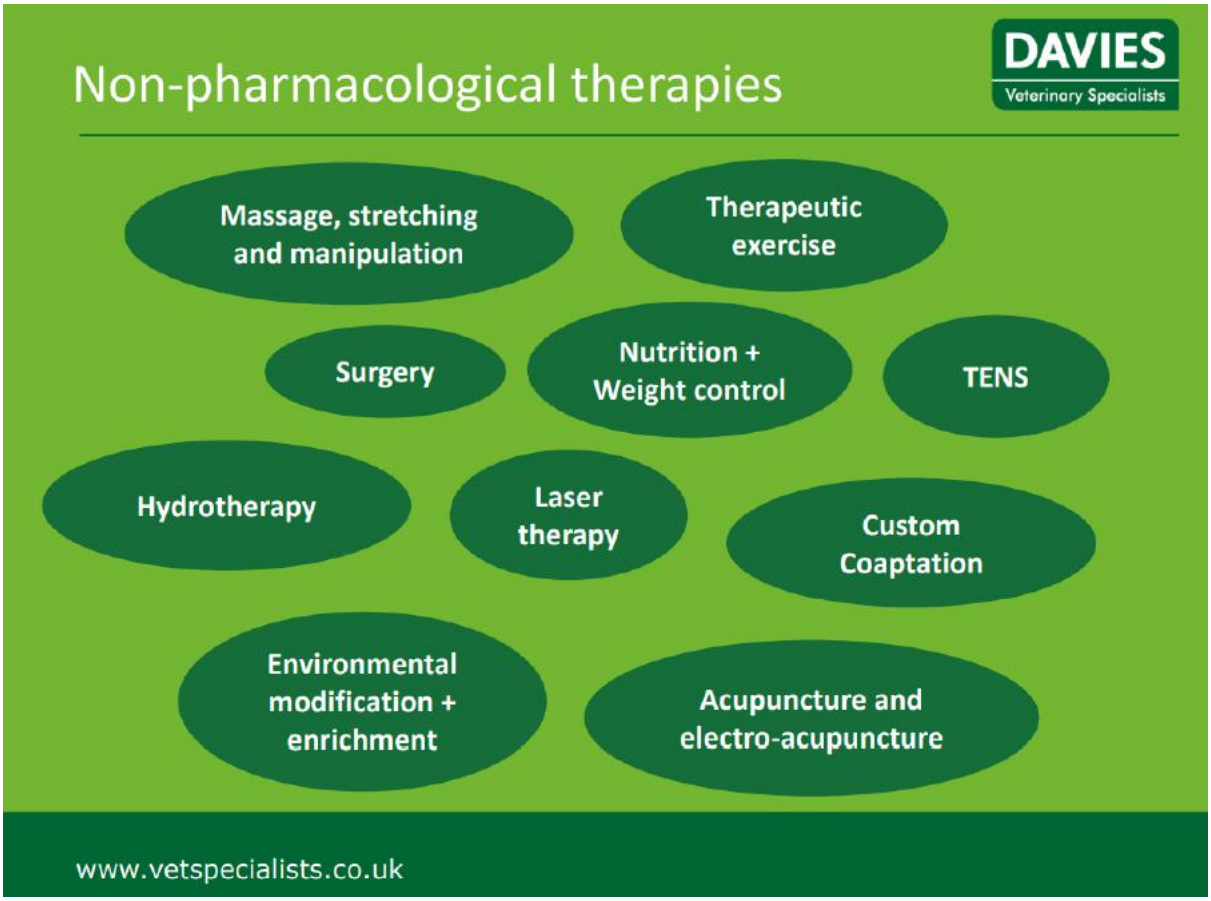
Managing cancer pain – nonpharmacological approaches.
Some of these approaches are designed only to address QoL rather than pain specifically but in enhancing this, pain may be reduced (e.g., hydrotherapy in amputees).



Interventional Procedures to manage cancer pain
These most typically involve interruption to or modification of nerve conduction with the aim of diminishing pain from a target area. The nerves involved include those of the peripheral, autonomic, and central nervous system. The procedures may be considered as non-destructive or destructive. In non-destructive procedures, nerve blockade or modulation is achieved by the deposition of reversible pharmacological agents. These may be provided by bolus injection and most commonly involve local anaesthetic agents often supplemented by depot steroids. Alternatively, catheter placement allows for the continuous delivery of agents. When placement is adjacent to peripheral or autonomic nerves similar agents are used. The destructive procedures involve the use of chemical agents (alcohol 50–100% and phenol 6–10%), physical methods of heat (radiofrequency) and cold (cryoablation) and surgery. These procedures remain uncommon in Veterinary Oncology

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From chronic inflammation to Feline Low-grade Intestinal Lymphoma: a new model of lymphomagenesis

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Feline chronic enteropathies include both inflammatory and neoplastic lesions. Gastrointestinal lymphoma is by far the most frequent digestive tract neoplasia in cats. Among the different lymphoma subtypes, low-grade intestinal T-cell lymphoma (LGITL) is an indolent disease, affecting elderly cats, and its incidence has significantly increased over the two past decades. Gastrointestinal lymphoma is defined by the infiltration of neoplastic lymphocytes within the gastrointestinal tract with variable involvement of extra-intestinal sites including draining lymph nodes, liver, spleen, and pancreas.
A challenge for both veterinary clinicians and pathologists is to distinguish lymphoplasmacytic enteritis (LPE) from LGITL: in fact, clinical signs, laboratory results, and diagnostic imaging findings lack specificity. Moreover, these two diseases are characterized by a heterogeneous distribution of the lesions whose severity may vary along digestive segments. No real consensus has been established considering biopsy sampling: gastroduodenoscopy allows biopsies, but distal jejunal lesions may be missed by this technique, leading to misdiagnosis. In contrast, all the digestive tract is reachable by surgery, even if rare life-threatening complications can occur. Finally, histology, immunohistochemistry and clonality features are also known to overlap. Clonality testing has been considered as a key step to ultimately differentiate LGITL from LPE.
This presentation will focus on different pending questions:

1- Is it an issue to differentiate LGITL from LPE?
2- What is the gold standard for diagnosis (Histology + Immunohistochemical analysis? May clonality allow reclassifying "equivocal" cases?)
3- What arguments support a potential *continuum* between LPE and LGITL?
4- If a corticosteroid treatment is prescribed prior to definitive diagnosis, what would be its impact on LGITL follow-up?

1- Is it an issue to differentiate LGITL from LPE?

According to the "One-Health" concept, feline LGITL has recently been validated as a relevant model for human indolent T-cell lymphoproliferative disorder of the GI tract (GI-T-LPDs).
Indolent T-cell lymphoproliferative disorder of the gastrointestinal tract is a rare human primary gastrointestinal T cell lymphoma, recently included in the 2016 revision of the World Health Organization classification of lymphoid neoplasms. LGITL was first compared to monomorphic epitheliotropic T-cell lymphoma (MEITL), in view of the associated infiltration of the lamina propria and epithelium by medium-sized monomorphic neoplastic T cells. However, the clinical, laboratory, histological and immunohistochemistry features of LGITL are very similar to those of GI-TLPD. As a result, feline LGITL was cited as a relevant model of human GI-T-LPD in two recent publications.

Researchers have long used animal models to better characterize human diseases. The mouse has several advantages and is the most frequently used animal model. That said, this model is primarily limited by the need to induce tumors. Furthermore, genomic instability and heterogeneity in tumor cells and microenvironment are not spontaneously reproduced in

mice models. In contrast, naturally occurring tumors (sarcomas, osteosarcomas, or lymphomas) in dogs are well-depicted models for different human cancers. They share many characteristics -biological behavior, histological appearance, genetics features- and response to conventional treatments. The canine genome was sequenced in 2005, whereas the complete feline sequence was more recently published. Hence, comparative oncology with feline models is now developing because the cat may be a good model of different neoplasia such as thyroid carcinoma, mammary carcinoma, or oral squamous carcinoma. These neoplasia are more frequent in cats than in humans, and the response to treatment is much more predictable in cats than in laboratory animals.
As a result, for the purpose of both prognosis evaluation and therapeutic adaptation, it is a real issue to differentiate LGITL from LPE. Furthermore, deciphering the underlying mechanisms of lymphomagenesis is essential for the future development of targeted therapies. According to the “One-Health” concept, this approach would be beneficial to both species.

2- What is the gold standard diagnosis (histology + Immunohistochemical study? May clonality allow reclassifying “equivocal” cases?)

To better define the frontiers between intestinal inflammation and indolent intestinal lymphoma in cats, recent studies have established more precise and even novel histopathologic, phenotypic, and molecular criteria. We prospectively analyzed clinical, paraclinical data and full-thickness small intestinal biopsies from 22 domestic cats diagnosed with LGITL and 22 cases diagnosed with LPE. A novel extensive histopathological and molecular study including T-cell receptor clonality analysis was performed on all samples. Separate assessment of the epithelium and the lamina propria was achieved. All biopsies were blindly reviewed by a human pathologist specialized in gastrointestinal disorders and by a veterinarian pathologist.
Differentiation criteria between feline LGITL and LPE included villous atrophy, lymphocytic cryptitis, depth of infiltration, apical-to-basal gradient of cellularity, nest and plaques identification and fibrosis extent within the lamina propria revealed by Trichrome de Masson’s staining. CD3 and Ki67 expression levels in lamina propria and intra epithelial lymphocytes were validated and significantly increased in LGITL cases as compared with LPE cases. All LGITL were PhosphoStat3- and PhosphoStat5+ in contrast to LPE: thus, dysregulation of the JAK-STAT pathway has been highlighted. This pathway is known to regulate lymphocyte development, differentiation, and proliferation and it has recently emerged as a major oncogenic mechanism in several T & NK leukemia and lymphoma subtypes.
Clonality assessment revealed monoclonal TCRγ rearrangement in 82% of the T-LGIL cases and in 40% of the LPE cases while 30% showed monoclonality in a polyclonal background. Given the clonality results, the current gold-standard tests should still be a combination of conventional histopathology and immunohistochemistry and no equivocal case should be reclassified according to the clonality results.

2- What arguments support a potential *continuum* between LPE and LGITL?

Concurrent LPE has been evoked in up to 60% of LGITL cases and it has also been suggested that LGITL might develop from LPE. A recent prospective study compared the distribution and severity of the lesions within the digestive tract in feline lymphoplasmacytic enteropathy or low-grade intestinal T-cell lymphoma. The results showed that LGITL shows a heterogeneous repartition within the digestive tract. Moreover, the jejunum is systematically involved in this disease. Misdiagnosis is possible if biopsies are only performed by gastroduodenoscopy, supporting the relevance of jejunal surgical biopsies. Furthermore, signs of concurrent inflammation were found in more than 65% of LGITL cases.
Several arguments were pointed out to support a continuum between LPE and LGITL:
- The duration of clinical signs is statistically longer in cats diagnosed with LGITL compared with LPE cases.
- A significant epitheliotropism may be identified in both LPE and LGITL

- LGITL small lymphocytes appear according to an "apical-to-basal" gradient suggesting a chronic endoluminal antigenic stimulation. No LGITL case emerge from the depth of the mucosa.
- "Patchy lesions" and nests are hallmarks of LGITL, even in a polymorphic background
- Persistent cellular polymorphism infiltrates are regularly found in some TLGIL cases
- Only minimal differences are seen between LPE and LGITL intestinal microbiome and there is high similarity with the microbiome dysbiosis seen in human IBD.

A new model of lymphomagenesis has thus emerged and suggests a continuum between inflammatory enteropathy towards low-grade T-cell intestinal lymphoma. In this model, the JAKSTAT pathway activation leads to the recruitment of monoclonal lymphocytes within the intestinal epithelium in LGITL cases.

3- If a corticosteroid treatment is prescribed prior to definitive diagnosis, what would be its impact on LGITL follow-up?

As previously mentioned, a continuum between LPE and LGITL is strongly suspected. These two types of feline chronic enteropathies are known to be highly “corticosteroids responsive”. Some clinicians recommend empirical treatment rather than obtaining biopsies in cats after ruling out other differentials. However, corticosteroid administration prior to final diagnosis (e.g., gastrointestinal sampling) may modify the appearance and the analysis of the GI tract lesions and may alter the response to treatment (chlorambucil) if a LGITL is finally diagnosed. This feature is called “corticosteroid-induced resistance”. Chlorambucil, a nitrogen mustard derivative, is metabolized in the liver to its active metabolite, further metabolized in the liver to other inactive metabolites that are excreted in the feces and in the urine.

To date, there is no supporting evidence that cats with LGITL treated with prednisolone alone will not respond to chlorambucil later. That said, the prognosis of the two diseases is quite different. In a recent retrospective study, previous administration of glucocorticoids and lack of improvement over three first months of treatment were significantly associated with a decreased survival time.

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The process of metastasis, predicating the pattern of neoplastic behaviour

Nicola Read

Oncogenesis is the term for the development of cancer, the name given for the process by which normal, healthy cells transform into cancer cells. Oncogenesis is the result of a complex series of events beginning with a single cell which has acquired malignant properties through cellular DNA damage that leads it to become unresponsive to the highly regulated signalling cascade of the cell cycle. The survival and proliferation of a cell with malignant properties has the potential to create a population of clones with similar genetic errors.

Most literature describes the six hallmarks of cancer as the properties a cell must acquire in order to become cancerous or malignant. This is (i) self-sufficiency to growth signals; (ii) insensitivity to anti-growth signals; (iii) evade apoptosis; (iv) have limitless replicative potential (v) be able to sustain angiogenesis; (vi) the capacity to invade tissues and metastasize

A colony of cells which are multiplying uncontrollably, will require nutrition and oxygen to survive. Some cells develop the ability to secrete growth hormones which stimulates new blood vessel formation that helps strengthen their increasing numbers. These newly formed vessels then have the potential to act as a conduit of spread if these cells then go on to develop an invasive capabilities; this can allow the mutated cells to move to other parts of the body, a process which we know as metastasis. Once a neoplasm goes on to recolonise throughout the body, there becomes further competition for nutrients, leading to exhaustion of the body's regulatory systems and eventual death.

There are three ways in which a tumour can spread to another part of the body: (i) via the lymphatic system known as lymphatic spread; (ii) via the blood, a process known as haematogenous spread, and (iii) translocation or seeding throughout a cavity or tissue body. There is often a pattern of behaviour between the type of neoplasm and its method of metastasis.

Sarcomas are cancers that begin in the mesenchymal (connective, supportive) tissues such as bone, cartilage, fat, muscle, tendons and blood vessels; their route of spread is via the vasculature where they can circulate and recolonise to any other part of the body. Invasive mesenchymal cells usually breach their protective boundary, break off from their primary mass and travel, usually to the lungs, liver, spleen, kidneys, brain and heart where they try to anchor and form metastatic lesions.

Carcinomas are neoplasms of parenchymal (epithelial) tissue which cover the internal organs (digestive, urinary and respiratory tract, the skin, the superficial layers of most organs, the body cavities etc.). Carcinomas also arise from glandular tissue, which then are prefixed as adeno- carcinoma; both carcinomas and adenocarcinomas usually spread by the lymphatic route. Small clusters of cancerous cells penetrate the lymphatic vessels and travel to the sentinel (local) lymph node, where they recolonise, then with the potential to move on to the next lymph node in the chain. Some carcinomas can penetrate the plural and peritoneal cavity and recolonise in large numbers very quickly throughout a tissue layer, this seeding can be naturally or accidentally induced via biopsy or surgery.

Knowing what type of tumour the patient has, together with understanding the process of metastasis helps predict the pattern of neoplastic behaviour; this knowledge allows our veterinarians to plan the staging process, evaluate the burden of disease then present options to the owner on how best to treat their companion.

The importance of nursing in radiotherapy (RT)
Understanding what we do, to best deal with side effects.

Dr. Jérôme Benoit
EBVS European Specialist in Veterinary Diagnostic Imaging and Radiation Oncology

Radiotherapy is one of the three main discipline of oncology, with **surgery** and **chemotherapy**. RT in animals has a long history and an extensive literature.

What is true for humans, also apply to our patients. More and more of our patients will benefit from this modality of treatment which can be used in three settings :

Adjuvant RT (usually after surgery, sometimes before), to kill tumours cells at the level of the surgical margins —> DEFINITIVE « CURATIVE » INTENT / LONG TERM CONTROL

RT alone when tumours are naturally sensitive to radiation (no or little benefit of surgery) —> DEFINITIVE « CURATIVE » INTENT / LONG TERM CONTROL

Palliative RT, when tumours are not resectable and/or already metastatic, and/or possibly associated with a local discomfort —> PALLIATIVE INTENT / SHORT TERM CONTROL

Side effects are part of any radiotherapy course and should be understood by the clinical team and always discussed with the clients. Expected RT induced side effects (to be treated) must be differentiated to RT complications (to be prevented).

Side effects may vary depending on anatomical locations, treatment volumes (size), and the RT protocol (intensity, fractionation, total dose).

The VRTOG grading system for acute and late side effects must be referenced and for each case the grade of side effects must be documented in patients medical files.

Acute side effects
are common and appear within 7-14 days and involve skin (dermatitis) and mucous membranes (mucositis : conjunctiva, GI, gums…). Acute side effects resolve within 2-4 weeks after RT is completed. Treatment usually involves a symptomatic approach :

NSAIDS (or corticosteroids - to be avoided if possible)
Pain killers (tramadol, gabapentine…)
Topical ointments (greasy « keep clean and moist », eg Chlorexhidine and Flamazine)
Buster collar
Esophageal feeding tube

Note that RT for brain tumors is usually associated with a corticosteroid treatment before, during and after RT (at reducing doses) to prevent peritumoral edema.

Late (delayed) complications
are rare and appear 6 months to years after RT. It involves late responding tissues (eg. vessels, nerves, muscles, bones, lens…) and appears as fibrosis, necrosis, and carcinogenesis (new cancer in the RT field). These lesions are usually irreversible and there is no specific treatment in most cases.

General rules to remember :
The higher the radiation doses, the more acute side effect (malignant tumors require more dose than benign tumors)
The shorter the radiation protocol, the more acute side effects (some tumor types respond better to more intensive - shorter courses of RT)
The larger the dose-fraction, the more risk for delayed complications

At an institution where RT IS NOT available, oncology nurses may be in contact with clients whose pet is going for RT or had RT at another centre. It is important to reassure clients about RT indications, results and safety. It is also important to fully participate of the follow-up of RT cases after RT is completed. The aftercare after RT is an important period so clients must be well informed, and local care should be appropriate, so the patient is best supported and complications can be avoided.

At an institution where RT IS available, oncology nurses may already be working as part of the radiation oncology service and nurses and clinicians will be more used of dealing with RT side effects. However, RT clients often travel from far for RT and don't have the option to come back for the recheck visits. In such cases, it is important to keep contact with RT clients and be available for them, the local GP, or the local oncologist (and team) to give advice. Email communication with exchange of photos is usually very useful to assess the severity of side effects and the appropriate treatments.

