Kristie Amobi: Hello. My name is Kristie Amobi and I work for the product division with Xstrahl. Welcome. I'm pleased to introduce today's speaker, Dr. Olivier Keravel from Eiffelvet who will tell us about his experience in veterinary oncology referral and how he treats patients using orthovoltage radiotherapy. Dr. Keravel was trained in oncology in the early '90s in France at the Gustave Roussy Institute and in the United States at The Ohio State University. Dr. Keravel created Eiffelvet in 2014, a referral veterinary clinic dedicated to oncology only.

Although oncology is a growing specialty in the veterinary care, it can be expensive and time consuming for veterinary practices to implement radiation treatment equipment to support cancer therapy and pain management. Dr. Keravel has shared cases in the past and will present a few new cases today. He will also provide more information about his decision to use orthovoltage therapy instead of a linear accelerator. Thanks again for your attention. And with that, I'll hand it over to Dr. Caravel.

Dr. Olivier Ker...: Thank you, Kristie. Hello, everyone. Thanks again to Xstrahl for inviting me to talk about orthovoltage. Please forgive me if my English is not perfect. I will try to do my best.

So, the last time, the last webinar, I tried to show you that orthovoltage was the device, among others, to do radiation therapy in cats and dogs. And in this webinar, I'd like to show you, to emphasize the specificity of orthovoltage and somehow maybe, I believe, sometimes even better for radiation therapy than linac, for instance.

So, the first question of course, as the last webinar is why orthovoltage? I will try to go a little more deeply into why I've been choosing orthovoltage. First of all, you have veterinary experience in the field and while I was training at Ohio State during a VCS meeting at that time where only 30 or 40 in the room, I met Alice Villalobos and she became a close friend of mine. She just retired. She taught me a lot and I kind of dedicate her this lecture.

And since I was eager to learn during my stay in the United States, I spent time in her clinic in LA. It was at that time one of the first private clinic dedicated to oncology only. And I realized when I was treating a pet with cancer in Alice's clinic more than anywhere else because everything was arranged to be able to take care of the pets and not only giving medication but also looking at the quality of life, preparing the owner for their grief and orthovoltage was part of this clinic and was perfectly fit in to go forward and preserve the quality of life. Remember that, at that time, there was no other solution basically than orthovoltage. It was not the same machine. There's a learning curve since then in the veterinary field about radiation therapy.

So, what we do today with orthovoltage might be a little different from what Alice was doing, but she was, at this time, already practicing, for instance, intraop radiation therapy with orthovoltage. She was using it for palliative, for bone cancer, et cetera, et cetera. So, there is some experience in the vet field

with orthovoltage and most of the articles unfortunately originated in this period, '80s, '90s. So, whether or not we can rely a hundred percent on these articles, I don't think so.

And then, after my training at Ohio State, I came back in France and I end up opening an imaging facility because there was not city at this time in France. It was back in '19.

I opened the imaging facility in 2000. So, I started training during the year 1996, 1997, and I got my training in human medicine with human radiologists either in CT scan or MRI. And while I was spending time in neuroradiology at the Tenon Hospital, which is a big hospital within Paris, I had the chance to meet an old radio oncologist, radiation therapist. He's now retired for a long time. And I then discuss with this guy and we end up talking about orthovoltage because he used to have an orthovoltage that he had to abandon when the cobalt machine came in. And then when the cobalt came in, the nuclear authority asked to the radiotherapist to be able to give precise dosimetry on a millimeter basis, which was not possible to do with orthovoltage. It's still not possible to do dosimetry with orthovoltage like we do with linac.

So, he went on using the cobalt unit and the linac, of course. And so, I was discussing with him and he said, "Well, this millimeter basis is okay, I can understand why to preserve the normal tissue to be as efficient as possible and more as precise as possible. But sometimes when you deal with margins in oncology, the orthovoltage was pretty good."

And, with this, Alice Villalobos, this guy in Tenon Hospital, this orthovoltage stayed in my mind. And when I decided to open a new facility back in 2013, I had to choose and then comes in the ethical considerations because this is something that I've always done in my career, trying to put myself in a situation where I don't depend from the bank. Meaning if I had to choose for a linac, the price of linac, I do not know how to make money with this linac in France, in Paris with the price of the building, the shielding.

So, if you do an oncology consult and you have somewhere back in your mind that you need to pay your loan, you may end up not being totally free in what you're going to propose as a protocol, what is best for your patient because you have the loan to pay and then you may end up pushing towards a solution which is probably more expensive than another or more demanding for the patient than another because of the loan. Maybe if it's not conscious, it's not direct in your mind. But still, to me, that's important. So, in this sense orthovoltage, because the price of the shielding is smaller because the size of the room needed for orthovoltage is smaller, was adapted to these considerations.

And then you have to think of the ease of use of the machine, but not for you but for the pet and how long is the anesthesia, et cetera, et cetera. And as a vet community, we want to propose some options of treatment that could be used

in as many patients as possible and used by as many clinics as possible. Because the problem with linac because it is so expensive, is that you end up treating very well a very small percentage of your patient and maybe we may try to promote a solution which can allow us to propose and treat more patients and these ethical consideration. I think orthovoltage is interesting because real life when you're using radiotherapy, again, it needs to be affordable to as many client and clinics as possible. And we are often going for palliative treatment just to preserve quality of life, not necessarily curative intent protocol, an interop solution because all the soft tissue tumors when you go for carcinologic surgery, then you will need to try to control the tumor bed as well as possible at interop, as I will show you, is a very interesting solution.

So, all this taken together, the answer is probably orthovoltage 300kv. So, we went for that. But, remember, it is a radiotherapy device, so you still need expertise to use this machine. You don't need to consider orthovoltage like a low cost radiation therapy device. You need expertise.

So, some general consideration, I would go quickly over some things that I've went through during the previous webinar, but still it's interesting to keep in mind. First of all, radiation therapy is a local treatment. Okay. You may have abscopal effect. I never encounter it. It's only in the literature that you find cases where you irradiate the primary tumors and you see the mass going away, but that never happened to me. So, it's a local treatment.

You need CT scan. I told you later that when I came back from Ohio State, I got trained in imaging and the question was CT or MRI. It is still, that's another question. It's like maybe orthovoltage versus linac. I think CT scan will allow you to do very correctly 90% of your indication in imaging and CT scan on a topographic point of view is absolutely excellent and necessary for radiation therapy. So, we prefer in oncology at Eiffelvet use CT scan first line.

Just a few rule of thumbs. Remember that sarcomas are radio resistant. So, we're going to talk about fibrosarcoma in cats, but remember that, generally speaking, all the sarcomas, cats and dogs, are, macroscopically speaking, radio resistant. But on the contrary, mast cell melanoma carcinoma, very radio sensitive. And look at your past report because you have always many indication there and you know that when you do radiation therapy, you're going to hit the DNA and when the cell is going to go into mitosis, it's going to die. So, you need a good mitotic index. The more mitosis you have, the more efficient you'll be with your radiation therapy. It's not always curative, but that's okay. That's okay. We told that. We discussed this already. I'm a vet, I'm not a human MD, and I will aim specifically at the quality of life rather than the duration of life. You can use it for palliative, analgesic, osteosarcoma, you can use it for palliative when you know you're not going to cure, but you give comfort, quality of life, nasal cavity, all cavity tumors, mediastinal tumors, and you can use it, of course, during or after surgery.

So, as an external radiation therapy solution, we decided at Eiffelvet to go for orthovoltage. We know there are some indication we will not be able to propose to our patient. For instance, a glioma in a Boxer, for instance, a nasal cavity tumor in a German Shepherd. That goes well back to the cribriform plate. Of course, we will recommend linac, but orthovoltage on the other hand, despite the image of being superficial, in fact, could be an advantage on all the cutaneous and subcutaneous tumors in canine and in feline.

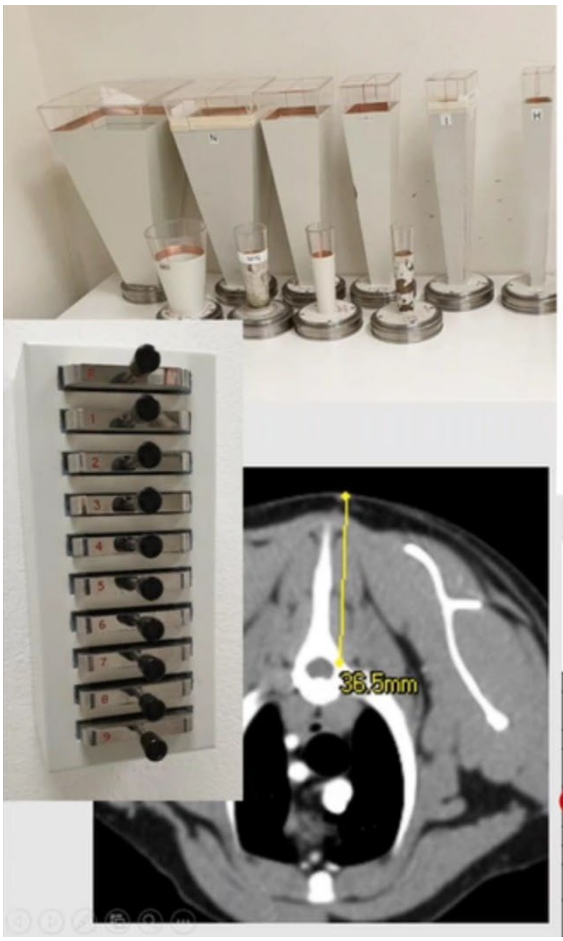
So, these are some cases we went through the last time. That's a palliative treatment hypofractionated on an osteosarcoma, that's a third ID'd lymphoma. We're doing a curative intent protocol and that's a curative intent protocol on the mast cell tumor. And this is an intraop radiation therapy with a cat under the sheet.

Okay. So, when you do radiation therapy, whatever the device you're using, you're facing side effects. Immediate side effects because by you're going to irradiate quick proliferative tissue, we're talking about the skin, we're talking about the mouth, the gingiva, we're talking about the digestive apparatus and the cornea. It does happen. You do not encounter immediate side effect if you go for hypofractionated palliative protocol. And that's a consideration for the owner because they know there will have much less chance to encounter this burn in the mouth of the skin that really impair negatively the quality of life of the patient. So, basically on the skin, the cat's skin is less sensitive to this immediate side effect than the dark skin.

For the leg side effect, well, you can have change of color in the hair like you see down on the right. This is for the slow proliferative tissue. So, when you do hypofractionated radiation therapy, palliative protocol, you're exposing yourself to more late side effects. And these side effects can be problematic. It could be fibrosis, it could be mainly in the bone, the famous bone necrosis that you can encounter. And that has been identified with orthovoltage in the early publication.

Now, again, the learning curve has changed over the last 30 years. So, at Eiffelvet, is it a concern for us having bone necrosis following hypofractionated radiation therapy with orthovoltage? Yes, it is. Is it something we often face? No, it's been a little 10 years and I could count on my two hands the number of cases where I suspected to have bone necrosis for my protocol and especially there were oral tumors, either squamous cell carcinoma in cats or melanomas in dogs. The problem is we should have done some biopsy to identify the bone necrosis from radiation therapy, which we did not because of course it's also the process of the disease to come back and probably to cause necrosis of the bone. So, how do we differentiate it just clinically or on CT? It's not obvious.

And in these patients where they had big, ugly tumors usually that come back, it's difficult to propose the client to do biopsy and to be able to tell them, "Well, that's not the tumor. That's, in fact, the radiation therapy." So, it can happen but we are careful.



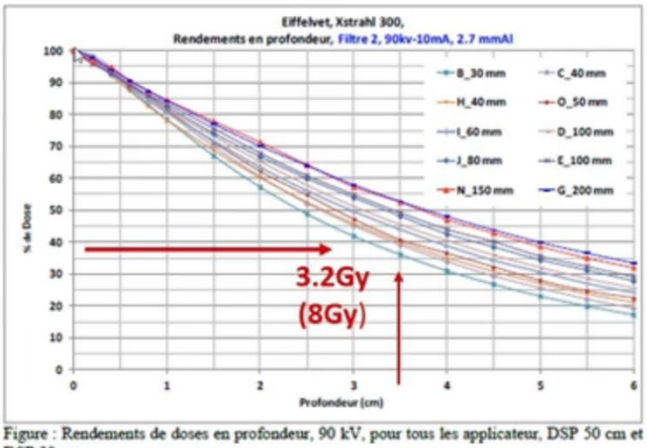


Figure : Rendements de doses en profondeur, 90 kV, pour tous les applicateur, DSP 50 cm et DSP 30 cm.