TABLE 1. VRTOG Acute Radiation Morbidity Scoring Scheme

Organ/Tissue	0	1	2	3
Skin/hair	no change over baseline	erythema, dry desquamation, alopecia/epilation	patchy moist desquamation without edema	confluent moist desquamation with edema and/or ulceration, necrosis, hemorrhage
Mucous membranes/oral cavity	no change over baseline	injection without mucositis	patchy mucositis with patient seemingly painfree	confluent fibrinous mucositis necessitating analgesia, ulceration, hemorrhage, necrosis
Eye	no change over baseline	mild conjunctivitis and/or scleral injection	KCS requiring artificial tears, moderate conjunctivitis or iritis necessitating therapy	severe keratitis with corneal ulceration and/or loss of vision, glaucoma
Ear	no change over baseline	mild external otitis with erythema, pruritis 2° to dry desquamation not requiring therapy	moderate external otitis requiring topical medication	severe external otitis with discharge and moist desquamation
Lower GI	no change over baseline	change in quality of bowel habits not requiring medication, rectal discomfort	diarrhea requiring parasympatholytic medications, rectal discomfort requiring analgesia	diarrhea requiring parenteral support, bloody discharge necessitating medical attention, fistula, perforation
Genitourinary	no change over baseline	change in frequency of urination not requiring medication	change in frequency of urination necessitating medication	gross hematuria or bladder obstruction
CNS	no change over baseline	minor neurologic findings not necessitating more than prednisone therapy	neurologic findings necessitating more than prednisone therapy	serious neurologic impairment such as paralysis, coma, obtunded
Lung	no change over baseline	alveolar infiltrate; cough requiring no treatment	dense alveolar infiltrate; cough requiring treatment	dyspnea

USEFUL READINGS

Radiation therapy in veterinary medicine: a practical review (I. Del Portillo, et al.) Companion Animal Vol. 25, No. 7 Oncology
Small Animal Clinical Oncology, Radiation Oncology (Chapter 13), Withrow and MacEwen's 6th edition
Toxicity criteria of the Veterinary Radiation Therapy Oncology Group (T. Ladue and M. Kay Klein) Vet Rad and Ultrasound, 2001, Vol 42, 5, 475-476

TABLE 2. VRTOG Late Radiation Morbidity Scoring Scheme

Organ/Tissue	0	1	2	3
Skin/hair	none	alopecia, hyperpigmentation, leukotrichia	asymptomatic induration (fibrosis)	severe induration causing physical impairment, necrosis
CNS	none	mild neurologic signs not necessitating more than prednisone therapy	neurologic signs necessitating more than prednisone therapy	seizures, paralysis, coma
Eye	none	asymptomatic cataracts, KCS	symptomatic cataracts, keratitis, corneal ulceration, minor retinopathy, mild to moderate glaucoma	panophthalmitis, blindness, severe glaucoma, retinal detachment
Bone	none	pain on palpation	radiographic changes	necrosis
Lung	none	patchy radiographic infiltrates	dense radiographic infiltrates	symptomatic fibrosis, pneumonitis
Heart	none	ECG changes	pericardial effusion	pericardial tamponade, congestive heart failure
Joint	none	stiffness	decreased range of motion	complete fixation
Bladder	none	microscopic hematuria	pollakiuria, dysuria, hematuria	contracted bladder

Neoadjuvant Radiotherapy in the treatment of Feline Injection Site Sarcoma (FISS)

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Introduction

FISS is an aggressive, infiltrative tumour requiring wide surgical excision. Multimodal therapy increases survival times. Radiotherapy has been utilised in the palliative, neoadjuvant and adjuvant settings. No consensus exists on optimal radiotherapy scheduling. Advantages in delivering radiotherapy prior to surgery include smaller treatment field, potential for tumour shrinkage and increased likelihood of obtaining clean surgical excision.

Materials and methods

Five cats with interscapular FISS measuring 3-7cm diameter, without metastatic disease, underwent advanced imaging, neoadjuvant radiotherapy, and definitive surgical excision. Diagnosis was by incisional biopsy/cytology. When available histology samples were reviewed pre and post radiotherapy. Radiotherapy was delivered on a Monday Wednesday Friday basis, total four fractions over 8-days, median dose 16Gy (range 14-20).

Results

Three cats received 6MV photons, one 15MeV and one 6MeV electrons. At the time of surgery, performed median 15 days (range 10-21) post radiotherapy, all tumours became more mobile and defined, and reduced in size in 4 cases. All cases underwent planned curative intent surgical excision with lateral margins of 3-8cm. Deep fascial margins included muscle and/or bone. Tumour free margins were obtained in all cases. Minor wound breakdown at 10, 16 and 31-days post-surgery required surgical repair in three cats, all healed uneventfully. One cat died of unrelated causes DFI 1493d/OS 1536d. One cat developed tumour recurrence, DFI 1628d/1954d OS. Two cats developed metastatic disease, DFI 541days/OS 585days and DFI 434/OS 465. One cat is alive/disease free, 188d post-surgery.

Conclusions

Neoadjuvant radiotherapy may enhance surgical excision of FISS by improving mobility and definition and reducing size.

Outcome and pattern of failure of a split protocol of RT and CCNU for canine non-visceral histiocytic sarcoma

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Introduction

Histiocytic sarcoma (HS) has a high metastatic potential that requires early chemotherapy administration and is highly sensitive to radiation (RT). The aim of this study is to evaluate the outcome and pattern of failure in dogs with non-visceral HS treated with a split protocol of RT and CCNU chemotherapy.

Materials and methods

Included dogs had non-visceral HS treated at OVC between 2013-2020 with RT (5 x 4 Gy) to primary and regional lymph nodes (LN) +/- distant metastasis followed by 4 doses of CCNU then a second RT protocol (5 x 4 Gy) +/- 2 additional doses of CCNU.

Results

Thirteen dogs were included. At presentation, 8 (62%) had evidence of LN metastasis, 5 (38%) of which also had distant metastasis (spleen (n=2), bone (n=2), lung (n=1)). Overall, 77% (10/13) completed the 2 RT protocols and 4 doses of CCNU. Seven (54%) received all 6 doses of CCNU. All dogs but 1 (alive at day 569) have died. During follow-up: 1 (8%) developed recurrence within the RT treated area, 8 (62%) developed metastasis and 3 died without clear evidence of recurrence or metastasis of HS. The median overall survival (mOS) was 521 days (95%CI: 283-678 days, range: 52-2450 days). The presence of metastasis at presentation was a negative prognostic factor (mOS: 406 days versus 824 days, HR: 4.32 95%CI: 1.28-14.57. p=0.003).

Conclusions

The split RT and CCNU protocol is reasonable for treatment of non-visceral HS even with metastasis as a 60% 1-year survival was found in dogs with metastasis.

Percutaneous cementoplasty as a palliative treatment for dogs with osteosarcoma using a new self-setting bone substitute

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Introduction

Surgical amputation or limb-sparing techniques, in association to adjuvant chemotherapy, are indicated to treat appendicular osteosarcoma; however, these are not always suitable. Percutaneous cementoplasty proposes an alternative for the palliative treatment of primary bone tumors. Our objective is to demonstrate the efficacy and safety of a novel self-setting bone substitute (BIOCERA-VET®OSA) that would provide pain reduction, mobility improvement, a lower risk of pathological fractures, and a better quality of life

Materials and methods

Tumor lesions of 12 dogs were filled percutaneously with the self-setting bone substitute with no other adjuvant therapy. During follow up visits at 1, 2 and 6 months; pain was assessed using veterinary and owner assessment questionnaires (VAS/CBPI). Whilst safety assessment considered the incidence of complications.

Results

Results showed a pain score decrease of at least 50%, at 1-month follow up in 67% of patients; and at 2- and 6-month follow up in 50 % of patients. A slight lower pain reduction was registered on the “pain severity” and on the “pain interference with the function” scores of the CBPI.
Quality of Life was improved in 78% of the dogs at 1 month follow up; and in 50% of the dogs at 2- and 6-month follow up. The cementoplasty technique using BIOCERA-VET®OSA showed a low rate of complications, each reported in different patients: superficial wound infection, swelling, pathological fracture.

Conclusions

Our results demonstrated that percutaneous cementoplasty with a self-setting bone substitute (BIOCERA-VET®OSA) lowered the pain and improved the quality of life of patients with appendicular osteosarcoma.

Pre-operative neoadjuvant vinblastine-prednisolone in canine mast cell tumours: a single-centre retrospective cohort study

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Introduction

Neoadjuvant chemotherapy can be used in canine mast cell tumours (MCTs) to optimise

surgical margins or to enable marginal excision in challenging locations. The objective of this study was to describe the outcome of dogs with cutaneous and subcutaneous MCTs treated with neoadjuvant vinblastine-prednisolone (NA-VP).

Materials and methods

Records of treatment-naïve dogs with cutaneous/subcutaneous MCT that received NA-VP were reviewed including signalment, intent of NA-VP, staging results, clinical response (ClinR), surgical data and histopathology reports. For dogs with post-operative follow-up >365 days, local recurrence (LR) and time to recurrence were evaluated. Possible predictive factors for complete excision (CE) and LR were analysed.

Results

Forty-four dogs were included: 19 (43.2%) received NA-VP to optimise surgical margins (NA-VP-SM) and 25 (56.8%) to enable excision (NA-VP-EX). Clinical response was documented in 18 dogs (40.9%). Thirty dogs (68.2%) underwent surgery (8 wide, 22 marginal): 18/19 (94.7%) following NA-VP-SM and 12/25 (48%) following NA-VP-EX. Five dogs (16.7%) experienced wound dehiscence. Complete excision was achieved in 14 dogs (46.7%). Only intended surgical margins (wide vs marginal, $p<0.01$, OR 17.55, 95%CI 2.53- 364.4) was associated with CE on multivariable analysis; neither ClinR nor mitotic count (MC) was associated with CE. Post-operatively, five of 24 dogs (20.8%) experienced LR after a median of 416 days (range 96-2683). None of the factors analysed predicted LR; notably, LR occurred in 3/11 (27.2%) completely excised MCTs.

Conclusions

In a pre-operative setting, NA-VP appears safe and could be beneficial in some MCTs. Prognostic factors such as ClinR, MC and CE should be interpreted with caution following NA-VP.

Retrospective evaluation of toxicity and response in 29 cancer-bearing cats treated with masitinib mesylate

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Introduction

Off-label use of tyrosine kinase inhibitors is frequently reported as a rescue treatment in cancer-bearing animals. Preclinical data suggested that masitinib mesylate is well-tolerated in healthy cats. The objective of this retrospective study was to report tolerance and response to masitinib mesylate in cats with cancer.

Materials and methods

Medical records of three veterinary hospitals were retrospectively reviewed. Cats with any confirmed cancer and adequate follow-up were included if they had received masitinib mesylate for at least 2 weeks. Toxicity was graded according to the VCOG-common terminology criteria for adverse events and response was measured according to RECIST criteria.

Results

Twenty-nine cats were included. Median dosage was 50mg per cats every other day. Median duration of treatment was 53,5 days. Toxicity occurred in 9/29 (31%) cats. Most adverse events were grade 1 (2 grade-1 vomiting, 3 grade-2 vomiting, 1 grade-1 diarrhea, 2 acute kidney toxicity, 2 grade-1 increased plasma ALT, 1 grade-1 lethargy). On the 24 cats with an available response evaluation, there were 12 progressive disease, 9 stable disease, 2 partial response, and 1 complete response. Objective response rate was 50%. Objective responses were observed in visceral mast cell tumor (1 CR, 2 PR, 1 SD), injection site sarcoma (2 SD), pancreatic carcinoma (3 SD), histiocytic sarcoma (2 SD), and adrenal carcinoma (1 SD). Interestingly, 2/4 (50%) of cats with stage-IV pancreatic carcinoma survived more than 6 **months**.

Conclusions

Masitinib mesylate is well-tolerated in cancer-bearing cats. Study in a larger cohort is mandatory to confirm these preliminary results.

Monitoring treatment response and disease progression in Canine lymphoma using serial plasma nucleosome concentrations

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Introduction

Changes in plasma nucleosome concentrations are reported to predict patient responses to chemotherapy in breast cancer, NSCLC, colorectal cancer and others in humans. Significant changes often precede clinical responses and treatment failures. Plasma nucleosome concentrations have also been shown to be elevated in dogs with lymphoma. The purpose of this study is to determine the utility of serial plasma nucleosome concentrations in predicting treatment response and progressive disease in dogs with lymphoma.

Materials and methods

Serial plasma samples were prospectively collected from 20 dogs with naïve lymphoma throughout treatment and monitoring. Samples were processed and stored at -80C and run in batches. The Nu.Q® H3.1 total nucleosome ELISA and TK1 assays were performed

according to the manufacturer's protocol. Results were plotted over time and medical record information from each case was paired for evaluation.

Results

Complete data sets were acquired on 20 cases. 80% were B cell and 20% were T cell lymphomas. 18 dogs had elevated nucleosome concentrations at diagnosis. The median plasma nucleosome concentration at diagnosis was 224.9 ng/mL and the median plasma nucleosome concentration at best clinical response was 76.48 ng/mL. Plasma nucleosome concentrations for all dogs returned to the healthy range during treatment. The median percent change was -93.9% from diagnosis to lowest recorded value.

Conclusions

Nucleosome concentrations mirrored the clinical response in patients and often increased before clinical detection of progression. Plasma nucleosome concentrations may be a useful tool for monitoring treatment response and disease progression in dogs with lymphoma.

Evaluation of Serum Amyloid A (SAA) and other clinical-pathological variables in cats with lymphoma

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Introduction

Serum amyloid A (SAA) concentrations have shown prognostic value in some feline neoplastic and non-neoplastic diseases, however, the association between serum SAA concentrations and prognosis in cats with lymphoma is unclear.

Materials and methods

Cats diagnosed with intermediate or large cell lymphoma between 2012 and 2021 with SAA data available were included. Associations between tumour site (nasal vs. non-nasal), stage, response to treatment and SAA concentration were evaluated using non-parametric statistics. Associations between SAA concentrations, stage and response to treatment with survival time were evaluated using Cox regression analysis. Patients with nasal tumours and those not receiving high dose chemotherapy were excluded from the survival analysis. Data are presented as median [range].

Results

Thirty-four cats were included. SAA concentrations were significantly higher in non-nasal vs. nasal lymphoma (38 [<0.3-797] µg/mL vs. <0.3 [<0.3-0.9] µg/mL; p=0.024). Responders tended to have higher SAA concentrations than non-responders (108 [<0.3-797] µg/mL vs. 16 [<0.3-255] µg/mL; p=0.07). SAA concentrations did not correlate with tumour stage. Median survival time for the population examined was 60 [2-1728] days. Responders had better survival time than non-responders (817 [89-1728] days vs. 30 [2-169] days; p<0.001) whereas tumour stage and SAA concentrations were not associated with survival time.

Conclusions

SAA concentrations are not a prognostic indicator in patients with lymphoma undergoing chemotherapy, although cats likely to respond to treatment are likely to have higher SAA concentrations at diagnosis. SAA concentrations are elevated in patients with non-nasal lymphoma compared to patients with nasal lymphoma, likely reflecting disease burden.

Safety and efficacy associated with 5 consecutive fractions of 4-5Gy in dogs with intracranial tumours

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Introduction

Definitive or stereotactic radiotherapy (RT) protocols are often used for treatment of canine intracranial tumours (ICTs). These may be declined due to expenses and/or high number of fractions. Palliative protocols using large dose per fraction have been described but are avoided due to risk of radiation brain necrosis. Five fractions of 4Gy is a frequently used palliative protocol for different tumours in dogs but has not been thoroughly evaluated for ICTs.

Materials and methods

Medical records of dogs with ICTs that were treated with IMRT or 3D conformal RT consisting of 5 daily fractions, each of 4-5Gy prescribed to the planning target volume were reviewed.

Results

Eight dogs with ICTs diagnosed based on imaging were included (5 meningiomas, 1 pituitary macroadenoma, 1 glioma, 1 nerve sheath tumours). Median follow up time was 380 days (180-1095 days). Four dogs were euthanatized 253, 401, 754 and 951 days after RT and 5 dogs are still alive. Complete resolution of neurological signs occurred in 7 dogs, and in 1 they remained static. In 4 dogs undergoing repeat advanced imaging, a partial response was detected based on RECIST. Only in 1 dog, steroid responsive early delayed toxicity was suspected.

Conclusions

This protocol appears safe and effective in management of canine ICTs and can be considered when definitive or stereotactic protocols are declined. A prospective study including a higher number of cases and longer follow up to further assess its tolerability and efficacy is warranted.

Choroid plexus tumors treated with radiotherapy alone or combined with ventriculoperitoneal shunt in dogs and cats: a retrospective descriptive case series

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Introduction

Intraventricular choroid plexus tumors (CPT) are rare, optimal treatment is not defined. Symptomatic patients often exhibit life-threatening hydrocephalus. With several months' time-to-effect after radiotherapy (RT), pets are at risk of herniation. This can be overcome by ventriculoperitoneal shunting (VPS).

Materials and methods

Client-owned pets with symptomatic, imaging-presumed CPT treated with definitive-intent RT or VPS/RT were retrospectively included. Duration until normalization of clinical signs, complications, tumor size evolution and outcome were documented.

Results

Eleven pets were included: 1 cat and 5 dogs with single-modality RT; 4 cats and 1 dog with VPS/RT. Neurological worsening was seen in 4/6 pets during single-modality RT and 2/6 died during RT (suspected herniation). All dogs with VPS normalized clinically by the end of RT at the latest; complications occurred in 3/5, all were successfully corrected surgically. Imaging follow-up in 8/11 pets showed marked decrease in tumor volume at one point in all pets surviving RT. Median survival time for RT was 49 days (95%CI:0;361) and 1103 days (95%CI:577;1628) for VPS/RT. Median time-to-progression was 16 days (95%CI:0;159) and 895 days (95%C:634;1156) for each group, respectively. Two pets in the RT group never fully recovered (died at 49 and 276 days), 2 dogs died due to intraventricular metastasis (1 year). In the VPS/RT group, 2 cats showed imaging-progression (30 and 46 months), 1 cat clinical progression (23 months), 1 dog was alive 33 months and 1 cat lost-to-follow-up 14 months after treatment.

Conclusions

VPS led to quick normalization and RT had a measurable effect on CPT. Combination therefore seems beneficial.

Retrospective study of 30 canine presumed intracranial gliomas treated with external radiation therapy (RT) between 2007 and 2018

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Introduction

Therapeutic responses described for RT treated canine gliomas notably vary among studies and clinical benefits of RT are not always clearly distinguished from that of supportive care. The objective of the study was to further document clinical response and survival after RT for intracranial gliomas and identify prognostic factors.

Materials and methods

Thirty cases of client-owned dogs from VSHSD records with presumed intracranial glioma treated with external RT as primary treatment modality between January 2007 and November 2018 were reviewed. Clinical response and tolerance to treatment were retrospectively evaluated using RECIST 1.1 inspired classification and VRTOG classification (grade 0 to 3) respectively. Prognostic factors were identified using log rank test and Cox proportional hazard model for survival and fisher exact test and logistic regression for clinical response.

Results

RT improved clinical status in 76,7 % dogs. Response to medical treatment before irradiation and age ? 8,8 years at diagnosis were significantly associated with a better clinical response to RT.
Overall median survival was 326 days (95% CI: 189-602). Survival prognostic factors were: clinical response to medical treatment before RT and RT protocol intent (definitive versus palliative).

Signs of radiotoxicity were reported in 7/30 dogs. The most common signs included mild oral mucositis (n=4) and mild to moderate transient decline of neurological status (n=3).

Conclusions

In this series, RT of intracranial gliomas proves to be more effective for younger dogs and for dogs able to respond to medical treatment. Our study suggests that priority should be given to definitive protocols where possible.

Combined lomustine / temozolomide-irradiation proves efficacy even in resistant canine glioma cells

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Introduction

Dogs with glioma could profit from combined chemoradiation. The alkylating agents temozolomide and lomustine penetrate the blood-brain-barrier and doses for dogs are established. We evaluated the benefit of lomustine-temozolomide-irradiation in chemo-resistant canine glioma cell lines and treatment-induced molecular changes.

Materials and methods

The canine glioma cell line J3T-BG was exposed to lomustine and temozolomide for a period of four weeks to generate drug-resistant subclones. Clonogenic survival and proliferation assays were used to evaluate single and double-drug combination +/- radiotherapy. Further, western blot and methylation-specific DNA-sequencing was used to investigate changes after long-term drug exposure.