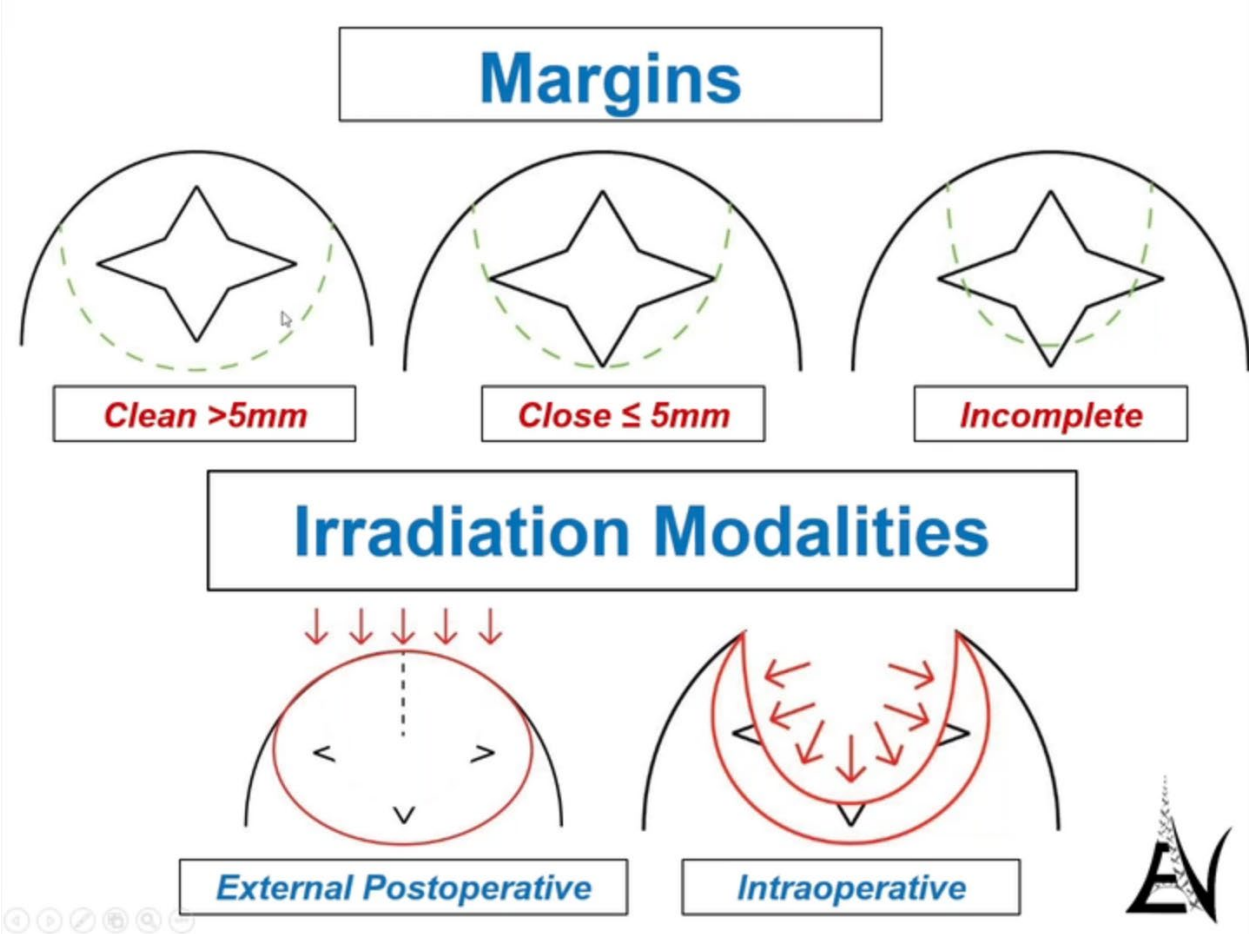
Tableau III – Tableau regroupant les doses de tolérance (HDV, dose maximale, dose moyenne) ayant fait l'objet d'un consensus fort ou relatif en 2007.

Ces niveaux de dose peuvent éventuellement être dépassés sous réserve d'une justification liée au contrôle local et à la survie du patient, après information et accord de celui-ci. Ces dépassements sont notamment possibles lorsqu'ils concernent des organes à risque dont les lésions radiques n'ont pas de conséquences vitales.

Organe sain (organe à risque)	Dose de tolérance
Parotide controlatérale	V26 ≤ 50% Dose moyenne < 30 Gy
Tronc cérébral	Dose maximale de 50 Gy
Articulation temporo-mandibulaire, notamment controlatérale	Dose maximale de 65 Gy
Moelle épinière	Dose maximale de 45 Gy
Larynx	Dose maximale de 50 Gy
Chiasma	Dose maximale de 54 Gy
Conduit auditif, oreille moyenne et interne	Dose maximale de 50-55 Gy
Œil	Dose moyenne < 35 Gy
Poumon sain	V20 ≤ 35 % V30 ≤ 20 %

When we do radiation therapy with orthovoltage, we have a physician that comes in regularly just to make sure that the dose we think we're giving is really the dose we deliver. You have a hundred percent of the dose at the skin depending on the type of filter and applicator you're going to use. Well, usually you go down to 50% of your dose down to four. You see, that's 50% of your dose here around three-and-a-half, four centimeters. So, if you put eight Gray here, then you're going to give 3.2 Gray there and then if you want to see what's going to be the problem with the middle line right there, well that's 36.5 millimeter. So, that's the dose you're going to give and that's the maximum tolerable dose for the spinal cord. So, for its tissue, you look at this and you build your protocol being careful of the potential side effects of normal tissue.

There are some other modalities for radiation therapy, for soft tissue sarcoma and fibrosarcoma in cats, especially in France, brachytherapy has been used because historically it's been developed. This is technique that's been developed in France back in the '70s, '80s, which we chose interop radiation therapy. I felt that because I'm not sure this brachytherapy technique is in fact very efficient most of the time. And I will explain you why.



These are the margins. This is the tumor with the star shape. When you try to get the margins with the carcinologic surgery, we want five millimeters all around the tumor. Remember this? This is important because that's something we want at Eiffelvet. Maybe in another clinic they'll go for three millimeter. The basic behind this is that the pathologist in veterinary medicine is going to cut vertically and transversely and look at the margins here, here, here, here. As in human, they're going to do tangential margins, they're going to do this way all around the tumor in different planes.

So, we know from different articles in the vet literature that you can reclassify some clean margins into dirty margins if you go for tangential margins, study in pathology, but it's just not possible practically because it's slow and expensive. So, usually they do one or two cut, one or two cut.

So, what if here you have the margins but you only have one or two millimeters? Maybe the next millimeter where you don't look at the margin, maybe you infiltrate it. So, this is why we are aiming at five millimeters all around. If it's close, less than five millimeter, then we're not a hundred percent happy. If it's incomplete, well, we're not happy at all.

So, the idea is that if you go for surgery and you know that you may left little piece of tumor in there, it's right there. Then you go for surgery, take what you think, what you can, that's the shooter and this is the volume you need to irradiate when you do external adjuvant postoperative radiation therapy, which is the standard of care for soft tissue sarcoma after surgery after CT.

But what if we do intraop? Because when you do intraop, you give exactly the radiation therapy where you want it to be and you spare the normal tissue and you give probably more dose of what is needed just directly where you need it.

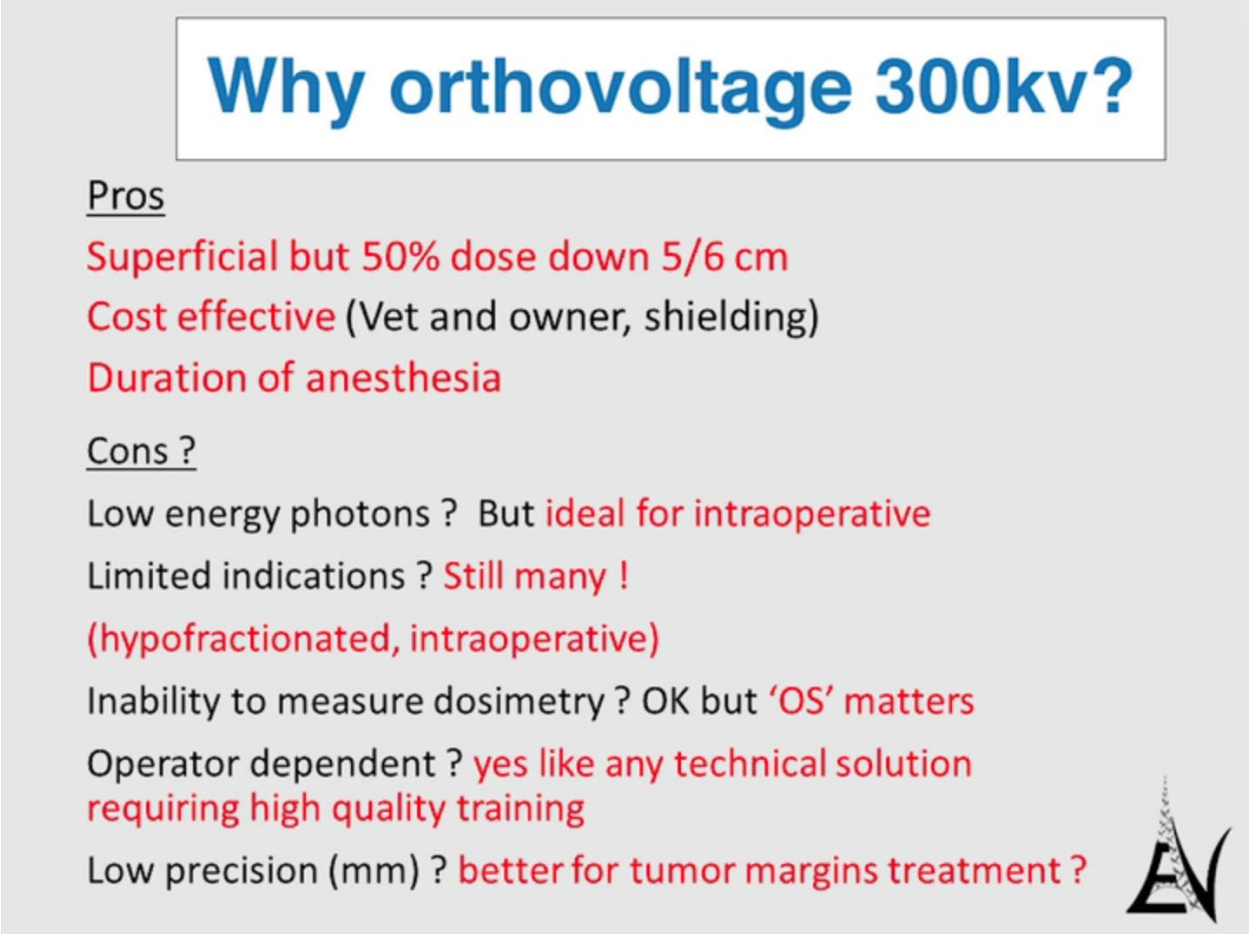
When you look at the brachy catheter, you can deliver a high dose of radiation on a small volume. So, for it to be efficient, you need your oncosurgeon to position the catheter while the iridium will go through during the surgery right there. Because if you rely on the scar to position your catheter as it has been done for years in France, well, how do you know you're at the right place? So, there are too many failure with this solution, no publication. So, that's why we really wanted to try intraop radiation therapy and orthovoltage was well fitted for that.

Okay, it's superficial, but as we said, you can sometimes have 50% of your dose down to five to six centimeter. It's cost effective for the vet, for the owner of the shielding. Duration of anesthesia is an issue when you go for treatment, especially if you do a curative protocol three to five times a week.

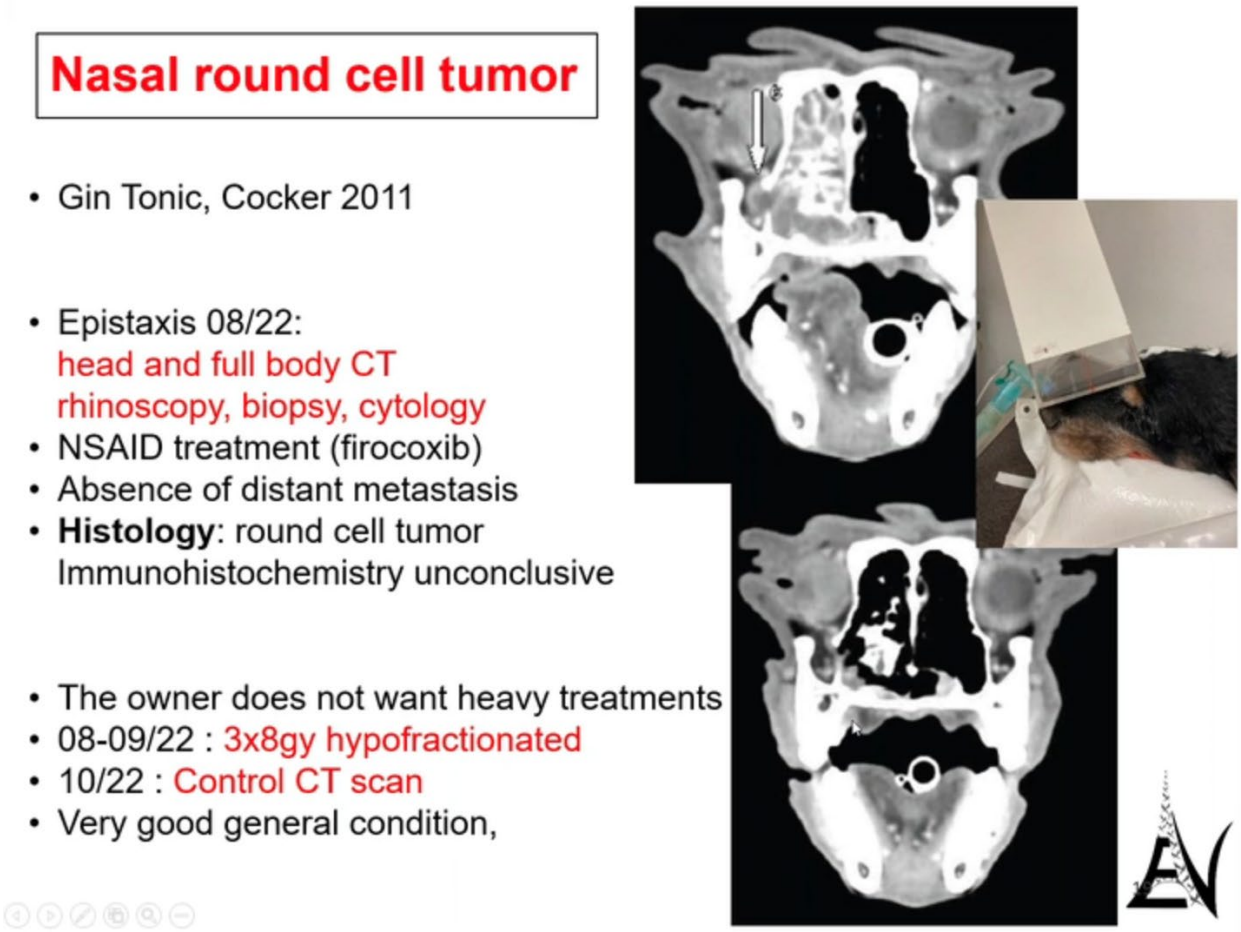
So, of course, these are low energy photons, but you will see that it might be ideal for intraop radiation therapy. Okay, limited indications, yes. There are some indication that you need to know your limit, but this is the case for any type of device. But there are still many indication you can deal with very correctly with orthovoltage, especially the palliative protocol and the interop protocol.