Results

After long-term drug exposure, both subclones show higher IC50 values against lomustine and temozolomide as well as cross-resistance. For the CCNU-resistant cell line, both, single-drug CCNU ($p=0.0006$) and TMZ ($p=0.0326$) treatment combined with irradiation (4Gy) remained effective. The double-drug-irradiation combination reduced the cell survival by 86% ($p<0.0001$), compared to 92% in the parental (non-resistant) cell line. For the TMZ- resistant cell line, only the double-drug combination with irradiation (4Gy) reduced the cell survival by 88% ($p=0.0057$). Single-drug treatment lost efficacy. The resistant cell lines demonstrated higher P-gp expression. MGMT-methylation profile analysis showed a significantly higher methylation rate in 2 and 4 CpG-islands ($p=0.01$-0.04) regarding the promoter and exon1 region, respectively.

Conclusions

In conclusion, the addition of lomustine to temozolomide-irradiation reduces tumour cell survival even in drug-resistant canine glioma cell lines. The mechanism of drug-resistance seems not to be primarily based on MGMT promoter status in the investigated cell lines and may well lie in P-gp upregulation.

Validation of A Novel Non-Invasive Imaging System for Detection of Malignancy in Canine Subcutaneous and Cutaneous Masses Using Machine Learning

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Introduction

Early diagnosis of cancer enhances treatment planning and improves prognosis. A previous study that we performed showed a preliminary proof-of-concept for the HTVet device. This device is a novel artificial intelligence-based thermal imaging system, developed and designed to differentiate benign from malignant, cutaneous and subcutaneous masses in dogs. We showed an accuracy of 88% in 45 dogs with 69 masses. The present study was performed to validate the use of the device in a much larger set of dogs.

Materials and methods

Prospective study from November 2021- February 2022 in private clinics in Israel. One hundred and eight dogs with 195 masses were included (182 benign and 13 malignant). Cytology was done in 182 cases, histopathology in six, and in seven cases both diagnostics were done. Each mass was clipped and heated by the HTVet device. The heat emitted by the mass and its adjacent healthy tissue was automatically recorded using a built-in thermal camera. The thermal data from both areas were then analyzed using an Artificial Intelligence algorithm. Definitive cytology and/or biopsy results were the inclusion criteria and later compared to those obtained from the HTVet system and used to validate the algorithm.

Results

The accuracy, sensitivity, specificity, positive predictive value, and negative predictive value of the system were 76%, 85%, 76%, 20% and, 99% respectively.

Conclusions

This novel system could be used to provide a decision-support tool enabling clinicians to differentiate between benign lesions and those requiring additional diagnostics.

Epidemiological and clinical characteristics of frontal sinus carcinoma in 39 dogs (2001-2021)

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Introduction

Reports on canine frontal sinus carcinomas (FSCs) are scarce. This study describes demographic and clinical characteristics of canine FSC and reports on the clinical experience and overall survival time following treatment with toceranib phosphate (TOC) and meloxicam.

Materials and methods

Retrospective review of medical records of 39 dogs with FSC (2001-2021).

Results

Median age at diagnosis was 10.6 years (range: 6.5-15.4 years). There was a male-to-female-ratio of 2.25:1. The most common breeds were Jack Russell Terriers (JRT) (n=7; 17.9%) and Rottweilers (n=3, 7.7%). Mesocephalic breeds (68.7%) were most commonly affected, brachycephalics accounted for 9.4%. The most frequent signs included facial deformation dorsomedial to the eye (84.6%), pain/head-shyness (41.0%), ocular (20.5%) / nasal (17.9%) discharge, and exophthalmos (12.8%). Duration of symptoms prior to diagnosis varied from a few days to 9 months. There were no neurological signs despite imaging evidence of cerebral invasion in most dogs (71.4%). There was no side predilection; no dog had nodal metastasis, but 10% had metastasis or concurrent neoplasia in the lungs. Tumour types included squamous cell carcinoma (59.9%), adenocarcinoma (10.2%) and unspecified carcinoma (30.8%). Nine dogs were treated with TOC (median 2.8mg/kg eod or 3 times/week) and meloxicam (0.1mg/kg, eod) (TOC-M). Subjective regressions of facial deformity were seen in 7/9 (77.8%) patients. Overall median ST with TOC-M was 191 days (range: 120-434 days).

Conclusions

FSC typically presents with facial deformation, but no overt neurological signs. Male dogs and JRT are overrepresented. The use of TOC-M in FSC appears promising and warrants further prospective evaluation.

Long-term outcome of macroscopic anal sac adenocarcinoma in dogs treated with single-modality definitive-intent radiation therapy

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Introduction

Radiation therapy (RT) can be used as a stand-alone treatment to reduce local/locoregional disease burden in canine anal sac adenocarcinoma (ASAC) where surgery is not possible or declined. Using image-guided intensity-modulated RT, acute toxicities were found minimal in a moderately hypofractionated definitive-intent protocol. Aim of this retrospective study was to assess outcome from single-modality definitive-intent RT for canine macroscopic ASAC.

Materials and methods

We followed dogs irradiated with 12x3.8Gy for macroscopic ASAC. Macroscopic disease was defined as either primary tumor and/or overt lymph node metastasis. Veterinary Radiation Therapy Oncology Group radiation toxicity criteria were used to assess radiation toxicities.

Results

Thirteen dogs with macroscopic disease were included, 6 had previously undergone surgery. Patients presented as follows: stage 1 (n=1), stage 2 (n=2), stage 3a (n=6) and stage 3b (n=4), (Polton et al.). Mean follow-up time was 562 days (range: 392-735 days). Six dogs developed progressive disease with a median time to progression of 908 days (95%CI: 388; 1428). Disease progression was local in 1, locoregional in 1, local and distant in 1, local, locoregional and distant in 1, and distant in 2 dogs. Median overall survival time was 597 days (95%CI: 351; 843) with 8/9 tumor-unrelated deaths. Late toxicities involved the skin, anal mucosa and lower gastrointestinal tract and were mild with alopecia, pigmentation change, dry desquamation, and rectal discharge (all grade 1). No genitourinary or spinal cord complications were detected.

Conclusions

In conclusion, canine macroscopic ASAC have a favourable outcome after single-modality, moderately hypofractionated RT.

Clinical validation of a multi-cancer early detection blood-based “liquid biopsy” test in dogs using next-generation sequencing

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Introduction

Cancer is the leading cause of death in dogs; however, no established screening paradigms exist for early detection. Liquid biopsy methods that interrogate cancer-derived genomic alterations in cell-free DNA fragments in blood are being adopted for early cancer detection in human medicine and are now available for use in veterinary medicine. Early detection of cancer in dogs may lead to improved outcomes.

Materials and methods

Blood samples from an all-comers cohort of 352 cancer-diagnosed dogs and 524 presumably cancer-free dogs were subjected to DNA extraction, proprietary library preparation, and next-generation sequencing. Sequencing data were analyzed using an internally developed bioinformatics pipeline, previously established in an independent cohort, to detect genomic alterations associated with the presence of cancer.

Results

In an all-comers cohort of cancer-diagnosed subjects, the overall test sensitivity was 55%. In a subgroup of cancer-diagnosed subjects with three of the most aggressive canine cancers (lymphoma, hemangiosarcoma, osteosarcoma), the detection rate was 85%; and, in eight of the most common canine cancers, the detection rate was 62%. The specificity of the test was 98.5%, corresponding to a 1.5% false positive rate. At least 2 presumably-cancer free dogs in the study received a Cancer Signal Detected result and were diagnosed with cancer after undergoing confirmatory cancer evaluations (6- and 7-months following collection of the blood samples, respectively).

Conclusions

A novel, multi-cancer early detection (MCED) liquid biopsy test has demonstrated the ability to identify cancer-associated genomic markers (in some cases months prior to the onset of clinical signs) in canine patients.

A living biobank of canine mammary tumors organoids enables genetic modifications and drug testing to better understand disease heterogeneity

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Introduction

Canine mammary tumors (CMTs) are the second most common neoplasia in dogs and for metastatic disease the treatment options are limited. Moreover, we have a poor understanding of the underlying biology that drives the growth of the heterogeneous CMTs. To facilitate functional analyses of essential factors driving the disease and testing of new therapeutic options, we aimed to establish a living biobank of CMTs using 3D organoid cultures.

Materials and methods

CMTs and non-neoplastic mammary tissue were collected from client-owned dogs undergoing mastectomy and cultured under organoid-forming conditions. Morphology and different molecular markers (vimentin, cytokeratines, p63, Ki67, HER2, estrogen and progesterone) were compared between the primary tissue and the organoids. Whole-genome sequencing and single nucleotide polymorphism genotyping were used for genetic characterizations. Genetic modification was conducted using the CRISPR-Cas9 technology. Cytotoxicity of carboplatin, cisplatin, doxorubicin and alpelisib was evaluated by CellTiter-Blue® viability assays.

Results

We established a long-term culture of 24 organoid lines from 17 patients, derived from carcinoma, adenoma and non-neoplastic mammary tissues. CMTs organoids capture the heterogeneity of the disease and recapitulate key histological and immunohistological features. Genetic characteristics including driver gene mutations affecting PIK3CA or KRAS are conserved. Moreover, we developed protocols to genetically modify CMTs organoids and to use them for drug testing.

Conclusions

We present a robust 3D in vitro model to get a better functional understanding of CMTs and to test personalized treatment options. This provides a new angle to study the molecular basis of canine mammary tumorigenesis and to find novel therapeutic targets to be exploited.

The mitotic regulator Polo-like kinase 1 as a potential therapeutic target for c-Myc-overexpressing canine osteosarcomas

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Introduction

Osteosarcoma is the most common primary malignant bone tumour in dogs and is characterized by an aggressive behaviour and poor patient's prognosis. Polo-like kinase 1 (PLK1) is dysregulated in various human cancers, including osteosarcoma, and induces c-Myc accumulation. The crosstalk between PLK1 and c-Myc coordinates cell proliferation, differentiation and apoptosis, and overexpression of both genes is associated with disease progression.
The aim of this study was to evaluate the potential prognostic role of c-Myc and PLK1 in canine osteosarcoma and to investigate the effects of selective PLK1 inhibition in vitro.

Materials and methods

Immunohistochemistry for PLK1 and c-Myc was performed on 53 canine appendicular osteosarcomas. A c-Myc and PLK1-overexpressing canine osteosarcoma cell line (D17) was

treated with BI 2536 for 24h (range 2.5-15 nM) to evaluate the in vitro effects of PLK1 inhibition by viability and apoptotic assays, q-RT-PCR, Western Blot, and flow cytometry.

Results

By immunohistochemistry, c-Myc overexpression was associated with a significantly reduced overall survival (P=0.003). No further correlations were identified for both PLK1 and c-Myc. Western Blot and RT-qPCR revealed that D17 expressed high protein and transcript levels of both PLK1 and MYC. When treated with BI 2536, D17 showed a substantial decrease in cell growth, inducing apoptosis and G2/M cell cycle arrest. Interestingly, under BI 2536 treatment, D17 showed decreased c-Myc protein levels.

Conclusions

Consistent with human osteosarcoma, these preliminary data outline the prognostic value of c-Myc expression in canine osteosarcoma and highlight the potential role of PLK1 as an antiproliferative therapeutic target for tumours overexpressing c-Myc.

Corticosteroids reduce interferon-gamma production in atezolizumab treated peripheral blood mononuclear cells of cancer bearing dogs

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Introduction

Immunotherapy with immune checkpoint inhibitors (ICI) is a promising treatment approach for canine cancer patients. Corticosteroids are known to have an immunosuppressive effect and to blunt efficacy of anti-PD-1/PD-L1 immune checkpoint inhibitor therapy in humans. As dog-specific marketed ICI are not yet available, atezolizumab, a cross functional anti-PD-L1 ICI, was used in this study. We investigated the functional in vitro response of peripheral blood mononuclear cells (PBMCs) derived from healthy and corticosteroid treated or corticosteroid naïve cancer-bearing dogs to PD-L1 blockade.

Materials and methods

PBMCs were collected from healthy, as well as cancer-bearing untreated and corticosteroid pre-treated dogs. 2x105 cells were polyclonally stimulated in biological triplicates with Staphylococcus Enterotoxin B and 10µg/ml atezolizumab or durvalumab (isotype control). Analyzed was fold change in IFN-gamma production (ELISA) compared to non-cross-reactive durvalumab.

Results

Checkpoint blockade leads to higher IFN-gamma output in PBMCs isolated from corticosteroid-naïve dogs (n=36; fold change 1.21 +/- 0.30) than in treated dogs (n=20; fold change 1.06 +/- 0.49) and even higher IFN-gamma production in healthy dogs (n=14; fold change 1.49 +/- 0.36; p=0.039 vs corticosteroid naïve dogs, p=0.015 vs treated; Welch's ANOVA, Games-Howell post hoc test).

Conclusions

Prior corticosteroid exposure dampens anti-PD-L1 induced IFN-gamma production in PBMCs of cancer bearing dogs, yet with considerable variability. Compared to healthy donors also cancer derived immunosuppression appears to reduce IFN-gamma production. Further analysis of patient and immune cell subsets is warranted and together with data from prospective studies could be used for future treatment decisions for anti-PD-1/PD-L1 immune checkpoint inhibitor in dogs.

Oncolytic virus therapy using genetically engineered Vaccinia virus encoding FCU1 protein in dogs diagnosed with malignant solid tumours

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Introduction

TG6002 is an oncolytic Vaccinia virus (VACV) expressing FCU1 protein which converts 5-fluorocytosine into 5-fluorouracil. Its oncolytic potencies rely on lytic replication, targeted chemotherapy and reversion of immunosuppressive tumour microenvironment. Objectives were to assess viral replication, 5-fluorouracil synthesis, tumour microenvironment modifications and response to treatment in dogs with malignant tumours.

Materials and methods

Thirteen dogs (7 soft tissue sarcomas, 2 mammary adenocarcinomas, 2 urothelial carcinomas, 1 mammary sarcoma, 1 colic adenocarcinoma) received three, weekly, intratumoral injections of TG6002 (5.10^6-5.10^7 PFU/kg) with oral 5-fluorocytosine. Viral genome was assessed by qPCR in blood and tumour biopsies. Serum and intratumoral 5-fluorouracil concentrations were measured by high-resolution mass spectroscopy.

Histological and immunohistochemical (CD3, CD8, FOXP3) analyses were performed on tumour biopsies at days 0 and 38. Antiviral immune response was monitored. Response to treatment was assessed by computed tomography scan after 38 days.

Results

Viral genome was detected in blood (7/13) and tumour biopsies (4/11). Viral replication was confirmed for 6 dogs. Median intratumoral concentration of 5-fluorouracil was 314 pg/mg (5 biopsies). 5-fluorouracil was not detected in blood (13/13). Increase in necrosis (6/8), downregulation of regulatory T lymphocytes (5/5) and upregulation of CD3 (2/4) and CD8 (1/5) lymphocytes were noticed in tumour biopsies. Anti-VACV (10/13) and neutralizing (12/13) antibodies were detected from day 7 with maximal titers at day 21. Among 11 dogs assessed at day 38, 1 partial response, 7 stable diseases and 3 progressive diseases were noticed.

Conclusions

This study confirms replicative properties, targeted chemotherapy and reversion of immunosuppressive tumour microenvironment in canine tumours treated with TG6002

HER-2 CAR-TILs as adjuvant treatment in dogs with spontaneous high grade and high stage malignancies

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Introduction

Chimeric antigen receptors (CARs) are recombinant receptors for tumor antigens, which redirect the specificity and function of T-lymphocytes. Here we generated CAR-T cells using a CAR binding the common tumor antigen HER-2 and autologous tumor infiltrating lymphocytes (TILs) from companion cancer bearing dogs with poor prognosis. To investigate feasibility and tolerability of these so-called CAR-TILs we treated four companion dogs.

Materials and methods

Cases were selected by routine staging and diagnostics at the University Animal Hospital in Uppsala. HER-2 expression was confirmed by immunohistochemistry. Two dogs with squamous cell carcinoma and two with malignant melanoma were recruited. Cytoreductive surgery was performed and TILs extracted, expanded ex vivo and equipped with HER-2 CAR construct in vitro. Dogs were pretreated with toceranib and then received a tolerance dose of one million CAR-TILs followed by a 10-100 fold higher dose two weeks later. The two first dogs received CAR-TILs alone; the remaining two dogs received daily sc injections with interleukin 2 (IL-2) following their second CAR-TILs. Toxicity was recorded according to the VCOG-CTCAE v 1.1 and tumor response was monitored.

Results

CAR-TILs treatment had minimal toxicity. IL-2 treatment required transient dose reduction due to gastrointestinal toxicity. Two dogs died from tumor progression, one died of ruptured pyometra and one is still alive.

Conclusions

Even if response was not a primary endpoint in the study, it is notable that one dog with stage 3 melanoma was still in remission >370 days after initial diagnosis. CAR-TILs therapy is therefore a promising therapy in dogs, and perhaps humans, with aggressive cancer.

Immunohistochemical evaluation of cyclooxygenase-2 expression in feline nasal epithelial tumours

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Introduction

Cyclooxygenase-2 (COX-2) is upregulated in some human, canine, and feline tumours. In cats, COX-2 expression has been reported in epithelial tumours such as oral squamous cell carcinomas, transitional cell carcinomas, and mammary carcinomas. However, data regarding COX-2 expression in feline nasal epithelial tumours are scarce. The purpose of this study was to detect by immunohistochemistry COX-2 expression in nasal epithelial tumours in cats.

Materials and methods

Formalin-fixed, paraffin-embedded biopsy samples from feline epithelial nasal tumours were retrospectively assessed for COX-2 expression by immunohistochemistry. Biopsies from cats previously treated with non-steroidal anti-inflammatory drugs were excluded. Immunohistochemistry was performed with a monoclonal rabbit antibody. Feline renal macula densa was used as positive control. Immunoreactive score was determined as the product of a semi quantitative estimation of immunolabelled neoplastic cells (0: no positive cells, 1: <10%, 2: 10-30%, 3: 30-50%, 4: >50%) and intensity of labelling (0: negative, 1: weak, 2: moderate, 3 intense). Scores from 0-1 were considered as negative, 2-3 as low, 4-8 as intermediate, and greater than 8 as high COX-2 expression.

Results

Nasal biopsies from 18 cats were included (9 adenocarcinomas, 7 carcinomas, 1 squamous cell carcinoma, 1 mucinous carcinoma). COX-2 immunoreactivity was negative for all samples.

Conclusions

Although overexpression of COX-2 has been found in a high proportion of canine nasal epithelial tumours, these results suggest a lack of COX-2 expression in feline nasal epithelial tumours. Additional studies, such as evaluation of PDGFR or VEGFR expression, are justified to investigate relevance of other treatments as alternative to radiotherapy.

Use of single agent Lomustine as first-line treatment in canine high grade multicentric lymphoma

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Introduction

Multiagent chemotherapy is considered the most effective treatment for canine high-grade lymphoma; however, due to cost and time requirements, single agent protocols have also been described.
Previously reported median survival time (MST) in 17 canine multicentric lymphomas of unknown immunophenotype receiving Lomustine combined with Prednisone was 112 days.
The aim of our study was to evaluate the outcome of canine high- grade B and T-cell multicentric lymphomas treated with Lomustine and Prednisolone as first line treatment. Identification of prognostic factors contributing to response to treatment was of secondary interest.

Materials and methods

Cases of high-grade lymphoma treated with Lomustine and Prednisolone were included in the study. Response to therapy, time to progression (TTP), disease free interval (DFI) and MST were retrospectively described.

Results

Twenty-five cases were included. Twelve (48%) were T cell, 9 (32%) were B, in 4 (16%) the immunophenotype was unknown. Complete remission (CR) was identified in 11 cases (44%), the DFI for patients achieving CR was 130 days, the TTP was 46 days and the MST was 143 days. Stage and substage were significantly associated with MST while hypercalcemia was significantly associated with DFI.

Conclusions

Dogs with high-grade multicentric lymphoma treated with Lomustine and Prednisolone have lower response rates, DFI, TTP and MST compared to dogs receiving multiagent protocols. This protocol is not a replacement for multiagent chemotherapy; however, it could be considered an alternative if time and cost are factors, providing therapeutic benefit greater than Prednisolone alone.