Okay, you cannot do those imagery, but this is something very important you have to keep in mind. What matters the most is the overall survival and, well, that's something I really believe. We need collectively to think about this because what if we develop some very fancy protocol, very expensive protocol for the owner and what if it doesn't make that much of a difference for the overall survivor? So, this is something really we need to consider.

Operator dependent. Yes, it is operator dependent, but any radiation therapy protocol is operator dependent. And this is like for any technical solution requiring high quality training. Low precision in millimeter. That's a difficult matter to assess when you do radiation therapy because you don't want side effect in normal tissue but you want to control the tumor. And as this old radiotherapist told me, "Well, it was not that bad orthovoltage to control the tumor margins." So, in total, orthovoltage is an interesting solution.

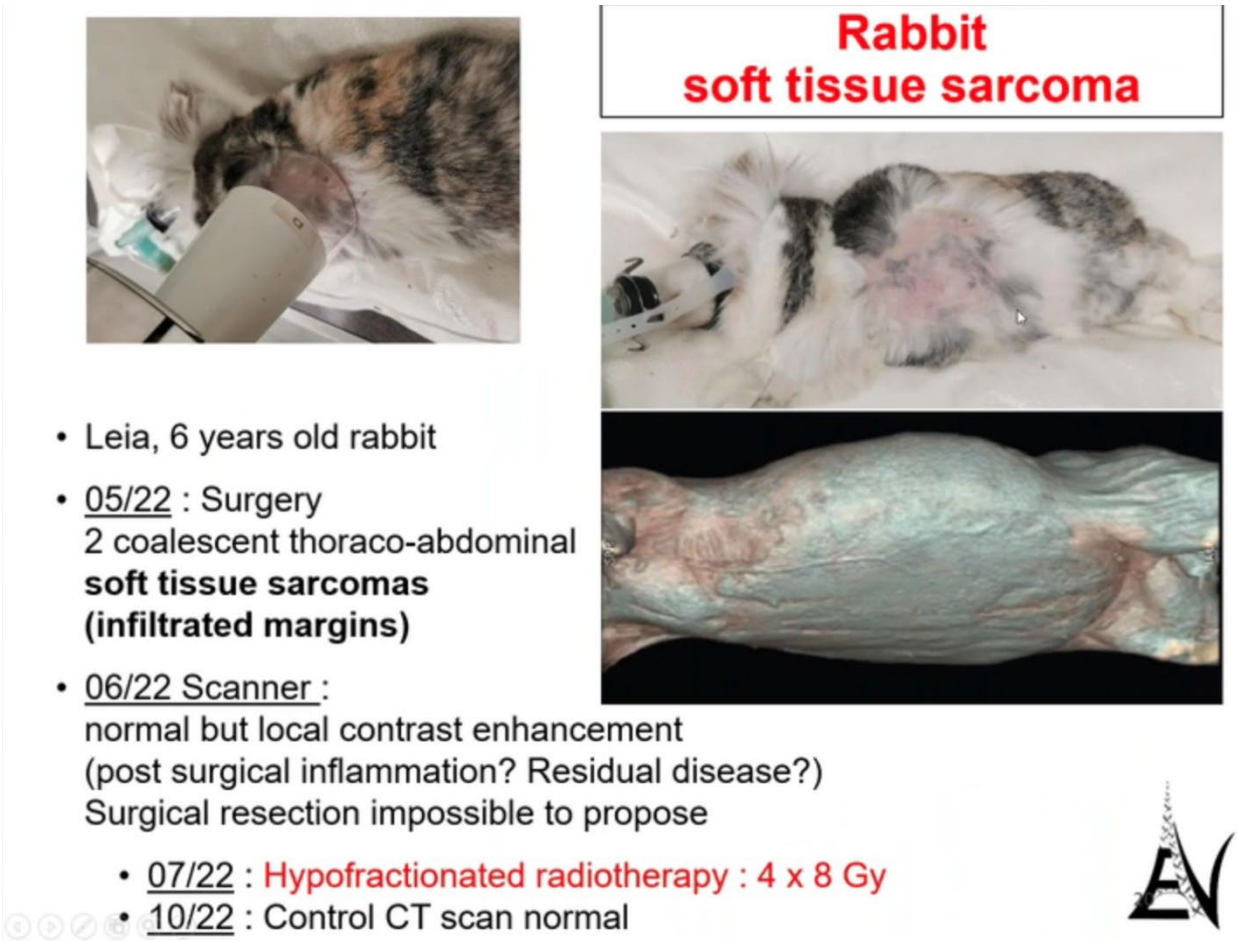


So, the way we use it at Eiffelvet, these are the protocols. I'm now going to go into the curative intent protocol. I will more focus today on what I think is very interesting with orthovoltage is the palliative protocol. So, usually we do three to four stations of 8 Gray, 10 Gray and we use the same dose for intraop radiation therapy.

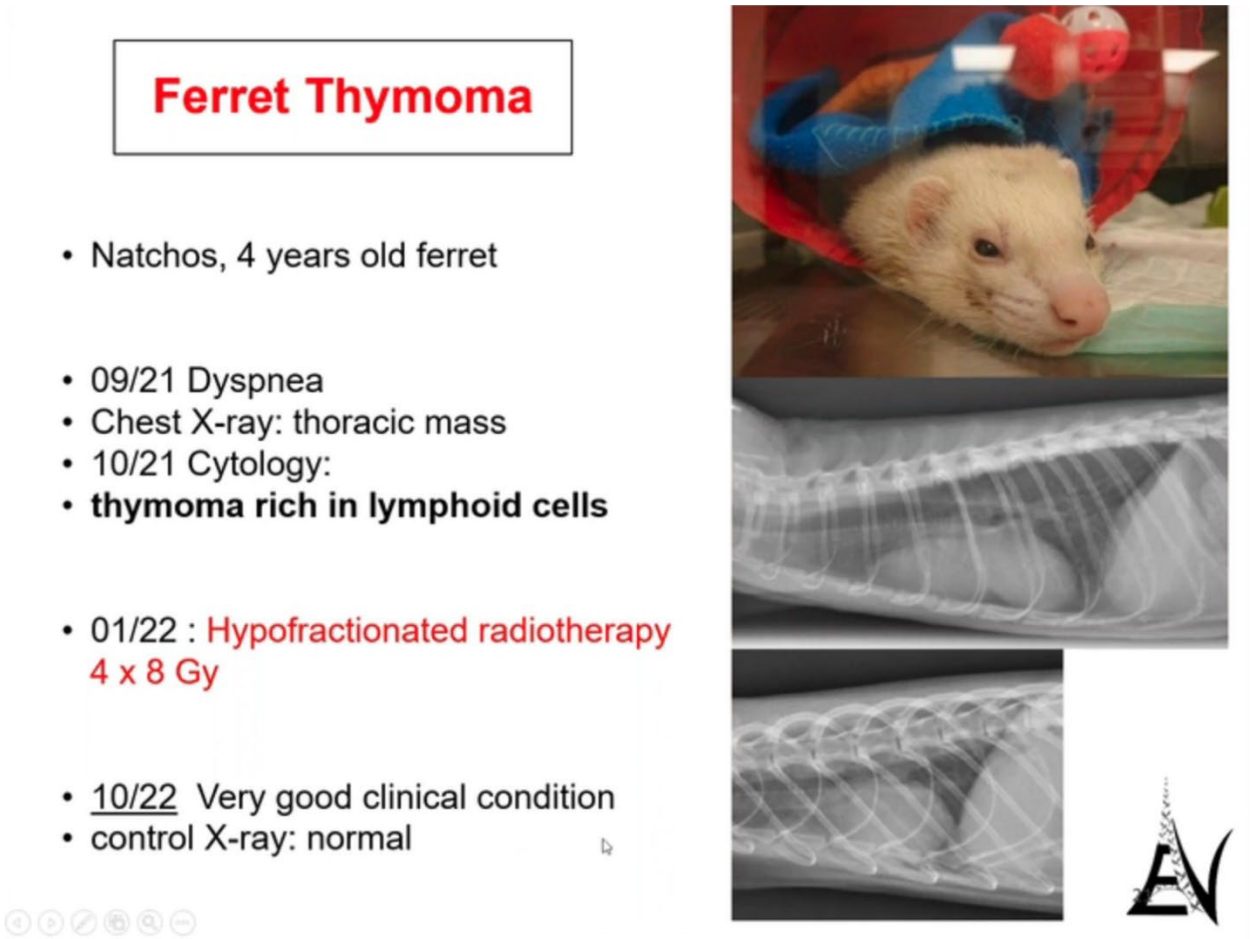


So, I just decided to give a few examples different from the one that I presented you during the last webinar. Gin Tonic, nice name, nasal round cell tumor. It's a dog with epistaxis. So, we do the CT head and full body, we do rhinoscopy to get our biopsy. We do cytology of the lymph nodes. We give him NSAID, well, firocoxib, because of the antiandrogenic effect. There's no distant metastasis. We wait for the histology. Comes back round cell tumors. But even with immunohistochemistry, impossible to conclude. And the treatment on the owner on the other end doesn't really want heavy treatments. The dog is already 11-years-old. So, we go for three session of hypofractionated radiation therapy and we do a CT scan in October 2022. So, very recent.

Look. You don't get rid of all of the tumor but you have a very good response. Dog is happy, the owner is happy. That doesn't mean it's going to last forever, but it's a nice thing to have a happy dog and a happy owner in your practice. And, of course, when we proposed this protocol, we knew it was a round cell tumor so we knew we had every chance for radiation therapy, even with small dosage to be efficient because it's more sensitive to radiation therapy than would be a sarcoma or a nasal carcinoma in there.



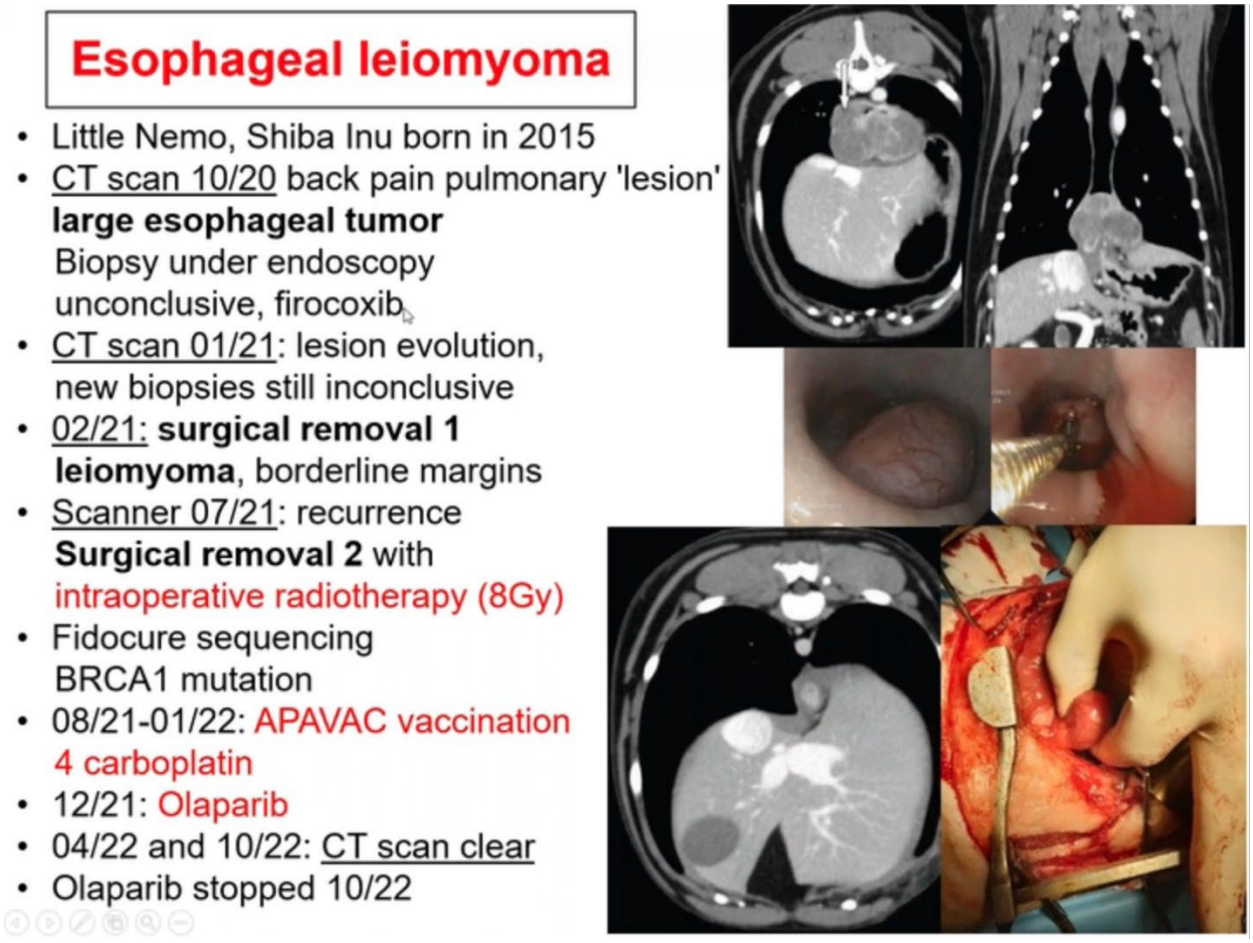
So, that's a rabbit. And this bunny had two soft tissue sarcomas, coalescent right there. This is where the surgery was done. So, unfortunately, infiltrated margins, it was difficult on this bunny to go back for surgery. So, we did the CT, we had local contrast enhancement, but you see it's a month after the surgery. Always difficult to know when you have enhancement in the region of the scar tissue. Is it just normal postsurgical inflammation healing or is it residual disease? Anyhow, you have infiltrated margins. Difficult to go back for surgery in such a small baby. So, we propose hypofractionated radiation therapy for dosage and that was back in July and we've done a controlled CT in October, which was normal. So, we happy with this bunny. Again, happy bunny, happy owner, happy vet.