CT-Guided microwave thermal ablation and cementoplasty as a part of the management of appendicular osteosarcoma in a dog

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Introduction

Appendicular osteosarcoma is a common cancer in dogs. The combination of local control (amputation, limb-sparing surgery or radiation therapy) and adjuvant chemotherapy is considered the standard of care. The objective of this case-report was to describe the feasibility and the clinical outcome of a dog with stage-1 osteoblastic appendicular osteosarcoma treated with the combination of microwave ablation (MWA) and cementoplasty.

Materials and methods

A 14-gauge MWA antenna (ceramic, 200 mm, ECO system) was inserted using computed tomography (CT) guidance in the lesion of the left distal radius. Three ablation cycles of 5 minutes (60 W) were performed using a Saberwave ECO-200G generator (frequency 2,45 GHz). Following the MWA procedure, bone cement (Bioceravet Osteosarcoma, Theravet, Belgium) was injected to consolidate the ablated zone. Adjuvant chemotherapy consisting in 6 doses of carboplatin, and an autologous vaccine were prescribed. Response was evaluated according to RECIST criteria by CT evaluation at 2 months and at 7 months after the initial treatment.

Results

24-hours after MWA, the dog was pain-free and no side effects of the treatment were identified. CT measurements revealed a decreased by 13 % and 25% of the longest diameter of the osteolytic lesion at 2-months and 7-months respectively, corresponding to stable disease according to RECIST criteria. Even if a pathologic fracture of the treated area was diagnosed three months after the MWA, the quality of life of the dog remains excellent.

Conclusions

The combination of MWA and cementoplasty could potentially represent a new limb-sparing option, as a part of multimodal management of appendicular osteosarcoma in dogs.

Intratumoral viroimmunotherapy for treatment of canine intracranial hemangioma

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Introduction

Intracranial hemangiomas are rare tumors in dogs. The gold standard treatment in humans is the total resection, and it has also been reported in a dog as a possible curative therapy. If total resection is not achieved, prognosis worsens due to tumor progression. Use of oncolytic virus has shown promising results in non-surgical tumors. We describe the use of canine oncolytic adenovirus ICOCAV15 in a dog with an intracranial hemangioma in which total resection was not possible.

Materials and methods

A routine craniectomy for parcial resection of a frontal intra-axial hemangioma with intratumoral inoculation of ICOCAV15 was performed in a dog. After 18 months, due to tumor progression, a second inoculation was performed. In both procedures tumor samples were obtained to analyze CD3, IBA1 and Calprotectin by immunohistochemistry. Hemogram, biochemistry and flow cytometry from blood samples were analyzed to ensure safety and to quantify the immunophenotypes. Follow-up by magnetic resonance imaging was performed to evaluate tumor progression.

Results

No signs of life-threatening consequences derived from the treatment were detected. Tumor sample obtained at the second intervention showed higher infiltration of CD3. According to RAVNO criteria the dog presented a stabilization of the disease for 18 months, followed by a tumor progression. Currently, after two inoculations of ICOCAV15, the canine patient is stable after 26 months from the initial diagnosis.

Conclusions

The increased immune infiltration, long survival time and the lack of secondary effects suggest that oncolytic adenovirus ICOCAV15 should be further explored as a novel therapy for canine intracranial hemangiomas.

Toceranib phosphate (Palladia®) treatment in a hyperthyroid cat with mediastinal metastasis from a thyroid carcinoma associated with chylothorax

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Introduction

Feline thyroid carcinoma (TC) causing hyperthyroidism is uncommon (~2%) but highly metastatic. Cardiomyopathy, vena cava thrombus, compressive effect and metastasis can cause chylothorax in feline TC. Toceranib phosphate (TP) has demonstrated clinical benefit

(CB) in canine TC. This case report describes CB to TP treatment in a hyperthyroid cat with TC, mediastinal metastasis and chylothorax.

Materials and methods

The patient was previously diagnosed with hyperthyroidism, controlled during 1 year with dietary management (y/d Hill's). Presented initially with tachypnea and attenuated respiratory and cardiac sounds. Thoracic and cardiac imaging revealed pleural effusion secondary to a mediastinal mass. CT showed a left thyroid gland mass (1.6cm), left cervical lymph node enlargement (1cm), mediastinal mass (4.3cm) and a right hepatic nodule (1.2cm). Fine needle punction showed a neuroendocrine population compatible with TC. T4 was elevated 5.1 µg/dL (0.8-4.7).

Treatment included TP (2.75mg/kg 3 times per week) and thiamazole (2.5mg BID). Complete bloodwork, physical exam, T4, UPCR, arterial pressure and thoracic X-rays were performed every 3 months and CT every 6 months.

Results

A CT scan 4 weeks after starting treatment showed stable disease while chylothorax and clinical signs resolved. T4 normalized 3.8 µg/dL (0.8-4.7) and has remained within normal limits. No adverse events were described and owners reported good quality of life. Chylothorax was controlled during 500 days with the patient alive at 590 days.

Conclusions

This case provides the first description of CB to TP in a mediastinal metastatic TC causing chylothorax suggesting therapeutic potential for feline TC.

Palliative treatment of primary bone tumours in dogs: a case series of 6 dogs treated with bedinvetmab, zoledronate and analgesics

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Introduction

Primary bone tumours in dogs can be treated with surgery or palliation. Palliative treatment is aimed at controlling cancer-induced bone pain with radiotherapy, bisphosphonates and analgesia (NSAIDS, opioids). Pain control is a well-documented challenge. The life-limiting factors of palliative treatment are uncontrolled pain and neoplastic progression, leading to euthanasia.

The most frequent primary bone tumour in dogs is osteosarcoma which expresses nerve growth factor (NGF), contributing to nociceptor activation and central sensitisation. Binding of NGF to tyrosine kinase receptors (TrKA) promotes pain, osteoclastogenesis, bone reabsorption and inhibits cellular apoptosis; blockade of NGF can control tumour-induced pain and potentially have an anti-tumour effects as shown by in vitro and in vivo studies. Bedinvetmab is a canine-specific monoclonal antibody which targets excessive NGF.

Materials and methods

Six dogs with primary bone tumours were treated with zolendronate (0.1-0.2mg/kg every 4 weeks), bedinvetmab (0.5-1.0 mg/kg every 4 weeks) and additional analgesia (ketamine, NSAIDs, paracetamol, amantadine and gabapentin). One dog received additional chemotherapy.

Results

Dogs achieved clinical benefit with the aforementioned protocol with noticeable improvement in their quality of life for a median time of 118 days. Three dogs are still alive with an optimal quality of life 2, 9 and 11 months after diagnosis.

Conclusions

These preliminary results suggest an advantage of bedinvetmab inclusion for dogs that cannot receive treatment with surgery and/or radiation therapy for financial or medical reasons. All dogs showed an improvement in quality of life and a subset of dogs achieved prolonged survival compared to previously investigated palliative treatments.

Clinical presentation of frontal sinus squamous cell carcinoma in the dog and response to treatment with radiation therapy

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Introduction

Primary frontal sinus squamous cell carcinoma (SCC) is a very rare neoplasm both in people and in dogs. In dogs, frontal sinus SCC is mainly described as an extension of nasal SCC. The treatment of choice for dogs with nasal SCC is radiotherapy with or without surgery. There are no reports in veterinary literature on the treatment and outcomes in dogs with frontal sinus squamous cell carcinoma treated primarily with radiotherapy.

Materials and methods

Medical records of dogs with diagnosis of frontal sinus squamous cell carcinoma were reviewed. Data collected included signalment, presenting complaint, clinicopathologic and diagnostic imaging findings, treatment, therapeutic response, date of death or last follow-up

Results

Six cases of primary sinus SCC in dogs were treated by means of radiotherapy. Three of the dogs were treated with a coarsely fractionated protocol with one dog alive at the time the study was performed, 18 months after completing the treatment. The second and the third of these dogs survived 18 and 6 months from the time of diagnosis. Three further dogs were treated with a more fractionated protocol (Monday-Wednesday-Friday schedule). These dogs survived 2.5, 4.5 and 7 months after completing the radiation course.

Conclusions

Despite the small number of cases and variation in the radiation protocols used, the treatment outcomes in these six dogs suggest that radiation therapy is a viable treatment option for dogs with primary frontal SCC, and that coarse fractionation might be an appropriate approach if more fractionated protocols are not possible.

Preliminary results of vitamin D receptor expression in canine haemolymphatic neoplasms

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Introduction

In human medicine, it has been described how some of the cells involved in haematological malignancies highly express the vitamin D receptor (VDR). Thus, it has been suggested that the VDR could be used as a therapeutic target. However, to date, there is no information on VDR expression in canine lymphomas and leukemias.

Materials and methods

Animals suspected of having lymphoma or leukaemia were evaluated by flow cytometry with the following panel:

CD34/CD45/CD21/CD3/CD5/CD4/CD8/MHCII/Ki67/VDR

Peripheral blood from healthy dogs was used as a control.

Results

VDR expression was evaluated in 30 dogs: 22 B-cell lymphomas (22/30; 73.3%), with an average percentage VDR expression on CD21+ of 64.72% (+/- 24.88%) and 78.17% (+/- 20.59) on CD5+ cells ; 3 T-cell lymphomas (3/30; 10%), VDR expression on CD5+ of 59.60% (+/-23.28%) and 64.25% (+/- 0. 35%) on CD21+; 2 acute leukemias, (2/30; 6.67%) VDR expression on CD21+ of 91% (+/- 11.46%) and 99.2% (+/-0. 57) in CD5+ cells; 2 chronic leukemias (6.67%), CD21+ VDR expression of 58.85% (+/- 57.91%) and 86.7% in CD5+ cells; 1 reactive lymphadenitis (1/30; 3.3%), CD21+ VDR expression of 20.4% and 10.5% in CD5+ cells.

Conclusions

All animals with neoplastic processes showed elevated VDR expression in both tumour cells and non-tumour lymphocytes present in the sample. However, both peripheral blood lymphocytes from healthy dogs, used as controls, and reactive processes did not show significant VDR expression. However, further studies with a larger number of animals, especially with a larger number of controls, are needed to evaluate the role of VDR in canine haemolymphatic neoplasms.

Metronomic chemotherapy in dogs and cats with malignant neoplasms, a retrospective study of 78 clinical cases

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Introduction

Metronomic chemotherapy (MTQ) which consists of administering low doses of chemotherapeutic compounds, continuously and for large periods of time, have been arise as a new paradigm in cancer treatment in veterinary oncology. We aimed to evaluate the MTQ effects in dogs and cats, on toxicity, tumour response and survival time.

Materials and methods

48 dogs and 30 cats diagnosed with spontaneous malignant neoplasms of several histological types (mammary, prostatic, hemangiosarcoma, osteosarcoma, fibrosarcoma, amongst others), treated with cyclophosphamide (10 to 15 mg/m2) or chlorambucil (4 mg/m2), alone/combined with Cox-2 inhibitors or toceranib, between May 2016 to August 2021, were included.

Results

In general, MTQ was well tolerated and was shown to be beneficial in increasing the survival time of patients (368 days for dogs and 511 days for cats). However, it did not demonstrate efficacy in stabilizing the neoplastic disease, compared to what is described in the veterinary literature. In dogs, after six months of treatment, 42.1% (n=16) achieved progressive disease, 31.6% (n=12) discontinued treatment 15.8% (n=6) achieved stable disease, 5.3% (n=2) became disease-free and 5.3% (n=2) died. Regarding cats, 51.9% (n=14) achieved progressive disease, 11.1% (n=3) discontinued treatment, 3.7% (n=1) achieved stable disease and 33.3% (n=9) became disease-free (cases of adjuvant MTQ in mammary tumor cases).

Conclusions

From the present work, it is concluded that MTQ is easy to apply, with low associated toxicity and well tolerated by owners and pets. However, more research is needed to elucidate its clinical benefit and to define specific therapeutic guidelines, making its use more frequent, methodical and safe.

Plays size a role? Tumor diagnoses in giant, standard, and miniature schnauzers

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Introduction

Predispositions of squamous cell carcinomas (SCC) and melanomas are well known in giant schnauzers (GS) and standard schnauzers (SS), but not in miniature schnauzers (MS) indicating an influence of size within the schnauzer breeds. The aim of the study was to compare the frequencies of histopathological tumor diagnoses in GS, SS, and MS.

Materials and methods

Data sets from histopathological routine diagnostics submitted to LABOKLIN GmbH & Co. KG (2016–2019) were analyzed retrospectively. Included were samples from 1,276 schnauzers (631 GS, 378 SS, and 267 MS) from which final tumor diagnosis was reported.

Results

Benign neoplasms were significantly more common in MS than in GS (p=0.001).
The most common diagnoses in GS were SCC (22%), melanocytic tumors (19%), benign skin/ hair follicular tumors (BSHFT,16%), and mammary tumors (10%). SS had 30% SCC, 12% mammary tumors, 10% BSHFT, and 4% melanocytic tumors. MS showed 21% mammary tumors, 11% BSHFT, 9% melanocytic tumors, and 3% SCC.
There were various significances in the frequency of distinct neoplasms in the size variants of the schnauzer breeds. For example, GS and SS showed significantly more SCC than MS (p<0.001). MS developed more often mammary tumors than SS (p=0.001) and GS (p<0.001). Melanocytomas and melanomas were more often reported in GS than in MS (p=0.004 and p=0.037) or SS (p=0.023 and p<0.001).

Conclusions

Frequency of tumors of GS, SS, and MS vary. Within the group of schnauzers, size and/or genetic background may have an impact on tumor occurrence. Further studies are needed for more detailed evaluation.

Estrogen receptor alpha expression in different canine lymphoma subtypes

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Introduction

Intact female dogs are believed to be less susceptible to lymphoma than other gender groups. Interaction of hormones and estrogen receptors (ERs) might play a key role in that phenomenon. This study aimed to show ER alpha (ERa) expression on lymphoma cells depending on the phenotype and indirectly to determine the correlation between ERa expression and gender status.

Materials and methods

54 samples of lymph nodes or peripheral blood from canine patients with confirmed hematopoietic malignancy were analyzed by flow cytometry (FC) with a standard diagnostic panel and recombinant Anti-Estrogen Receptor alpha antibody [E115] (PE) was evaluated on neoplastic cells, in two separate research centers in Poland and Italy. Based on the results of FC cases were grouped in B-cell lymphomas (n=43), T-cell lymphomas (n=9) and acute lymphoblastic leukemias (n=2).

Results

The medium percentage of ERa positive cells was 60.9±22.0 for BCL (n=24) and 51.8±12.0 for TCL (n=4). No ALL case tested positive. 32 cases were further evaluated in terms of sex and gender status. Among BCLs 66.7% (n=6) of spayed and no intact female cases expressed ERa. Regarding males, ERa expression was demonstrated in 50.0% (n=2) of neutered and 44.4% (n=4) of intact BCLs male cases. Among TCLs 50% (n=2) of spayed and an intact female patient demonstrated expression of ERa, whereas 50% of neutered and an intact male patient were negative.

Conclusions

Different lymphoma subtypes may vary in terms of expression of ERa with a prevalent expression in B cell lymphoma. The possible meaning of ER in terms of prognosis and therapy remain to be elucidated.

Clinical response evaluation of oral melanomas treated with electrochemotherapy

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Introduction

Oral melanoma (OM) is the most frequent malignant neoplasm in canine oral cavity. Surgery and radiotherapy are the main treatments and other approaches have so far shown little efficacy. Electrochemotherapy (ECT) consists of the combined application of electrical pulses together with a cytotoxic agent, thus achieving high intracellular concentrations.

Materials and methods

Nineteen dogs with OM were included in this retrospective study, diagnosed by biopsy (57.9%; 11/19) or cytology (42.1%; 8/19). All dogs were treated with ECT using intravenous bleomycin (15000 UI/m2). Fourteen dogs (73.7%; 14/19) received just one ECT treatment, and 5 two treatments (26.7%; 5/19) separated by 30 days.

Results

Nine were in mandible (47.6%; 9/19), 7 in maxilla (36.8%; 7/19), 2 in tongue (10.5%; 2/19) and 1 in lip (5.3%; 1/19). Two dogs had clinical stage I (10.5%; 2/19), 3 stage IIa (15.8%; 3/19), 3 stage IIb (15.8; 3/19), 4 stage IIIa (21%, 4/19), 6 stage IIIb (31.6%; 6/19). Clinical response was evaluated one month after the first treatment. Eight dogs showed CR (42%; 8/19: 100% stage I, 50% stage IIa, 66% stage IIb, 25% stage IIIa, 33.3% stage IIIb), 9 PR (47.4%, 9/19: 25% stage IIa, 33.3% stage IIb, 75% stage IIIa, 66.6% stage IIIb), 1 PD (5.3%; 1/19: stage IIb) and 1 dog was lost. All dogs at stage I remain alive at the time of writing this abstract.

Conclusions

ECT looks a good palliative treatment for OM when surgery or radiotherapy is not possible, showing a good response, especially in early stages. However, prospective studies are needed.

A case report of feline nephroblastoma treated with nephroureterectomy and adjuvant chemotherapy

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Introduction

Nephroblastoma is a congenital renal neoplasm, uncommonly reported in cats, characterized by aggressive behavior and short survival times. To the best of authors' knowledge, adjuvant chemotherapy has never been reported.

Materials and methods

A 15-month-old neutered female Highland Straight cat was presented for the accidental finding of a large painless abdominal mass in the right lumbar region, compatible with right nephromegaly associated with no symptoms. Bloodworks and urinalysis were unremarkable. Abdominal ultrasound revealed a 46.5x53.4mm mass with irregular vascularization and inhomogeneous echo-structure in the right renal pelvis, associated with a thrombus in the right renal vein running inside the caudal vena cava (CVC) for 16mm suggesting a renal tumor associated with CVC invasion.

Results

A nephroureterectomy and caval thrombectomy was performed. The histopathological diagnosis was nephroblastoma. A post-surgery total-body CT revealed the persistence of a 12mm thrombotic lesion in the CVC. Five actinomycin-D (0.4-0.5 mg/m2/3weeks) were administered. Five months after surgery, a recheck CT revealed disease progression with a 12x9x22mm mass with inhomogeneous enhancement involving the CVC wall. Owner declined further therapy. Nine months after diagnosis, the cat was euthanized for sudden restrictive dyspnea associated with chylous pleural effusion. Necropsy showed a large mass involving retroperitoneal fat, CVC and the right lateral lobe of the liver and a 5mm metastatic nodule of the left lateral hepatic lobe. No thoracic metastases have been found.

Conclusions

This case report confirms the aggressive behavior and poor prognosis of feline nephroblastoma. Adjuvant chemotherapy with actinomycin-D after nephrectomy could improve survival in cats with advanced stage nephroblastoma.

Subgrouping canine grade II mammary carcinomas by Ki-67: a potential diagnostic tool

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Introduction

Most of canine mammary carcinomas (CMCs) have a good prognosis after surgery, and only a few cases have a poor outcome. The histopathological grade of malignancy of CMCs (grades I, II and III) (Peña et al., 2013) has proven to be a useful tool to select those cases with poor disease progression and cancer-related death, which are mostly dogs with grade III tumours. Nevertheless, some dogs with grade II tumours can also have a malignant outcome. The aim of this study was to evaluate if Ki-67 proliferation index could help to identify grade II CMCs with poor prognosis.

Materials and methods

After mastectomy, histopathological diagnosis and grading (H&E), Ki-67 index was calculated in grade II CMCs by immunohistochemistry (n=24). Epidemiological, clinical, and histopathological variables were included for univariate and multivariate statistical prognostic study with a two-year follow-up.

Results

Ki-67 was associated with tumour-related death ($p=0.046$). Using onward steps multivariate analysis, age, histopathological diagnosis, Ki-67 index, and a Ki-67 cut-off (21.6%) were selected as independent variables associated with overall survival ($p<0.05$). Ki-67 was associated with other prognostic variables with a significant level of $p<0.08$.

Conclusions

Ki-67 allowed separating grade II CMC into two subgrades, grade IIa and grade IIb, with grade IIb being more malignant in terms of prognosis than grade IIa. Further studies, including larger case series, are necessary to fully validate these two subgrades in the diagnostic and clinical settings.

Extracellular vesicles-derived microRNAs in felines with spontaneous malignant mammary tumors

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Introduction

Extracellular vesicles (EVs) are used by cells in physiological and pathological conditions for intercellular communication. EVs synthesis was proved to increase in different oncologic diseases, with cancer cells derived-EVs containing tumor-specific molecules, such as miRNAs, which are important in carcinogenesis and tumor progression. This study aimed to evaluate the expression of miRNA-20a, miRNA-24, miRNA-126 and miRNA-210 in circulating EV of felines with malignant mammary tumors.

Materials and methods

The expression of EVs-derived miRNAs was determined in plasma samples of 20 queens with malignant mammary tumors and of 15 healthy control cats. miRNAs were analyzed in surplus plasma samples that were collected for clinical purposes in all diseased and healthy queens included in the study. The EVs were isolated from the plasma fraction and characterized by nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM). The selected miRNAs were extracted using a commercial kit and quantified by real-time PCR.

Results

Expression of EV-derived miRNA-20a and miRNA-210 was significantly higher ($P<0.05$) in queens with mammary tumors than in healthy controls animals. Expression of miRNA-24 and miRNA-126 was higher in healthy queens than in diseased animals, although the differences were not significant.

Conclusions

Differences in expression of the selected EV-derived miRNAs were detected between queens with mammary tumors and healthy controls. Our results suggest that these miRNAs, particularly miRNA-20a and miRNA-210, might be implicated in tumorigenesis of feline mammary carcinomas, and that could be potential clinically useful biomarkers for screening, diagnosis, early detection of disease progression and prognosis, and eventually potential therapeutic targets of the disease.

Calcium electroporation of canine solid tumors – a feasibility study

Pernille Holst

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Introduction

Calcium electroporation is based on the technique of electrochemotherapy and characterized by substitution of chemotherapeutic drugs with supra-physiological concentrations of calcium delivered directly into tumors followed by electroporation.
The aim of this work was to evaluate adverse events and feasibility of a calcium electroporation treatment protocol prior to a randomized clinical trial comparing efficacy of one versus two calcium electroporation treatments of canine solid malignancies.

Materials and methods

Two dogs were enrolled, one with a 0.06 cm3 stage IV interdigital melanoma and one with a 2.13 cm3 stage I subcutaneous mast cell tumor.
Calcium electroporation was applied to each tumor twice with a 37-day interval. Prior to treatment and at day 7, 14, 30, 37, 67, 180, and 360 post-treatment, tumor size and adverse events were recorded, along with assessment of serum biochemistry, canine owner- reported quality of life score (CORQ) and behavior-based pain score.

Results

Both dogs experienced a partial and long-lasting (360 days) response. The volume of the melanoma was reduced from 0.06 cm3 to 0.025 cm3 (58% reduction) and an enlarged locoregional lymph node normalised in size. The mast cell tumor volumen was reduced from 2.13 cm3 to 0.33 cm3 (83% reduction). One dog experienced a grade 1 skin toxicity, and no changes in CORQ, pain score or serum biochemistry parameters were identified.

Conclusions

The two dogs treated with calcium electroporation had a long-lasting clinical benefit of the treatment and did experience none or mild adverse events when treated with a veterinary calcium electroporation protocol.

Treatment of equine sarcoids using intratumoral Bleomycin in combination with Tumour Specific Electroporation

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Introduction

Tumour Specific Electroporation (TSE) is a patented technology for tumour electroporation. It combines a four electrode multi-dimensional electroporation with a gradually decreasing pulse train (Dynamic Field) delivered to spare healthy tissue, reduce futile local toxicity and optimize efficiency of the chemotherapy drug.

For electrochemotherapy (ECT) the agent Bleomycin has a strong safety profile, however with no previous reported success in equines.
Evaluation of intratumoral Bleomycin followed by TSE was evaluated for equine sarcoids

Materials and methods

One equine with nine cutaneous/infiltrative sarcoids underwent one treatment session performed with intratumoral injection of Bleomycin followed by TSE; 8 brief, electrical pulses starting at 1000V and then gradually decreasing to 600V, delivered with needle electrodes in a square configuration, 12mm distance. Tumour volume range 0.7cm3-13.1cm3.

Results

Achieved current was > 3.8A in 8/9 lesions. Complete remission (CR) of 9/9 sarcoids within an observation period of 13 weeks. Local toxicity was grade 1-4.

Conclusions

One session of intratumoral Bleomycin followed by TSE was adequate to achieve CR of all treated sarcoids within a short term observation period. This is the first reported successful treatment of equine sarcoids with ECT and Bleomycin, and first reported treatment with TSE in companion animals. Together with the drugs' lower toxicity this combination will allow for a much wider use as the primary option for treating sarcoids.

My patient has cancer what are the options

Nicola Read

This session will outline some of the options we have when caring for animals with oncological disease and signpost the participants through the contents of ESVONC's second nurse congress. An overview of the basic principles of conventional veterinary interventions will be presented together with some novel and emerging treatments that are becoming available. This introduction lays the foundation for our host of speakers and readies the nurse-technician for our first day of congress.

A diagnosis of cancer is often devastating news for any family but there are options available which have the potential to allow our companion animals to live a longer, more comfortable life. Some veterinary oncology treatments aim for a cure, most intend to push the disease into a state of remission, and with some the intention is to simply support the animal. Any veterinary intervention aims to reduce the clinical signs, pain or discomfort associated with illness, yet for animals diagnosed with cancer there are many elements to consider and there often isn't usually just one, straight forward decision to be taken.

Whenever possible, surgical excision of the tumour together with ample tissue margins and removal of the local draining lymph node is the preferred method when the goal is to cure the patient of cancer; however due to the location, size or co-morbidities of the patient, this may not always be possible and neo-adjuvant treatment with another intervention may increase the rate of success. Despite the best surgical technique some neoplasms can infiltrate deep into tissue layers or create microscopic colonies (metastases) in other parts of the body therefore adjuvant treatment with another intervention may be necessary to try to eradicate the cancer completely.

Radiation therapy is the treatment of choice for solid tumours, most neoplasms of the head and neck, the brain and the nose. Surgical resection of tumours in these areas carries a high risk of further morbidity and disfigurement which owners are often averse to. Radiation therapy can be used as a palliative- (pain relieving) or curative-intent; the treatment exerts its effect by killing cancer cells or slowing their growth by damaging DNA. There are three main types of radiation therapy: (i) external beam radiotherapy which uses a large external source of radiation to selectively deliver high-energy radiation to the tumour; (ii) brachytherapy which uses smaller radiation sources applied directly or implanted within the tumour; (iii) nuclear / biologically targeted radiotherapy which required a radioisotope to be administered to the patient where it then accumulates within the tumour. A typical course of radiation therapy consists of multiple doses (fractions) of radiation delivered over several days, this is so that healthy tissue in the path of the energy beam is allowed to heal. Treatment can cause adverse side-effects, commonly inflammation, oedema and mucositis which are usually related the dose. Recent advances in veterinary oncology have seen a newer adaptation of external radiation therapy where each fractional treatment is broken down into smaller doses which are then delivered via multiple angles to the tumour, therefore improving tumour control and reducing complication rates.

Chemotherapy treatment uses drugs that specifically target rapidly dividing cells, which is often the case in cancer, yet there are other rapidly renewing cells normally present in the body that can be affected by this treatment. Chemotherapy has the advantage of treating cancer systemically, i.e. addressing disease throughout the body and those cancers with a high metastatic potential; it can also be used to try to shrink tumours to a manageable size which then make surgical cure more possible. Chemotherapy drugs can be categorised as to when they exert their cytotoxic (cell destroying) effect on the dividing cell, depending on what part of the cell cycle it is in; therefore, how the neoplasm responds to a particular chemotherapy drug will depend on the type of cancer, its size, spread and location.

Chemotherapy is not suitable for all types of, or slow-growing cancers. Animals do not receive the same intense doses or protocols used in human medicine and so the focus is to achieve tumour control without a significant impact on their quality of life, which may require different drugs from different classes being used at once. Side effects including myelosuppression and gastrointestinal toxicity are sometimes seen, and are more likely with multi-drug protocols, however these are usually limited and can often be medically managed. Ever increasingly, cancer care is being embraced within the veterinary profession and methods of multi-modal care is being translated from examples in human medicine. Interventional radiography can facilitate delivery of targeted chemotherapy drugs and stent obstructive tumours; there are also less-invasive methods of treating superficial neoplasms being utilised which otherwise would have required aggressive or disfiguring surgery. Much investment into comparative drug therapies including immunotherapies, targeted molecular therapies and metronomic therapies are being investigated in veterinary patients, where results of tumour control and less adverse effects are being reported, therefore modelling a change of practice for some case of cancer management. It is time to acknowledge that veterinary oncology is evolving!

One important thing to remember is that whatever the cancer, the stage (otherwise known as volume of disease) and the predicted behaviour of the cancer plays a significant factor in selecting treatment. For animals with advanced cancer, medical management takes on the form of palliative care to try to control the impact of disease and manage pain as much as reasonably possible, so to enhance quality of life and positive interactions for the remaining time that they have. Measuring these signs and behaviours will be discussed over the conference as well as how we as care-givers play a valuable role by providing greater accessibility and support to clients wishing to treat their companion's oncological disease.

What impact can nurses have on our patients' pain?

Dr. Clark

Overview of the presentation:

- Impact of chronic pain in oncology patients
- Measuring pain and QoL
- Barriers to good chronic pain management
- What can nurses do that vets can`t?
- Practical ideas for clinical improvement
- Patients in the clinic – nurses as patient advocates

Pain is a devastating clinical effect of many forms of cancer, affecting the quality of life of patients and caregivers. It is a multidimensional problem that includes physical, psychosocial, and emotional components. Despite the development of novel analgesics and updated pain guidelines, cancer pain remains undermanaged, and some human patients with moderate to severe pain do not receive adequate pain treatment. In veterinary oncology there is almost no data assessing analgesia provision in chronic cancer pain.

Chronic pain has wide-ranging impacts which affects the quality of life (QoL) of the individual, whether that is a person or an animal. Adequate pain management requires a multidimensional approach toward assessment and management. Barriers to optimal cancer pain management are also multidimensional in nature and include patient, clinician, and system-related factors. These have been rigorously detailed in human practice, and whilst we have little research data, many of the same barriers apply to our practice.

Given the multidimensional nature of cancer pain and the multifaceted barriers involved, effective pain control mandates multidisciplinary interventions from interprofessional teams. Educational interventions for owners/caregivers and clinicians may improve the success of pain management, which is where Veterinary Nurses can play an important role.

Clinician related barriers to optimal pain management:

- Poor pain assessment
- Lack of knowledge

- Reluctance to prescribe certain drugs
- Fear of adverse effects
- Fear of legal/administrative constraints
- Access to controlled drugs
- Discrepancy between self-evaluation and knowledge
- Lack of specialists

System related barriers to optimal pain management:
- If caregiver or clinician does not report pain, there is no mechanism that mandates assessment
- Cost of analgesics
- Lack of access to wide range of analgesics
- Difficulty in accessing services to enable nonpharmacologic pain management
- Impact of distance on ability to access pain-related services
- Lack of coordination across multiple care providers
- Lack of support from specialists in pain and palliative care
- Lack of staff time to attend to pain needs of patient

Owner/caregiver related barriers to optimal pain management:
- Caregiver placebo effect (want to see a response)
- Cognitive, not understanding that pain is part of the disease process
- Concern regarding disease progression/ euthanasia discussions
- Adverse effects of analgesics
- Fatalistic belief regarding cancer pain
- Concern about distracting clinician from cancer care as this is most important
- Medication compliance

Practical ideas for clinical improvement:
Where can nurses play a role:
Assessment of pain
- Taking the time to talk to owners/caregivers and obtaining information that might not be revealed to clinicians
- Using validated QoL and HRQoL tools (see next presentation)
- Getting owners/caregivers to understand the “caregiver placebo” effect
- Providing multidimensional evaluation, including physical, functional and psychosocial aspects. E.g., This might include assessment and understanding of change in exercise limitations in some animals and ideas to keep them engaged and happy.
- Being involved in the ongoing assessment of pain with regular follow-up appointments, including video or telephone contact.

Management of cancer pain
- Ensuring availability and efficiency of access to appropriate analgesics
- Presenting easily accessible data for clinicians in respect of analgesics to avoid lack of knowledge as a barrier to prescribing - adequate dosages, routes, and schedules
- Monitoring the outcome after starting analgesics
- Anticipating and communicating with clients about analgesic adverse effects
- Treating adverse effects of analgesics
- Becoming involved in non-pharmacological pain management modalities and provision of comfort
  - E.g., managing and helping owners manage oral mucositis, discussing and delivering (legislation dependent) acupuncture.
- Helping facilitate collaboration with other specialties for a multidisciplinary approach
- Education of owners/caregivers and family members on pain and analgesic medications

- Informing caregivers that most cancer pain can be alleviated, while also setting realistic expectations and pain goals
- Providing owners/caregivers with pain and medication diaries

What can nurses do that vets can`t?
Human cancer patients who report being given the name of a specialist cancer nurse are more likely to describe better care experiences. Having the personalised support of a specialist
cancer nurse enables people to get support for their physical and emotional health needs because of having cancer
Among people recently diagnosed with cancer in the UK who did not receive enough support from a specialist cancer nurse during their diagnosis or treatment, almost half (44%) said this led to at least one of the following medical impacts:
- Being unsure about what side-effects of treatment they should be looking out for
- Ending up in A&E
- Being unsure if they were taking their medication correctly

Cancer pain is a multidimensional symptom with both physical and nonphysical components. Adequate pain control requires a multidimensional approach toward assessment and management.
We have emphasised the assessment and management of chronic cancer pain thus far, but many patients who already have some chronic pain, either resultant to their cancer, their cancer treatment, or a co-morbidity e.g., osteoarthritis will undergo relatively invasive procedures whilst hospitalised.

Patients in the clinic – nurses as patient advocates:
Procedural pain is an unpleasant sensory and emotional experience that arises from actual or potential tissue damage associated with procedures. Human patients rate the following procedures with higher pain intensity: MRI, radiotherapy, and lumbar punctures. We tend to anaesthetise our cases for these procedures, but oncology patients in are subjected daily to potentially painful procedures causing distress and discomfort. This could be as innocuous as placing a cephalic IV catheter in a dog with severe elbow osteoarthritis; or performing an FNA on an area of suspected tumour regrowth near a previous surgery – this patient may well have a degree of peripheral and central sensitisation secondary to the previous surgery. Veterinary Nurses can see these cases and ensure that appropriate steps are taken to try to mitigate the patient`s pain as much as possible. In the case of the dog with OA, consider a saphenous catheter if appropriate, or consider lying the dog down in lateral recumbency to reduce elbow flexion and using EMLA cream on the skin – which might be sensitised.
Even with various forms of local anaesthesia and analgesia, bone marrow aspiration (BMA) is associated with pain in most people who undergo the procedure, some of whom even label it as “unbearable.” According to the position statement issued by the American Academy of Paediatrics, “For procedural pain that is predictably severe and for which local measures give inadequate relief, such as for bone marrow aspirations, the use of systemic agents is required to bring pain to acceptable levels.” Because of the acute pain caused by BMA, the procedure is most often performed under general anaesthesia or heavy sedation in children, and this should be the same in our cases. BMAs are thought to cause minimal inflammatory pain (so is not NSAID responsive); instead, the pain reported by humans is characterized as sharp, intense, and piercing, humans report significant “sucking” pain during aspiration, which is not prevented by a subcutaneous lidocaine block or oral NSAID administration.

In each clinic there will be different barriers to optimal pain management – dependent upon the interaction of the system, the clinicians, and the owners/caregivers. I would suggest that you try to instigate THREE practical ideas for clinical improvement in your clinic! The rewards are worth it!

Additional reading:

Kwon JH (2014) Overcoming Barriers in Cancer Pain Management. Journal of Clinical Oncology Volume 32, Issue 16

Villegas Estévez FJ, López Alarcón MD, Beato C, et al (2021) Procedural pain in patients with cancer: a Delphi expert management consensus. BMJ Supportive & Palliative Care. Published Online First: 01/07/2021. doi: 10.1136/bmjspcare-2020-002668

Health Related Quality of Life in Oncology cases - advocating for your patients

Dr. Clark

Overview of the presentation:

- Why quality of life (QoL) in companion animals?
- What exactly is QoL?
- What is health related quality of life (HRQoL) assessment?
- Can we use QoL and HRQoL assessment in oncology cases?
- How can I advocate for my patients – practical implementation….

Why assess quality of life in companion animals?
"How will I know when my dog is suffering too much and it's time to call it a day?", "I just don't know whether I should put my cat through it." These are familiar questions from clients in our daily clinical work. They represent owners or perhaps more appropriately, carers, contemplating the QoL of their companion animal in different ways, when making decisions about clinical care options including euthanasia. As Veterinary Surgeons and Veterinary Nurses we have an ethical duty to consider the QoL of the animals under our care.
To promote the welfare of companion animals by providing appropriate guidance for clients, it is necessary to first understand what QoL is, how best to assess it, and what to do with the information. Clients and clinicians have been answering these questions to the best of their ability for many years. They have drawn upon their previous experience, scientific knowledge, and empathy to make these judgments in an informal way. The question is, what, if anything can be gained through formal, systematic assessment of quality of life within our clinical practice?

What is quality of life (QoL) and HRQoL and how does they apply to the oncology patient?
The World Health Organisation (WHO) defines quality of life as "an individual's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards, and concerns". According to this definition, QoL is a personal, private, and subjective experience of how an individual feels about his or her own life, rather than an external view of how others perceive it to be. It is determined not only by one's life circumstances but also what uniquely matters to them and their ability to enjoy life. As QoL is a concept which comes from within, assessments should be made from the perspective of the individual in question. However, this is impossible within veterinary medicine. QoL assessment tools in humans are designed to be self-reported where possible (patient-reported outcome measures (PROs)). In those unable to self-report, such as young children or the cognitively impaired, an observer or proxy (i.e., parent/clinician) is required to assess QoL on the individual's behalf (observer-reported outcome measures (OROs))
Animals cannot directly express how they feel, all QoL assessment tools are classified as OROs. Therefore, it is the responsibility of the veterinarian and owner to estimate a pet's QoL based on criteria deemed meaningful to that individual animal.
There is no consensus on the definition of QoL in veterinary medicine. It has many different proposed definitions, but broadly represents the aspects of an animal's life that make life better or worse for that specific animal. Note that the term often goes undefined in publications where it is measured (Belshaw et al. 2015).
In an article outlining QoL assessment, Mullan (2015) describes it as:

"…. synonymous with the term "welfare" and has been widely discussed as being dependent on one's philosophical viewpoint and may both influence, and be influenced by, scientific research. However, there is a consensus that the term "quality of life" refers to much more than simply health".