That's a ferret. Natchos has a mass on chest x-rays right there. You can see it. Cytology is done. It's a thymoma, rich lymphoid cells. So, you have every chance to be efficient with radiation therapy no matter what type of machine you're going to use, of course.

And where we decided to go for hypofractionated radiation therapy, 4 x 8 Gray, simple protocol for small anesthesia and 10 months later, we just got news from the Natchos. Very good clinical condition and that's a controlled x-ray at the vet. Well, there's probably still something there, but the mass somehow is controlled so far and you have a happy ferret, happy owner. So, we happy with that.

That's the last clinical case I'm going to show you today. I'm going to present you all the datas, but that's an interesting case, very interesting case and it's more aim at illustrating the advantage of a multimodal protocol when you're dealing with a difficult tumor to treat.



Little Nemo has a mass. Symptoms are not very clear. We end up identifying at the CT scan an esophagus tumor, distal esophagus right here near the diaphragm.

So, we try to do a biopsy under endoscopy under firocoxib, again for the anti-androgenic effect. Looks aggressive on the CT. Unfortunately, our biopsy are inconclusive. So, we tried some other biopsy because the lesion is growing but it's still inconclusive. So, we go for surgery. We know we're not going to be carcinologic in this location. Unfortunately it comes back approximately five to six months later.

So, we go back for surgery. But this time we decided to go for interop radiation therapy. We decided to sequence the tumor. We've been doing it at Eiffelvet with FidoCure since 2020 and we sequenced so far 35 cases. And for Little Nemo, we found a BRCA1 mutation in favor of platinum derivative chemotherapy and a tyrosine kinase inhibitor called olaparib. So, we went for that on a benign tumor like leiomyoma. Something strange for me with my 30 years of experience. But we've done it and we've also done a vaccination, it's an APAVAC solution. It's a biotech in France. You get fresh tissue, you froze it, you

mix it with the lab, try to re-inject some tumor protein, different from what's been taken from the body and try to provoke an immune response.

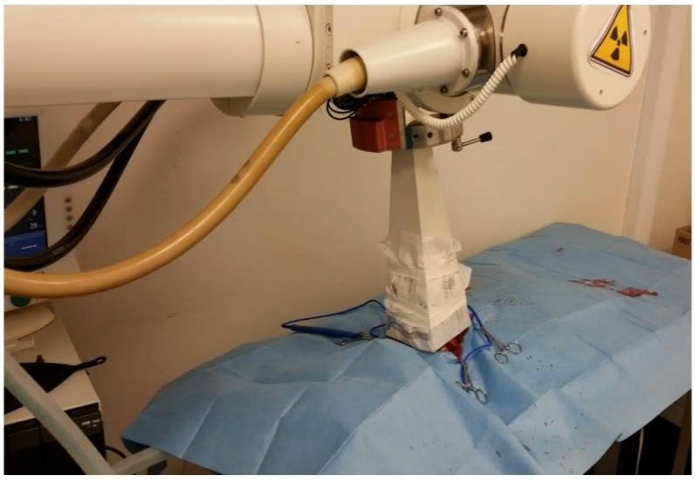
So, we've done a two CT control, the last one was in October, just last month. And it was all fine and the dog is fine. And with two years down the road, we stopped olaparib after 10 months in October '22.

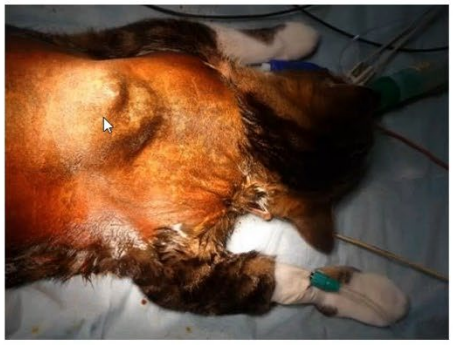
So, difficult to say in this case if it'll never reoccur. But two years down the road in a dog with cancer, it's always very satisfying and difficult to say if it's the APAVAC vaccination, the carboplatin, the olaparib, the intraop radiation therapy, the learning curve, our surgeon went back to surgery second time. But this is what is a multimodal therapy when you try to take into account everything you know in this field for a tumor where you don't have miracle solution to propose.

So, intraop radiation therapy that we've used in Little Nemo, the case just before. The idea is very simple. It's just optimizing the control of the tumor in the tumor bed where you have the higher chance to get recurrence.

And when we do carcinologic surgery, it could be a mast cell, it could be a soft tissue sarcoma, melanomas, carcinoma. It could even be nasal cavity through the soft palate when it's feasible and basically at Eiffelvet with our experience, if on the CT the surgeon is unsure of being able to control the tumor to get the margins, then even if it's first line, we then recommend intraop radiation therapy. If it's a recurrence, no matter what you have on CT, because we know that histology will be more aggressive, we'll propose interop radiation therapy.

And you have many cases in the real life where the owners don't have that much money. The cat has renal insufficiency or CMH or the dog has CMD. So, no matter what you want to do, a curative interop protocol with to have anesthesia is just not feasible. So, then you need to think about intraop radiation therapy because it's your only chance to be able to give a little bit of a radiation in this patient.