QoL could be considered to encompass three elements:
1) positive and negative experiences and feelings (on a spectrum ranging from very negative (e.g., severe pain) to very positive (e.g., playfulness)
2) physical fitness and health (encompassing elements such as challenge posed by diseases, animal's physical ability to cope with its environment)
3) naturalness (free from mutilations or extremely unnatural body shapes, with the ability to carry out natural behaviours and experience elements of natural environments)

Health-related quality of life (HRQoL) is a more specific term, defining the effect of a medical condition on an individual's health. In oncology, HRQoL defines the effect of cancer and its treatment on body function and well-being. This by default should include the impact of chronic pain associated with the cancer, something that is often neglected. Information about HRQoL can help making treatment decisions, provide prognostic information, improve owner–vet interaction and evaluate the impact of new treatments in clinical trials. In human medicine, QoL is considered one of the main goals, and an important decision-making factor, in clinical trials and cancer treatment. However, in veterinary medicine, this field has only begun to gain importance in the past two decades. Questionnaires have been published for assessing QoL of dogs with several conditions including:

- chronic pain
- neurological and musculo-skeletal pain
- spinal cord injuries
- cardiac disease
- obesity
- diabetes mellitus
- epilepsy
- skin disease

Can we use QoL and HRQoL assessment in Oncology cases?
No distinct guidelines are currently available for QoL and HRQoL tool design and appraisal which is clearly problematic, although the amount of interest in this area is encouraging. A recent study (Fulmer et al. 2022) assessed nine published generic QoL assessment tools designed for use in dogs and cats. Each tool was unique in terms of structural design, psychometric evaluation, and statistical analysis. Common items for both dogs and cats included those regarding activity level, the desire for caregiver interaction and appetite. In addition, common items for cats included those regarding mood and grooming.
Compared to human health care where different clinical outcome assessments available for many medical conditions, development and research of these tools within the veterinary profession is relatively embryonic. The potential for QoL tools to improve the welfare of the animals that attend the clinic, means that these existing tools should be built upon, refined, and tested. Validity, reliability, and responsiveness are key properties of HRQoL instruments as is utility (user friendliness).
Disease specific instruments (e.g., relating to lymphoma) may be more sensitive to clinical change, but generic instruments are useful to quantify a range of impacts related to disease and its treatment and may be the only choice when a patient has more than one condition, a situation encountered commonly in veterinary medicine, and in oncology where pain and the disease burden coexist. Instruments either generate a single index score which indicates that a patient is better or worse or a profile of scores which offers more information and may be more sensitive to group differences and to changes in health status over time (Reid et al. 2018)
Implementation in the veterinary clinic

Veterinary professionals are increasingly demanding an evidence base for their actions but the use of QoL assessment within the clinic requires a degree of consideration and planning before implementation. There is an acknowledged lack of information regarding positive outcomes of using many of the veterinary assessment tools, but this need not be a barrier to their use, assuming there are unlikely to be any negative consequences.
The International Society for Quality-of-Life Research in humans has produced useful guidance on implementing patient-reported outcomes in clinical practice, which are equally applicable to the veterinary setting and are detailed below in a practical outline. Whatever form the assessment takes, it would seem important to support clinicians in facilitating carers to find solutions to improve welfare and to evaluate the whole process.

1. Identify the goals for collecting QoL assessment in your practice.
- Is it to screen for deficiencies (e.g., in pain management) across the whole population or is it to track and monitor the progress of individuals?

2. Select your patients, setting and timing of assessments.
- Will you use it in all cases, and all species, or for example a subset that you have a need for more data/specific concerns about e.g., osteosarcoma patients.
- Will the assessment be filled in prior to the appointment, can you access it remotely (online) or will it be completed in the clinic at the time of the appointment? Will it be filled in directly by the client or will a nurse/vet ask the questions?

3. Determine the questionnaire to use.
- Choosing a published tool that has some degree of validation and peer review is desirable. This may be determined by your population/case selection.
- Disease specific instruments may be more sensitive to clinical change, but generic instruments are useful to quantify a range of impacts related to disease and its treatment and may be the only choice when a patient has more than one condition - remember this includes pain!
- Instruments either generate a single index score which indicates that a patient is better or worse, or a profile of scores which offers more information and may be more sensitive to group differences and to changes in health status over time.
- If this more scientifically robust approach is not practicable, tools such as the Villalobos (2011) an author developed HHHHHMM QoL scale are easy to use. Note that this scale has no validation or field testing so has some inherent weakness.
- Appropriate consistent questioning may also be considered, in effect, devising your own tool with reference to the published literature. In nearly all the assessed tools, activity, care-giver interaction, and appetite appear to be valuable QoL parameters for both dogs and cats. In addition, mood and grooming behaviour also appear to be important aspects of a cat's QoL.

4. Work out the practicalities of who/when/where… to keep things consistent.
5. Reporting results – how will these be recorded, stored, and processed?
- Needs to be practical and time efficient
- Data needs to be accessible for the next appointment (for the individual) to allow progression over time assessment, and to all clinicians involved to allow in group comparisons.

6. Interpretation of data
- It may be that comparisons are initially produced entirely internally (within the practice data set).
- There may be some published literature to aid interpretation.

7. Acting upon the data
- The autonomy of the clinician must not be compromised because they will be integrating data from multiple sources, but "red flags" should be addressed

8. Evaluating impact
- Practice/team meetings are the least formal way of approaching this and audit the most involved. Surveys of caregivers and staff might be helpful.

Chronic Pain assessment and HRQoL take home messages:

- The contemporary approach to pain measurement in people and animals seeks to measure the affective (emotional) component of the pain experience using structured questionnaires with formal scoring methodology.

- Measuring pain is crucial for effective pain management and a requirement of evidence-based medicine.
- Important to measure the affective ('how it makes you feel') component of the pain experience, not just intensity.
- In acute pain the veterinary surgeon or nurse is the animal's proxy while in chronic pain the owner assumes that role.
- We measure chronic pain in man and animals by quantifying its impact on health — related quality of life (HRQL).

Quality of life assessment overall:
- Is extremely important however it is undertaken…
- should be personal to each dog/cat
- has implicit limitations that can be minimised but not eliminated
- must engage assessors
- reports should be explicit about what and how they assess

Specific references:

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Mullan S (2015) Assessment of quality of life in veterinary practice: developing tools for companion animal carers and veterinarians. Vet Med (Auckl). 6:203-210 https://doi.org/10.2147/VMRR.S62079
Reid J, Nolan AM, Scott, EM (2018) Measuring pain in dogs and cats using structured behavioural observation, Vet. J. 236:72-79.

Further reading

There is no comment on the suitability of these tools for your specific caseload but please note the points about validity, reliability, and responsiveness as key properties of HRQoL instruments. Utility (user friendliness) is paramount and note the benefits and pitfalls of generic versus disease specific tools.

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Management of various drains

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Tracheostomy tubes
Placement of a tracheostomy tube can be performed quickly and simply, and requires a minimal amount of equipment. In the majority of cases, a tracheostomy can be performed on a stable, anaesthetised and intubated patient. Rarely is it necessary for a tracheostomy tube to be placed in the conscious, cyanotic patient as an emergency cut-down.
A tracheostomy tube should only be considered for short-term maintenance of an airway, and definitive treatment of the laryngeal condition should be actioned as soon as possible. Tracheostomy bypasses the normal protective mechanisms of the lower respiratory tract and bacterial colonisation of the trachea is inevitable. In addition, the presence of the tube interferes with normal ciliary motion and coughing, which further compromises the defences of the lower respiratory tract. Proper care of the tracheostomy tube is therefore imperative. Aseptic technique and sterile equipment are required in all manipulations of the tracheostomy site to delay development of infection and productive secretions.

Post-operative Care
A tracheostomy tube should only be considered for short-term airway maintenance. Definitive correction of the airway disease should be actioned as quickly as possible. Close monitoring for airway obstruction or dyspnoea, oxygen supplementation as required, analgesia, antibiotic

Tube maintenance:
Aseptic handling of the tube and instrumentation is essential at all times. Aspiration of mucus from airway will be required on a regular basis (15 mins – 2 hourly). Inject 1-2 mls of sterile saline into the tube prior to suctioning the airway to help loosen secretions. Suction the tube and distal trachea with a sterile suction system. If the tube becomes heavily occluded with secretions, it should be replaced with a new sterile tube.
Following removal of the tube, the trachestomy site can be left to heal by second intention. Appropriate cleaning of the wound may be required.

Complications:
Complications with tracheostomy tubes will increase exponentially with the duration of use. Tube dislodgement, obstruction and dyspnoea; coughing/gagging; pneumonia; dysphagia, vomiting/regurgitation; aspiration; tracheal stenosis; tracheocutaneous fistula; laryngeal nerve injury.

Chest drains
Thoracostomy tubes are available from several commercial suppliers. They consist of a tube made of PVC or silicone, together with a metal stylet to facilitate placement. Tubes come in a range of sizes. The correct size for an individual patient may vary on the type of effusion present, but a usual rule of thumb is to place a tube approximately equal to the diameter of the main stem bronchus. A wide bore may be required for thicker inflammatory secretions. Other equipment that will be required includes a scalpel blade, three-way tap, appropriate adaptors from the tube to the three-way tap, syringe, tube clamp and suture material. Some method of providing adequate security of the various connections to the chest drain is also essential to prevent dislodgement.

Complications of chest drains

When appropriate care is taken with the placement of chest drains, it is unusual for significant complications to occur. Potentially, a chest drain trocar can bruise/perforate the underlying lung tissue, causing pneumothorax and/or haemorrhage. Because there is usually air of fluid present within the pleural cavity, this can act as a reasonable buffer to prevent such injury from occurring.
Following placement of the chest drain, the most common complications include pain, stoma infection/irritation, subcutaneous emphysema, and poor 'seal' of the stoma due to an inadequate subcutaneous tunnel (particularly in lean animals, or due to poor placement). Catastrophic pneumothorax may occur if the patient interferes with the tube, due to dislodgement of sealing bungs, or by biting through the tube.

Chest drains can be very uncomfortable for some patients (especially cats), either due to direct pleural irritation, impingement on intercostal nerves, or other reasons. Appropriate use of opiate analgesia may be necessary. Regional anaesthesia may be of value in other patients.

Prevention of stoma infection requires careful attention to asepsis during placement and handling of the drain. The use of protective dressings to prevent contact contamination of the area during hospitalisation should also be considered. Latex or polyurethrane drains are likely to induce a minor cellular reaction if a drain is left in place for over 5 days due to chemical irritation of the tissues. This is usually self-limiting, but may be the cause of some discomfort for the patient.

Subcutaneous emphysema can develop if there is an insufficient seal about the surface of the drain, either as a result of air leaking directly from the pleural space (with a pneumothorax) or by direct extension from the skin surface. This is unusual, but may occur when a drain has been left in situ for a prolonged period (with progressive necrosis of intercostal muscles about the pleural wall stoma), or if an inadequate subcutaneous tunnel was performed at the time of tube placement.

The importance of creating a good subcutaneous tunnel at the time of chest tube placement cannot be understated. In very lean animals, it can be difficult to achieve a good seal, however. Without adequate tunnelling, there is a potential for atmospheric air to track along the chest drain during respiratory movements. In addition, if there is a poor 'seal about the skin/tube interface as a result of poor tunnelling, during aspiration of the chest drain large volumes of air may be obtained giving the impression of pneumothorax, when in fact this air is simply being drawn from the atmosphere. This complication can sometimes be difficult to differentiate – although if >1000ml of air has been removed from a dog, with no signs of dyspnoea or change in respiration pre- and post- drainage, then it is likely that the air being withdrawn was not within the pleural space.
Urinary diversion with Tube Cystostomy
Tube cystostomy diverts urine away from the urethra while maintaining bladder drainage. This eliminates the potential for submucosal or subcutaneous leakage of urine from the edges of the urethral repair, and allows healing of the urethral epithelium to occur with minimal scarring and fibrosis. Healing is usually complete within 7-10 days, when normal urination can resume.

Tube cystostomy has also been employed for prolonged periods as a palliative aid when the urethra is irreversibly obstructed due to neoplastic disease. Patient and owner tolerance was excellent, and some patients lived for many months longer than they would have if urinary diversion had not been employed. Complications of tube cystostomy include urinary tract infection, uroabdomen and peritonitis, premature removal, and leakage of urine from the stoma following removal.

Urinary infection should be expected to occur in all cases as a consequence of disruption to normal bladder defence mechanisms. Treatment with antibiotic is usually not necessary, and ideally, should be delayed until the tube is removed. A urine culture should be obtained immediately prior to tube removal to allow appropriate selection of antibiotic to be made. However, in a recent study, infections resolved in all cases without complication following tube removal and treatment with a broad spectrum antibiotic.

The potential for tube dislodgment and premature removal (which could lead to leakage of urine into the abdomen) is avoided by careful placement of the tube into the bladder at laparotomy. Laparotomy is preferred because the very mobile bladder of dogs and cats makes placement by blind percutaneous means very difficult. In critical patients, a mini-flank laparotomy can be performed quickly (and oftentimes, under neuroeleptanalgesic sedation only) to accurately place the tube into the bladder.

Removal of the tube is straightforward. Mild leakage of urine from the stoma should be expected for up to 5 days after removal, but this is usually well tolerated.

Enteral feeding
The importance of providing effective nutritional support for traumatised, sick and patients recovering from major surgery cannot be overstated. The detrimental effects of starvation on immune status, wound healing, and rehabilitation have been proven time and again in numerous studies. Despite this evidence, and a general tacit acceptance of the importance of nutritional management, it is still not an uncommon event for a veterinary patient to be kept in hospital for days on end receiving intravenous fluids only. Nutritional management is a pro-active endeavour, and requires close co-operation and agreement between veterinary and nursing staff.

Evidence from the literature indicates that:

Effects of protein-calorie malnutrition can contribute to up to 85% of deaths in the human ICU
Human trauma patients fed 18 hours post-surgery have improved nitrogen balance and less septic complications when compared to those fed only 5 days post trauma
Early feeding (within 24 hours) post-GI surgery in humans reduces the risk of any type of infection and the mean stay in hospital.
Underfeeding of stressed, sick animals results in a significant loss of lean (rather than fat) body mass and reduction in healing and immunity - even in fat animals! This means that even obese animals should have nutritional support post-surgery. Most of their weight loss under stress will be lean body mass and anorexic obese cats are at high risk of developing fatal hepatic lipid sis.

Always anticipate the need for nutritional support. The earlier nutritional support given, the better. Critical patients can lose weight dramatically. If nutritional support is not commenced until obvious weight loss is noted (>10% body weight), it can often be very hard to reverse the trend.

Tube feeding
Tube feeding provides the opportunity for regular and consistent provision of nutritional supplementation to the convalescent patient. A variety of tube locations can be considered, with the choice dictated by the length of the time that supplemental feeding is anticipated, the size and nature of the patient, and the experience and confidence of the veterinary surgeon and nursing team. Various tubes configurations that are described include:

Naso-oesophageal
Naso-gastric (not recommended due to potential gastric reflux & oesophagitis)
Pharyngostomy (not recommended - better alternatives now exist)

Oesophageal
Gastrostomy
Enterostomy — duodenostomy, jejunostomy.

Notes on tube placement

a) Naso-oesophageal tubes
These are used for short-term nutritional support (< 1 week) where there is no pharyngeal, nasal or oesophageal disease that the tube would exacerbate. They have the advantage that they do not need a GA for placement. They are contraindicated in above conditions and especially in reflux oesophagitis where presence of tube in distal oesophagus not only encourages more reflux but also mechanically opposes healing of oesophagus.

Placement:
Premeasure tube to ensure placement in caudal oesophagus. Avoid placement into stomach, due to concerns with gastric reflux. Premeasure to 7th intercostal space from nose. Mark tube with piece of tape (pen tends to rub off).
Apply local anaesthetic to nose. In some animals, sedation may be necessary.
Lubricate tube with KY and advance into ventral meatus. Elevate head, and tip point of nose upwards to help direct the tube ventrally. In the dog, the slight resistance as the tube passes ventral to the maxilloturbinate is normal.
Hold animal's head normally as approach pharynx to prevent tracheal intubation. Allow animal to swallow and advance tube to previously measured mark.
Check tube is correctly positioned. Instil sterile saline or water into tube, then inject a quantity of air whilst auscultating over the left flank. Listen for bubbling in stomach. If still uncertain, inject some iodine contrast material into tube and X-ray.
Pass tube around the corner of the alar fold. The tube can then be passed along the edge of the mouth, or directly over the top of animal's head. Ensure the tube is not interfering with sight lines, else the animal will be vigorous in attempts to remove it. Secure the tube at several points with butterfly strips, suture or superglue
Put on E-collar and attach tube to collar.

Complications.
Gastric reflux/aspiration - usually not problem if small diameter tube and in distal oesophagus.
Tracheal intubation – either by direct placement, or following inhalation of regurgitated tube. Catastrophic injection of food into airway avoided by rigorous checking of placement prior to each feeding
Removal/interference by patient
Poor patient toleration
Blockage of tube, diarrhoea, metabolic upsets possible with any method of tube feeding.

b) Oesophagostomy tubes
Have recently returned to vogue. Have all the advantages of PEG tubes (easily placed, large bore tubes, well-tolerated, long-term use), but fewer complications with their use (particularly peritonitis from premature dislodgement of PEG tubes). The use of a percutaneous gastrotomy introducer, and ready-made gastrostomy tubes makes placement very straightforward. However, they can also be placed with long curved forceps and conventional tubing.

Placement:
Patient must be anaesthetised. Place in right lateral recumbency (left sided placement is more desirable)
Prepare midcervical region from angle of jaw to midthoracic region for aseptic surgery

Premeasure tube from its proposed insertion point on neck to the level of the 7th intercostals space. Mark this point with tape.
Pass a long-handled introducer via the mouth, and palpate the point of the instrument at the mid-cervical region
Make a small skin incision over the point of the instrument
Grasp the end of the oesophagostomy tube and pull the instrument, with the attached gastrostomy tube through the cervical skin, oesophagus, and out through the mouth. The tube is pulled out of the mouth until the pre-marked region is level with the cervical skin
The tip of the tube is now reversed back down the oesophagus. A rigid stylet may be placed into one of the side holes to help guide the tube into place.
The tube is secured to the skin with a Chinese finger trap suture. Don't tie it too tight and inadvertently occlude the tube.
The tube can be bandaged to prevent disruption by the patient.
An x-ray should be obtained to confirm correct placement of the tube.

c) PEG tube placement
Quicker and less invasive if not already doing a laparotomy, but do need a fibre-optic endoscope! Although gastrostomy introducers can be used to allow 'blind' placement, there is a significantly higher rate of serious (and life-threatening) traumatic injuries (such as ruptured spleen) with inexperienced operators - can easily push tube through visceral surface of stomach and damage or entrap the spleen.

Placement:
Clip and aseptically prepare an area of skin caudal to left costal arch.
Pass endoscope per os into stomach and inflate stomach
Insert 18G over-the-needle catheter into stomach through stab incision in shaved area of body wall.
Remove stylet and pass thick nylon suture through catheter.
Grab suture with biopsy instrument of endoscope and pull out of mouth.
Thread nylon back through rigid catheter case and then attach to a ready-made gastrostomy tubes complete with stent and rigid end.
Pull the whole assembly back into the stomach by gentle traction on the nylon where it exits the body wall.
Pull the feeding tube out through the body wall and secure it with a second stent outside and sutures. Cap and cover as before.

Complications:
Must be left in for at least 7 days before removal
Dislodgement/disruption may allow (catastrophic) leakage of food into abdomen (usually occurs first few days and more of a risk with PEG tubes as no sutures to internal body wall)
Stoma infection.
Interference/pulling tube out.
Blockage of tube, diarrhoea, metabolic upsets possible with any method of tube feeding.

d) Enterostomy tubes:
Only indicated if the stomach is non-functional. Indications are very infrequent. Enterostomy feeding is associated with more metabolic complications (vomiting/diarrhoea) as 'dumping' too much food into the intestinal tract can cause profound osmotic disturbance. There are also few, if any, veterinary diets that are specifically designed for enterostomy feeding.
Once the tube is placed (surgically), continuous administration of iso-osmolar fluid via an infusion pump is required. Intensive nursing is required.

General principles of tube feeding
Start gradually — introduce food over 2-3 days.

Calculate daily requirements. Aim to feed about 4-6 times daily (more frequently for small bore tubes (naso-oesophageal)
Prepare the total amount of food to be fed at a single feed.
Ensure this food is at room temperature or slightly higher — cold food may induce vomiting and rapid stomach emptying.
Ensure you have the appropriate connectors/syringes to attach to the tube.
Flush tube with 5-10 ml of water at body temperature (depending on size of patient)
With naso-oesophageal tubes, if there is any evidence of coughing/choking STOP. Check placement of tip of catheter is correctly beyond 7th rib in the oesophagus before continuing.
Give food SLOWLY
Flush the tube with 5-20mls of lukewarm water (depending on patient size) in order to clear debris and maintain patency.

Removal of tubes
The tube should be removed once the patient is consuming 85% of its daily energy requirements per os. In practice, this is easy to assess in gastrostomy tube fed patients but very difficult with naso-oesophageal tubes, particularly in cats as they inhibit the animal from eating normally.

Naso-oesophageal tubes are removed simply by removing sutures or tissue glue and pulling the tube out. Administering some local anaesthetic to the nasal cavity prior to removal may make this less traumatic although it is usually fairly straightforward.
Gastrostomy tube removal is dependent on what type of tube has been used. Some are cut and pulled quickly and sharply, others require a stylet to be introduced down their lumen prior to removal, this is to straighten the 'mushroom' so that the tube can exit the abdominal cavity easily.

The remaining stoma should be dressed with a light dressing e.g. Primapore and allowed to heal by secondary intention.

Calculating (estimating!) the amount of food to give

Calculate animals daily food requirement using basal energy requirement (BER).

BER (kcals/day) = (30 x BW(kg)) + 70 in dogs and cats over 2kg
BER (kcals/day) = 50x BW in small cats

Next, multiply BER by an "illness factor" to get illness energy requirement (IER) for 24hrs.

Illness factors:

Cage rest / hospitalisation	1.25
Post surgery	1.25 - 1.35
Cachexia (cardiac, cancer)	1.35 - 1.50
Trauma	1.35 - 1.5
Neoplasia	1.35 - 1.5
Sepsis	1.5 - 1.7
Burns and head trauma	1.7 - 2.0

3. Divide the IER by the caloric density of food to estimate quantity needed.

Compounding Pharmacies for Chemotherapy Agents

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General Information

The use of compounding pharmacies by veterinary oncologists for cytotoxic therapies has skyrocketed in the last 5-10 years. Similarly, the word salad of terms which apply to these compounding pharmacies (e.g. PCAB, USP 795/797/800, 503A vs 503B) has become difficult for veterinarians to keep up with. This lecture will define and offer real-world explanations for the terms and review the current literature in the context of variations in quality of drugs delivered for our patients, to allow practitioners to make the most informed decisions on when to compound and which compounding pharmacies to utilize.

Another important distinction to be made surrounds the definitions of potency vs purity. Potency is a measure of drug activity expressed in terms of the amount required to produce an effect of given intensity. Purity is a measure of the amount of "active pharmaceutical ingredient" (API) present in a sample. Furthermore, sometimes purity can also dive deeper to compare to those of related substances, impurities, residual solvents, etc.

General Information on Compounding

Compounded drugs are not FDA-approved, meaning the FDA does not review these drugs to evaluate their safety, effectiveness, or quality before they reach patients. The FDA has investigated many cases of serious patient injury linked to poor quality compounded drugs. For example, in 2012, contaminated drugs compounded by a Massachusetts pharmacy led to more than 750 cases of infection and more than 60 deaths of patients in 20 states. In response to this tragedy as well as numerous other serious adverse events, including deaths, linked to poor quality compounded drugs, Congress passed the Drug Quality and Security Act (DQSA). The DQSA enacted in 2013, made important updates to the Federal Food, Drug and Cosmetic Act (FD&C Act) regarding drug compounding. The DQSA added a new section called 503B to the FD&C Act, which established a new, voluntary category of compounders known as "outsourcing facilities." Unlike compounders operating under section 503A, outsourcing facilities are subject to cGMP (current Good Manufacturing Practice) requirements, and they may distribute compounded drugs either pursuant to a patient-specific prescription or in response to an order from a health care provider, such as a hospital, that is not for an identified individual patient (e.g., for office stock). Outsourcing facilities are inspected by the FDA according to a risk-based schedule, and must meet certain other conditions, such as reporting adverse events and providing FDA with certain information about the products they compound.

What are the Differences Between 503A and 503B (and is it Important)?

It is important for veterinarians to understand the differences between 503A and 503B compounding pharmacies. Unfortunately, some 503A compounding pharmacies will discuss with veterinarians and technicians that 503B compounding pharmacies are illegal and/or dangerous for veterinary patients, which could not be farther from the truth. It is extremely difficult to obtain 503B certification from the FDA, as it essentially means the 503B compounding pharmacy becomes a "manufacturer" similar to the animal and human health pharmaceutical companies, which operate under cGMP guidelines. The rules and regulations which apply to 503A and 503B compounding pharmacies could not be more different and all signs point towards remarkably improved quality assurances and quality controls for those agents obtained from 503B vs the lesser regulated 503A compounding pharmacies.

To further highlight these differences, 503A compounding pharmacies are only required to be in compliance with USP <795> and <797> (though USP compliance varies by state) as well as in compliance with state board of pharmacy regulations. They also must have environmental monitoring performed every six months and their Beyond Use Dating (BUD) may be assigned based on internal or external scientific literature showing stability, however the BUDs are theoretical unless the drug has undergone a validated stability study. Furthermore, their processes do not require validation, their testing is not required, except sterility and endotoxin for batches larger than 25 units. The medications acquired by veterinarians from 503A compounding pharmacies are not intended for clinic or office use, although some states allow for a small percentage of veterinary office use. Lastly, there are no

requirements for master batch records nor requirements for GMP and CFR part 211 record compliance.
The 503A pharmacies produce compounded medications that are theoretically meeting strength, purity and potency, but are not required to be potency tested. If they are potency tested, the value of a potency test that is not validated may not accurately reflect potency. These medications are designed to be customized for one patient, not bulk processes where risk is reduced. In addition, they are not subject to process validation, are subject to significant process variation and often have uncontrolled potency results. Furthermore, they are not subject to sterility or endotoxin testing unless batches meet minimum number of units to be tested. This means single item batches are NOT routinely tested for sterility, endotoxin or potency. Lastly, and importantly, medications made under 503A oversight are subject to data alteration since documents are not controlled. This means that only individual integrity prevents alteration of records and data to make a batch "pass" where it was failing.
In contrast 503B compounding pharmacies 503B MUST comply with 21 CFR Part 210 and 211 (cGMP) and an environmental monitoring program must be developed and performed, at minimum, per production shift in the ISO 5 primary compounding areas and weekly in the secondary compounding areas. They must maintain their own quality department as an independent entity of operations and sales with complete autonomy for investigations and releasing product. They are also required to register with each state board of pharmacy as applicable as well as the DEA and FDA and report their product list to FDA biannually. They must also validate every process according to cGMP, must produce multiple batches and submit them for testing and stability before a new product can be brought to market and the products and testing methods must be validated. Furthermore, all suppliers and vendors providing raw materials must be vetted and qualified before use and a quality agreement should be in place once vetted. Lastly, they must maintain approved master batch records and approved production batch records in full compliance with CFR part 211 for records and signatures.
It becomes very easy to see the vast differences between 503A and 503B compounding pharmacies as 503B produces MANUFACTURED drugs that are made in the same manner as other pharmaceutical companies, are subject to cGMP standards and are produced only with controlled documents and/or software that prevent alteration or modification. A full audit trail is permanent and MUST be readily available for inspection. They also have processes that are validated such as material sourcing, weighing, mixing, blending, filtration, filling, particulate inspection, leak testing, packaging, labeling and are tested throughout the process in addition to mandatory testing of every final batch. Furthermore, the medications are not released until the quality unit has reviewed the batch records with full batch transparency and all testing to verify the materials and final drug have met all defined quality attributes such as purity, stability, identity, sterility, endotoxin, etc.

What does the Compounded Cytotoxic Chemotherapy Literature Say on the Subject?
To date there are only a handful of veterinary studies available which have examined the heterogeneity in purity of compounded chemotherapy agents across a variety of compounding pharmacies. The first was a study from Robat et al (E-pub in 2016) that assessed purity and stability of compounded cyclophosphamide at baseline and 60 days from 5 pharmacies. Four out of the five were outside the acceptable range on first analysis, then all within range on the second analysis. The stability at 60 days was acceptable in all but one sample. The second study was from Burton et al (2016) and they compared neutropenia in dogs treated with FDA approved lomustine (CCNU) vs. compounded lomustine. All dogs treated with FDA approved lomustine became neutropenic whereas only 25% of dogs treated with compounded lomustine became neutropenic. The actual amount of compounded lomustine API from the 5 different pharmacies ranged from 50% to 115% of the intended or prescribed amount. The third study by Burton et al (2017) examined compounded chlorambucil, melphalan and cyclophosphamide from 6 compounding pharmacies. Purity was assessed at baseline and 6 weeks later. It ranged from 58% to 109% for melphalan and 71% to 104% for chlorambucil. Furthermore, between 25% to 50% of the samples were <90% of labelled strength at baseline and 6 weeks. Purity of

cyclophosphamide ranged from 92% to 107% with all samples within +/-10% of labelled strength at both time points.
A fourth study has been performed that was presented in abstract form at the 2019 VCS meeting (PJB was the senior author). This study examined chlorambucil and cyclophosphamide across 8 compounding pharmacies. It also examined within batch heterogeneity by testing 2 samples each from the 2 cytotoxic chemotherapy agents across the 8 compounding pharmacies. The results from that study will be presented in detail at the lecture, but the synopsis is that there was remarkable heterogeneity in levels of API across the 8 compounding pharmacies as well as at times, significant heterogeneity in delivered API within some of the compounding pharmacies.

Are there issues with compounding outside of the oncology space?
There are a handful of veterinary studies available which examine the use of compounded medications outside of the oncology space. The results are mixed with one study evaluating itraconazole and finding remarkably lower concentrations upon compounding to another study evaluating levetiracetam and finding the compounded formulation to have 15-30% higher PK values. Another study evaluating chloramphenicol use in horses found the compounded versions to have significantly reduced Cmax, AUC and bioavailability compared to the approved product. An additional study evaluated compounded doxycycline on receipt and after 21 days of storage and found tablet compounded doxycycline content on day 1 and 21 respectively to be 89% to 116% and 86% to 112%, respectively. They also found doxycycline content of compounded chews and liquids to be acceptable in 5/15 samples at day 1 and in 0/15 samples at day 21.

Summary
It is becoming easier to understand from the 503 A vs B oversight/regulations and published purity data that compounding can have significant implications and not all compounding pharmacies are created equally. As veterinary oncologists, we understand and embrace that the prescribed dose and delivered purity of cytotoxic chemotherapy agents are of paramount importance based on the extremely narrow therapeutic and toxicity windows of these agents. This author ardently believes that veterinarians MUST arm themselves with the most up to date information to make the very best decisions around when to use compounded agents and then which compounding pharmacy to utilize to deliver those agents for our patients.

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Oncology therapeutics side effects & how to stop 'em

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Clients of pets with cancer are now expecting the same level of care for their pets as they receive from their physicians and oncologists. This predicates that veterinarians and veterinary technicians treating pets with cancer have an increased understanding of the possible complications of cancer therapy. This review will concentrate on developing an increased understanding of the side effects of chemotherapy in order to best prevent and treat these side effects, thereby leading to an increased quality of life for those pets.

Most chemotherapy agents have a "BAG" (Bone marrow suppression, Alopecia, and Gastrointestinal) of side effects. The reason why these side effects are so common after chemotherapy administration is because these tissues contain cells that are rapidly growing and therefore inherently sensitive to chemotherapy. Therefore, cells such as those found in the bone marrow, as well as gastrointestinal epithelial cells and hair follicle cells that rapidly turnover are quite sensitive to chemotherapy, whereas cells that slowly turnover, or do not turnover (e.g. spinal cord, muscle, etc.) are generally extremely resistant to most chemotherapy agents.

The "B" in a "BAG" of side effects stands for bone marrow suppression, or myelosuppression. Almost all chemotherapy agents are myelosuppressive, however, at standard doses, corticosteroids, L-asparaginase, vincristine and bleomycin are not myelosuppressive. After a myelosuppressive chemotherapy agent is administered, the neutrophil count and platelet count may decrease, and the low point of the count for either of these types of cells is called the "nadir." The neutrophil and platelet nadir for most myelosuppressive chemotherapy agents is ~ 7-10 days, and this can be predicted based on the half-lives of neutrophils and platelets. The half life of neutrophils, platelets and red blood cells is 6 hours, 6 days, and ~ 120 days, respectively. Therefore, after myelosuppressive chemotherapy administration, neutropenia is the first to occur, and then thrombocytopenia, whereas anemia due to chemotherapy in veterinary patients is extremely rare, because of the longer half-life of red blood cells. There are exceptions to this ~ 7-10 day nadir rule, which include cisplatin in the dog (days 7 & 17), carboplatin in dogs (day 10-14) and cats (day 21; and such is why an every 28 day therapy cycle is recommended in cats), as well as those chemotherapy agents that are orally administered on a somewhat continual basis such as melphalan, chlorambucil and others.

In order to ensure that the nadir does not drop below safe levels, a pre-treatment cbc/platelet count is required within 12-24 hours before EVERY myelosuppressive chemotherapy

administration. For most myelosuppressive agents, a good rule of thumb is the presence of > 2,500 neutrophils and > 50,000-75,000 platelets. Similarly, after the first administration of a myelosuppressive chemotherapy agent, a “nadir” cbc/platelet count is performed ~ 7-10 days after the drug is administered, but occasional later nadirs are possible with Carboplatin for example. If the neutrophil count is 1500-3000 or greater, this is to be expected and subsequent doses of this drug should continue at the same level. If the “nadir” neutrophil count is < 1500 but the pet is not sick and does not have a fever, prophylactic broad spectrum antibiotics (Clavamox, TMP-S, etc.) are instituted for 5-7 days and considerations are made for a decrease in the next dosing of that chemotherapy agent. If the pet has < 1500 neutrophils, has a fever and/or is sick (vomiting, diarrhea, etc.), then this represents an oncologic emergency, and hospitalization, blood/urine cultures, emergency fluid support and IV antibiotics are indicated. In addition, the dose of that chemotherapy agent upon the next administration will typically be reduced by ~ 25-30% to reduce the chance of subsequent severe neutropenia. The astute clinician will also remember that neutrophils are necessary for the production of a fever, therefore, a lack of a fever does not rule out sepsis.

The “A” in a “BAG” of side effects stands for alopecia or hair loss. This is a rare side effect of chemotherapy; however, it can occur in any breed of dog. Chemotherapy-associated alopecia is primarily seen in breeds with continuously growing haircoats such as Old English Sheepdogs, Terriers, and Poodles. More commonly, dogs tend to have partial alopecia with hair loss over exposed areas such as the face, shoulders, and the back. It is important to remember that shaved areas are particularly slow to regrow while on chemotherapy, and therefore these areas should be minimal and squarely shaved. Cats do not generally experience alopecia while on chemotherapy; however, loss of whiskers is extremely common. The hair/whiskers begin to regrow over the course of weeks to months once chemotherapy is discontinued, and it is generally coarser and of a slightly different color than originally seen.

The “G” in a “BAG” of side effects stands for gastrointestinal. While gastrointestinal side effects of chemotherapy are not very common, when they occur it can be a serious side effect for the patient and client alike. Gastroenteritis manifesting as vomiting and or small bowel diarrhea is seen in ~ 10-15% of dogs and cats receiving most chemotherapy protocols (because the small bowel enterocyte is so rapidly turning over), whereas nausea is thought to be seen in ~ 50-75%. This side effect may be seen ~ 3-5 days after chemotherapy administration and lasts typically for only a few days. Chemotherapy-associated colitis is extremely rare because of the slower turnover of large bowel epithelial cells, however, 25-40% of dogs experience colitis after administration of Doxorubicin.

This author routinely sends home Cerenia (maropitant) for all patients receiving chemotherapy, however, the client is instructed to use it on an as needed basis. In addition, dogs receiving Doxorubicin are sent home with Cerenia to be used prophylactically on days 1-5 (Rau et al study to be presented in the lecture) and then as needed metronidazole and/or sulfasalazine. The clients are also educated to be better able to delineate when their pet is experiencing nausea, as this can be very difficult to discern when compared to overt vomiting or diarrhea.

In addition to the more common “BAG” of side effects discussed above, there are unique side effects to certain organ systems that are generally chemotherapy-agent dependent. This includes Doxorubicin cardiotoxicity, the nephrotoxicity of platinum agents (cisplatin & carboplatin), cyclophosphamide sterile hemorrhagic cystitis, neurotoxicity of vinca alkaloids and platinum agents, allergic and hypersensitivity reactions to L-asparaginase and other drugs, dermatotoxicity of Doxil (liposome-encapsulated doxorubicin) and Tanovea, prevention of doxorubicin-related extravasation injury with Zinecard, pulmonary fibrosis from Tanovea and lastly the hepatoxicity of CCNU (Lomustine) and occasionally other chemotherapy agents. We will also discuss the side effects of Palladia and Laverdia-CA1 in more detail in the oral discussion, as well as the rarely seen syndromes of acute tumor lysis and cranial vena cava syndrome.

CE Questions by Dr. P. Bergman

When discussing with a client the side effects of chemotherapy, it is easy for the client to understand and remember the acronym a “BAG” of side effects, which stands for bone marrow, alopecia and gastrointestinal. Why are these cell types inherently so sensitive to chemotherapy?

Their DNA is less resistant to the chemotherapy

They come into more contact with the chemotherapy

They break down the chemotherapy making them more sensitive

They are rapidly dividing and growing cells

Which one of the following chemotherapy agents is myelosuppressive at standard dosages?

L-asparaginase

Vincristine

Carboplatin

Bleomycin

The lowest point that a neutrophil and/or platelet count reaches after administration of a chemotherapy agent is generally:

12 hours

24 hours

2 days

5-7 days

14 days

The neutrophil nadir for cats treated with carboplatin is X days and the recommended time between carboplatin treatments in cats is Y days (please answer X / Y).

21 / 28

14 / 21

10 / 21

7 / 21

The recommended minimum number of neutrophils and platelets prior to administration of a myelosuppressive chemotherapy agent is:

5,000 neutrophils & 125,000 platelets

3,000 neutrophils & 125,000 platelets

2,500 neutrophils & 75,000 platelets

1,500 neutrophils & 150,000 platelets

Which one of the following breeds of dogs would be most expected to experience significant chemotherapy-associated alopecia?

Rottweiler

Standard Poodle

Golden Retriever

German Shepherd

Cocker Spaniel

What drug should be sent home with clients to be used on an as needed basis for possible colitis from Doxorubicin administration in dogs?

Tylosin

Sucralfate

Clavamox

Metronidazole

Doxorubicin can cause dilated cardiomyopathy after any number of administrations in dogs, however, it is seen more commonly after what cumulative dose?

100-140 mg/m2

180-240 mg/m2

300-340 mg/m2

> 500 mg/m2

What drug can be given IV just after IV cyclophosphamide (Cytoxan) in dogs to prevent or decrease the chance of sterile hemorrhagic cystitis?

Furosemide

Dexamethasone
Normosaline (0.9%)
Thiosulfate
A cat represents to your clinic 7 days after administration of a chemotherapy agent for constipation, and on workup you find the cat to have GI ileus. Which of the following chemotherapy agents most likely has caused this??
Carboplatin
Vincristine
Doxorubicin
Cyclophosphamide

Answers:
d
c
d
a
c
b
d
b
a
b

Owner Support and education

Inge Breathnach DipVn DipAVn (Small Animal) RVN

What are your clients expecting from the oncology nurse?

A diagnosis of cancer can have a huge impact on pet carers, due to the strong mutual bond between them and their pet. The human-animal bond (HAB) provides many benefits: comfort, security, psychological safety and mental well-being amongst them. When a pet is diagnosed with cancer, this may cause pet carers to need additional support and assistance.

The veterinary nurse is well placed to provide this support. Not only do they have a well-rounded clinical knowledge of disease processes, they can explain clearly what is happening to the pet, and can build trust between the veterinary team and the pet carer. They can ensure the carer receives accurate information and understands the treatment plan, as well as assisting with more practical aspects of the patient's day to day nursing in the practice and at home. They are usually hugely empathetic beings with good communication skills, and often have more time available to discuss any concerns with a worried pet carer.

Effective communication skills are vital to this role. Poor communication has been linked to adverse outcomes in human healthcare, and it is becoming increasingly more evident that this is true for veterinary patients too. While good communication can build trust, increase client satisfaction and build realistic expectations, poor communication can be detrimental for client, patient and veterinary business alike. It may damage the pet carer's trust in the veterinary team and the treatment plan, and may lead to suboptimal patient care.

Therefore, communication should be carefully tailored to achieve the specific goals for the conversation, using appropriate body language, tone and words to impart the correct message to the correct audience. Veterinary nurses should be prepared to actively listen, clarifying what the pet

carer needs or wants to know, while focusing on their goals of care for their pet. Grief and emotional needs can be addressed and support resources signposted for the carer as needed. The veterinary nurse can also advocate for the patient, especially in situations where the pet carer is finding it difficult to consider end of life decisions.

How can we provide practical support for carers?

First of all, it is important to build a bond with the patient and their carers. This can be done even before we see the patient the first time, with the use of pre-visit questionnaires on patient preferences or conversations regarding the carer's expectations of the visit. For anxious or fractious patients, conversations regarding how best to manage the patient visit can be hugely reassuring for the pet carers, and will start to build the bond of trust between nurse and client. Opening a clear channel of communication empowers the pet carer to ask questions and look to the oncology nurse for answers when they are struggling to understand what is happening.

At the first appointment, take some time to introduce yourself, and to explain your role in the team. Clients often struggle with the large volume of information given on initial diagnosis, and provision of written instructions or information means they do not need to rely on memory to recall what the plan for the patient is. Offering to discuss any particular areas of concern, or recapping what the diagnosis means for the patient, is also often appreciated. A telephone call a day or two after discharge, or after the first chemotherapy treatment, can answer any remaining questions and ensure health and safety instructions have been understood. Where a more prolonged stay is anticipated, such as for surgical or radiotherapy patients, daily photographs of the patient in the hospital are often greatly appreciated and reassure carers greatly.

The veterinary nurse can also play an essential role in education and preparation prior to oncologic surgery or radiotherapy. By sitting and discussing the procedure - the likely outcomes, and recognised side-effects - the likelihood of uninformed consent, or complaints afterwards, decreases. This can be done by showing the carer photographs of similar patients, discussing how any side-effects or complications would be addressed, or even facilitating a meeting with someone who has had the same procedure on their pet.

As the patient progresses with treatment, there are some simple measures the oncology nurse can take to maintain communication with the carer, and to provide support as needed. Medication charts and cancer/chemotherapy diaries can help pet carers track what they should be administering at home, and ensure they are tracking any adverse effects of treatment. Many pet carers worry that telling the veterinarian about side-effects of treatment will lead to cessation of treatment, and ultimately euthanasia. Because we already have opened a channel of communication with them, carers are more likely to discuss any concerns with us, and trust us to recommend and initiate supportive care as needed, without worrying that we will immediately condemn the pet.

We can also discuss quality of life assessment with carers from an early stage, rather than waiting until it has deteriorated. Assessing quality of life when the patient is still well, allows the carer to get used to the process of doing so, and to identify when intervention may be needed to improve or support the patient's health. Ultimately, when it comes to end of life decisions, these carers are often more prepared, and will not prolong suffering when quality of life has deteriorated. This relieves a huge amount of moral stress on the veterinary team, where a pet carer wishes to proceed with futile procedures or treatments, on a patient with poor prognosis and poor quality of life.

It is clear that the oncology veterinary nurse can provide immense support for clients, and should be facilitated and encouraged to do so. This is beneficial for the team, the pet carer, and the pet. It can also be hugely rewarding for them and provide significant job satisfaction.

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The role of social media in professional communication with clients and between professionals

Inge Breathnach DipVn DipAVn (Small Animal) RVN

Setting up support networks for nurses, and safe and appropriate use of this platform.
With over half the world's population utilising some form of social media in 2021 (oberlo.co.uk), having an online presence as a veterinary professional has become much more of a focus in recent years. Whether it is for practice marketing, signposting of support networks, or just sharing veterinary information, the use of social media is cost-effective and easy to use for most veterinary professionals. Platforms such as Facebook, Twitter, You-Tube, Instagram, TikTok and LinkedIn have made it easier than ever to share what we are doing, and how we are doing it!

However, when we look at the statistics, there are some truly worrying figures. Although 55% of people between the ages of 16-74 will look for health related information online, only 23% of these users verify health content to ensure it is accurate (Eurostat). This is of huge concern when it comes to veterinary content; pet carers are likely to be looking for information and advice on their pets online, but unlikely to be checking whether this information is truly correct.

We are unlikely to be able to stop pet carers looking for information online. Therefore it is vital that we engage with our pet carers and potential clients via social media, providing verified and evidence based sources of information. Not only does this benefit the patient, this can help build trust between the public and the profession, and help us build our businesses too.

Social media can also help veterinary professionals with:

- Sharing information on common conditions
- Education on preventative medicine
- Spreading information on disease outbreaks (e.g. Alabama Rot)
- Signposting help for pet carers – bereavement and support groups

- Signposting help for veterinary professionals – Vetlife, Samaritans
- Building networks with similar minded professionals - LinkedIn, recruitement, sharing knowledge, sharing new techniques and research.
- Building connections with others – preventing isolation and stress (especially in a pandemic!)
- Business objectives – building a client base, customer service, marketing, fundraising, carer to carer support.

However, it is important to be realistic. Social media can have disadvantages too. Internet 'trolls' may attempt to provoke you by leaving misinformation, offensive messages or upsetting comments on your posts. In a world where professional and personal lives commonly overlap, this can be hugely stressful on team members, and lead to a blurring of boundaries and a reduction in work-life balance.

We also need to be very mindful of the fact that everything posted online is in a public forum, and can be retrieved easily by others. As social media becomes more normal in the workplace, social media guidelines are more commonly employed by both veterinary professional bodies and individual employers, to ensure there are no potentially damaging breaches of confidentiality or situations where a legal concern may arise.

How do we make sure we gain the benefits from social media, and minimise the negative consequences?

- Set professional boundaries – These will be individual to the person using social media, and to the reason it is used. For example, a social media manager for a large veterinary group will have different engagement rules than a small veterinary related business, who may respond to comments or queries outside normal business hours. However, we should set boundaries in line with the service we expect to provide to the audience. We may wish to state hours of operation, discourage patient queries online, or post disclaimers regarding the information provided (such as 'this information does not replace advise given by the veterinarian overseeing your pet's health care').
- We should endeavour to always show respect and courtesy for each other and for other professionals.
- Provide evidence-based information - Ensure all information provided is correct, up-to-date, and verified. References should be provided if possible.
- Be aware, and abide by, guidelines – in your place of work, supplied by your governing body, and by the veterinary nurse or veterinary surgeon professional associations.
- Maintain patient confidentiality - Be aware that often carer consent for social media on consent forms is related to the use of that information by the practice, and not by an individual posting on their own social media account. If in doubt, the identifying information should not be posted.
- Take care with issues such as conflict of interest, sponsored or gifted posts, or recommendations. It should be made clear to the viewer/reader if you have any specific vested interests in promoting a product.
- Workplaces should always have clear social media guidelines. They should be easy to understand, and discussed carefully with staff members. If there are breaches of guidelines, it may be due to a lack of understanding rather than intentionally.
- Operate a zero-tolerance policy to harassment of staff. Block/mute/call out any bad behaviour by people online.
- Take care not to connect private social media to practice social media – It may potentially affect your own or your staff members privacy.

In conclusion, social media can definitely add huge value to the services you offer your pet carers and the wider community. However, please do think before you post:

- Could this post be misconstrued?
- Is this evidence-based information, and have I referenced appropriately?
- Is this in line with practice values?
- Am I breaking any guidelines/do I have permission to post this?
- Am I signposting resources as needed?

If you can confidently tick all of these boxes, you are likely to be providing an important source of information to a wider audience, while keeping yourself and your practice safe.

References and further reading:

RCVS social media guidelines: https://www.rcvs.org.uk/setting-standards/advice-and-guidance/code-of-professional-conduct-for-veterinary-surgeons/supporting-guidance/social-media-and-online-networking-forums/

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Specialising as an oncology nurse

Nicola Read

This will be an inclusive session to conclude the conference, participants are invited to share their knowledge, experience and ask questions as to where their learning has taken them so far and explore which areas they are keen to develop. We will be joined by Ms Susan Howarth, Principal Lecturer and Programme Director at Harper Adams University to discuss the further education pathways available to our nursing and technician colleagues

Some of the subjects we will be covering:
What skills help us within our clinics (practical, professional, administrative)?
What options are there currently in Europe for continued professional development?
Do we want recognition for specialising in oncology?
Are there any formal oncology nurse-technician qualifications available to me?
Is my education / are my qualifications internationally transferable?

This will conclude the nurses' stream for 2022, we hope to see you again in Alicante, Spain in 2023

Vomiting and diarrhea management in dogs and cats

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Key-points:
Vomiting and diarrhea are a frequent post-chemotherapy clinical sign.
In this context, if the owner calls the Vet Clinic/Hospital, the nurse is supposed to assess the relative seriousness of the situation and offer an appropriate return to his request.
Since nausea, vomiting and diarrhea can be due to a variety of problems, different tests might be done to help sort out possible causes: thus, a diagnostic workup is needed if the clinical background is unusual (i.e., vomiting or diarrhea do not appear in the predictable period after chemotherapy administration).
Even if vomiting and diarrhea are common disorders encountered in veterinary oncology, their appropriate treatment is most often associated with a positive outcome.

The most common signs of gastrointestinal upset in small companion animals are anorexia, nausea, vomiting and diarrhea. Many causes can to these acute conditions: food intolerance or poisoning, parasites and viruses, or a more severe cause including metabolic diseases (such as kidney/hepatic failure), pancreatitis, and any drug administration.
If a concurrent treatment (i.e., chemotherapy) is supposed to lead to the underlying problem, it can be as simple as temporarily prescribing medications and/or withholding the administration.

Chemotherapy and adverse side-effects.
Chemotherapy is prescribed to treat systemic neoplasia (hematologic malignancies), metastatic solid tumors, nonresectable neoplasia, cytoreduction.
Cytotoxic chemotherapy drugs show different mechanisms of action. The aim of the treatment is to kill fast-growing cells. No distinction is possible between normal fast-growing cells and fast-growing tumor cells. This is called "non-specific toxicity". For this reason, a maximum tolerated dose (MTD) is defined for of each medication. Even if side effects are not systematic, their incidence varies from 20 to 40%. Severe side effects represent less than 5% of cases. Gastrointestinal (GI) cells are particularly damaged by chemotherapy drugs: clinical signs are generally self-limiting and reversible. They include anorexia, nausea, vomiting and diarrhea. It is common to pre-treat animals receiving treatment.
Cisplatin and doxorubicin are the two most common cytotoxic agents associated with GI toxicity. Clinical signs most usually appear from 2 to 5 days after administration and resolve in a few days with adequate treatment. Cisplatin is known to generate nausea and vomiting during its administration, directly stimulating the vomiting center (CTZ).

How to evaluate the severity of the case?
The role of the nurse is:
to assess the severity of the case and to recommend a first treatment at home if needed.
to ask the owners if the animal shows a serious impair or abnormal general condition.
the discussion with the owner is essential: the nurse must ask the owner to bring back his pet at the clinic/hospital to be fully stabilized if a poor condition/emergency is suspected.

Your veterinarian needs important information about the pet's general status. It is important to note whether the animal walks and acts as usual, drinks normal amounts water and/or eats normally.
Assessing the pet's gastrointestinal activity is essential: appearance of the vomiting or diarrhea, amount, and frequency: photos and description may help. Potential blood detection is needed: fresh blood in vomiting can result from gastric ulcerations. Digested blood indicates a small intestine lesion or is noted in some metabolic diseases, while fresh blood in the stools confirms a colonic lesion.

How to avoid pitfalls? Diagnostic work-up (Clinical cases presentation).
In this context, the main pitfall is to consider that the general condition is related to the ongoing treatment, ignoring the disease that causes directly the gastrointestinal clinical signs at that time.
Common causes of vomiting, diarrhea hematemesis and abdominal pain are supposed to be background knowledge.
Table 1 and table 2 depict the etiology of these clinical signs. (From K. Simpson, OVMA, 2020 proceedings).

Causes of Vomiting	
Gastric	Gastritis , Ulceration, Neoplasia, Outflow obstruction, Foreign bodies, Motility / functional disorders
Intestinal	**HGE**, Inflammatory Bowel Disease, Neoplasia, Foreign bodies, Intussusception, Functional disorders
Intra-abdominal non-GIT	
	Pancreas Pancreatitis, Pancreatic Neoplasia
	Liver Hepatitis, Cholangitis, Biliary Obstruction
	Spleen Torsion
	Genitourinary Nephritis, Pyelonephritis, Nephrolithiasis, Urinary obstruction, Prostatitis, Pyomertra,
	Peritonitis
Metabolic / Endocrine	Uremia, Hypoadrenocorticism, Diabetic Ketoacidosis, Hperthyroidism, Hepatic Encephalopathy, Hypercalcemia, Septicemia
Infectious	Distemper, Parvovirus, ICH, Leptospirosis,Feline Panleukopenia, Heartworm (cat), FIP, Salmonella, FeIV/FIV related
Drugs	Digoxin, Erythromycin, Chemotherapy, Apomorphine, Xylazine
Toxins	Strychnine, Ethylene Glycol, Lead
Dietary	Indiscretion, Intolerance, Allergy
Neurologic	Vestibular disease, Encephalitis, Neoplasia, Raised intra-cranial pressure
Infectious	Distemper, Parvovirus, Infectious Canine Hepatitis, LeptospirosisFeline Panleukopaenia, FIP, Salmonella

Table 1: causes of vomiting

Causes of melena / hematemesis		
Gastrointestinal erosion/ulceration		
	Metabolic	uremia (?), severe liver disease, hypoadrenocorticism
	Inflammatory	gastritis, enteritis, **HGE**
	Neoplastic	leiomyoma, adenocarcinoma, lymphosarcoma
	Paraneoplastic	mastocytosis, hypergastrinaemia/ other APUDomas
	Vascular	A-V fistula, aneurysms
	Ischaemia	hypovolemic shock , hypoadrenoscorticism, thrombosis / infarction, reperfusion
	Foreign objects	
	Drug induced	non-steroidal and steroidal anti- inflammatories
Coagulopathies	thrombocytopenia, factor deficiencies, D.I.C.	
Ingestion of blood	oral, nasal, pharyngeal, pulmonary bleeding	

Causes of abdominal pain:	
Gastric	Dilatation/volvulus, ulceration,
Intestinal	Obstruction, intussusception, rupture, torsion, acute enteritis, **HGE**
Pancreatic	Pancreatitis
Hepatic	Acute hepatitis, ruptured bile duct, hepatic neoplasia
Splenic	Torsion, ruptured neoplasm
Urogenital	Nephritis, pyelonephritis, ruptured bladder, ureteral / urethral calculi, pyometra, prostatitis
Peritoneum	Primary or secondary peritonitis (e.g. chemical - bile and urine : septic- ruptured viscus)
Musculoskeletal	Discospondylitis, prolapsed disc

Table 2: causes of melena/hematemesis and abdominal pain

Table 3 resumes the main causes of diarrhea. (From Tello Luis & Perez-Freytes Rossana "Fluid and Electrolyte Therapy During Vomiting and Diarrhea" Vet Clin Small Anim 47 (2017) 505–519).

Common causes of diarrhea in dogs and cats
Alimentary Disorders
• Food indiscretion
• Hypersensitivity
• Food allergy
• Food poison/toxicity
• Food change
• Excess of food
• Trash consumption
Metabolic/Inflammatory disorders
• Stress
• Sepsis
• Inflammatory bowel disease
• Pancreatitis
• Lymphangiectasia
• Stress colitis
• Hemorrhagic gastroenteritis
• Hepatitis
• Cholangiohepatitis
• Chronic kidney disease
• Hyperthyroidism
• Hypoadrenocorticism
• Exocrine pancreatic insufficiency
Neoplastic Disorders
• Carcinoma
• Lymphoma
• Intestinal stromal tumor
Medications
• Laxatives
• Chemotherapy
• Nonsteroidal anti-inflammatory drugs
• Antibiotics
Infectious
• Virus: parvovirus, coronavirus, distemper, rotavirus, feline leukemia, feline immunodeficiency
• Parasites: giardia, helminths
• Bacteria: *Campylobacter, Clostridium, Salmonella, Escherichia coli*

Table 3: Main cases of diarrhea in small companion animals

A complete physical examination is needed, even if the cause seems to be due to the ongoing chemotherapy administration. The assessment of hydration status is mandatory. Some diagnostic tests may be needed. These globally include:
Complete blood count (CBC): cytopenia is frequent during chemotherapy (myelosuppressive toxicity). Moreover, total leucocytes count is needed to exclude leukocytosis (infection).
Biochemistry panel (kidney disease, hepatic injury, pancreatitis…)
Fecal testing if needed (parasites, several samples may be needed if a diarrhea is reported).
B12 dosage (if a diarrhea is observed or if a GI disease is ongoing, it is essential to prescribe B12 complementation).
Abdominal ultrasonography: a crucial step to investigate all the gastrointestinal diseases. An extensive abdominal ultrasonographic evaluation is mandatory to assess all the abdominal organs (concurrent cholangitis, acute pancreatitis, chronic kidney diseases, urinary lithiasis, prostatic lesion…).
X-ray: exclude a gastrointestinal foreign body, check the lung diseases.

Chemotherapy and GI signs usual treatment.
Oncology case management implies adjunctive therapies as a means of improving the quality of life in veterinary cancer patients.

In the event of chemotherapy-induced gastrointestinal clinical signs, the following treatment can be instituted:
Liquid diet for a few hours.
Hyper digestible feeding in divided doses.
Fluids and electrolytes if needed (it is the most essential therapeutic measure in dogs or cats suffering from dehydration caused by gastrointestinal losses (vomiting and diarrhea). Fluid volumes are calculated based on the hydration status if the pet is poorly perfused. The rehydration phase is defined based on percentage of dehydration (dehydration % X BW [kg] X 1000 = mL of fluid deficit), ongoing losses (vomiting and diarrhea), and maintenance requirements. If the pet is febrile, 10% of maintenance fluid should be added. The objective is to restore 80% to 100% of the fluid deficit within a 24-hour period.
Pain management
Animals receiving cytotoxic medications need preventive antinausea/anti-emetic treatment 30 mins to 1 hours prior to administration:
Maropitant citrate: 1 mg/kg SC ou 2 mg/kg PO SID; (A study confirmed that the use of maropitant citrate for five days following doxorubicin administration notably decreased the amount and intensity of vomiting)
Metroclopramide: 0.5 mg/kg BID
Ondansetron hydrochloride 0,1-0,2 mg/kg slow IV injection every 6/12 hours.
Antacid treatments and gastroprotectants:
Proton pump inhibitors: Omeprazole/pantoprazole: 1 mg/kg PO BID
H2-blokers: Cimetidine: 5 mg/kg PO TID, famotidine
Gastroprotectants : sulcralfate, aluminium phosphate
Symptomatic diarrhea treatment include :
Diosmectite
opiate antidiarrheals (loperamide)
metronidazole (if strictly necessary, i.e. if bacterial translocation may occur)
Orexigens if needed : mirtazapin, cyproheptadine.
Adapted diet.

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antinfiammatoria
e **antidolorifica,**
per gestire a **360°** questa
patologia nel cane e nel gatto.

Boehringer Ingelheim

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