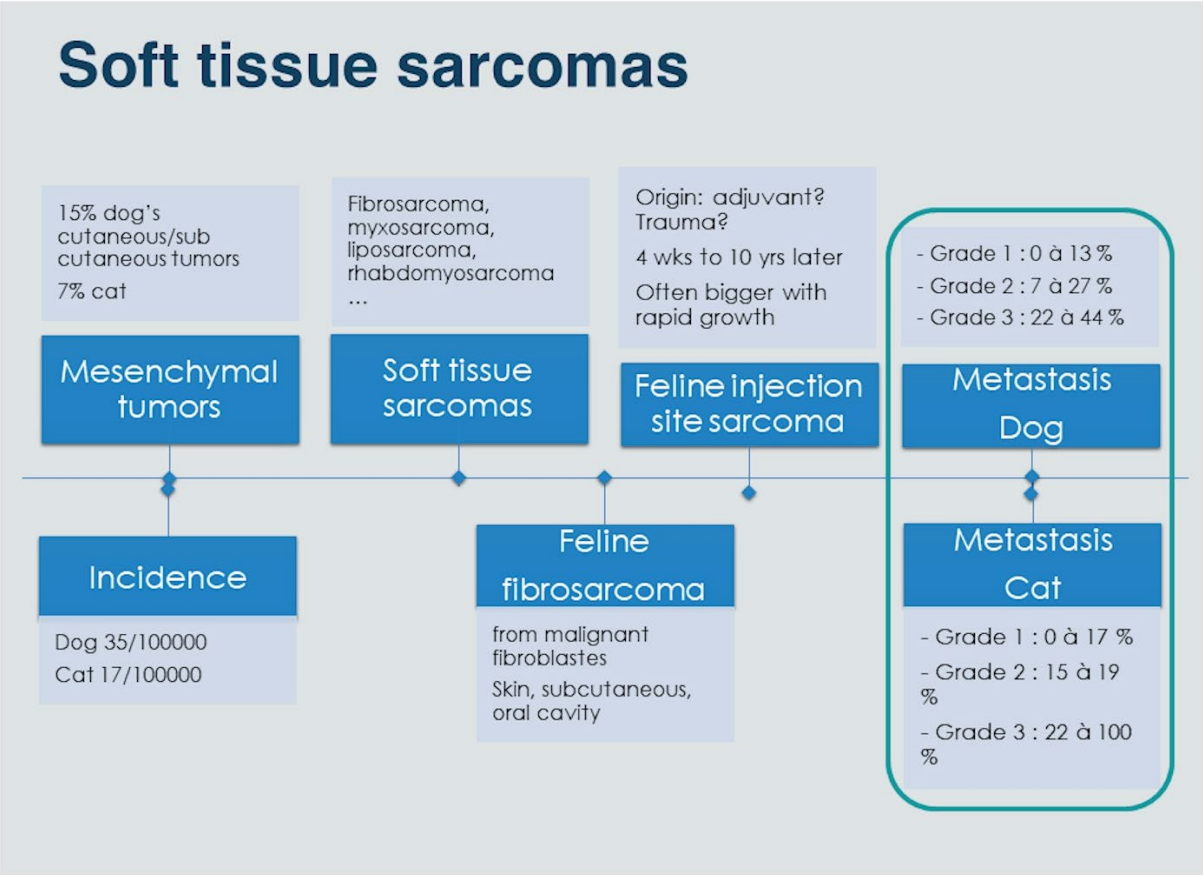


Intraoperative Radiation Therapy

So, that's a cat with a fibrosarcoma and this is the cat underneath the sheet. Basically the procedure at Eiffelvet with our team, we're now well trained altogether, the nurses, the surgeon, the radiotherapist, the oncology. So, it's a procedure altogether that takes us two hours. So, it's just one surgery, one radiation, two hours. Not that bad. That's the case I've already presented you, but this is a good case. We don't have that many cases that are relevant to this option for nasal cavity tumors. It needs to be a tumor that is probably more sarcoma than a carcinoma because it's calcified, more amenable to surgery, and it needs to be fairly localized. You don't want the tumor to be posterior towards the cribriform plate.

And then, what you can do is propose surgery through a soft palate, very well tolerated surgery. You're not going to be carcinologic. So, you propose to add interop radiation therapy. This, Hyanto, ended up having a chondroblastic osteosarcoma and the owner didn't want to do anything else. They didn't want to go for curative intent protocol radiation therapy. So, we didn't have in fact any other choice to propose them. And basically, with one procedure, one anesthesia for surgery, one radiation, the dog was okay for nearly a year and a half. So, only with firocoxib. So, it's not a miracle, but it's good quality of life for a year and a half.

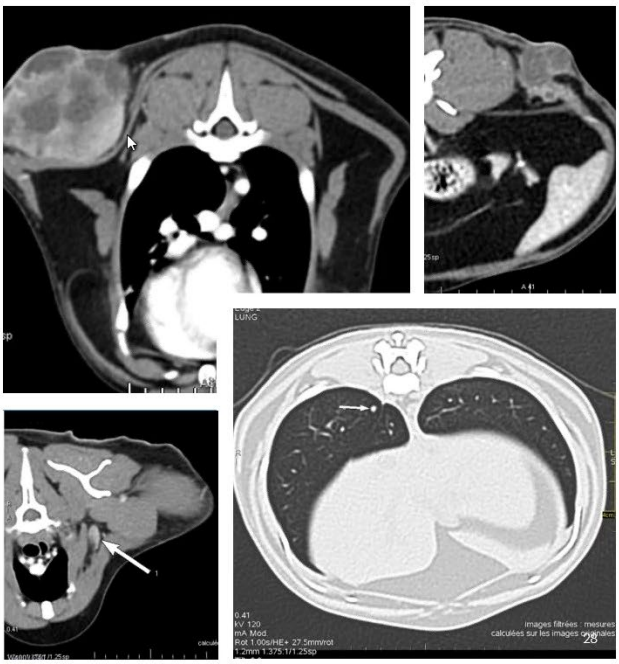
So, now I want to develop the interop radiation therapy. This is, as you will see, very interesting with orthovoltage, but I want to go through soft tissue sarcomas in general first. Well, this is classic, but you know that within the mesenchymal tumors you have the soft tissue sarcomas. Within the soft tissue sarcomas, you have the fibrosarcomas including for the feline. And among these sarcomas you're going to have your feline injection site sarcoma. But if it's inguinal, basically it's not a feline injection site sarcoma. If it's lumbar or interscapular, it is a feline injection site sarcoma.



The most important, this slide is the metastasis. Depends on the histologic grade of the tumor either in cats and dogs. But you see, while it's not zero and you have to be careful, it's not because it's a soft tissue sarcoma that you not encounter metastasis. So, the first step is extension assessment. You need to do your CT.

Step 1: Extension assessment

- CT scan
  mass and thorax
- Size, vascularisation, localisation, invasiveness ?
- Métastasis : lungs, lymph nodes ?
- Cytology ?



And when you look at this big tumor, in fact, for a surgeon, it's piece of cake even if it's big. Here you can see that it's infiltrative. It goes down all the way down to the peritoneum. So, you're going to have to remove piece of the peritoneum. You can tell from there that it's extending around. So, this is going to be very difficult to get the margin on this case. So, the CT gives you the size, the location, is it very vascularized, is it very infiltrative, invasiveness, et cetera. And you look at the lymph node, but that's a metastasis in the lung from a cat with fibrosarcoma. You look at the original lymph node ... Sorry, that's the lungs and that's the lymph node. Maybe I made a mistake in my English.

And when you have lymph node there, it's enlarged and it's hyperdense and it's white so you have to consider it could be metastatic. So, you stick a needle in there. Every time we do CT as an oncologist, we stick needle everywhere to make sure what we're dealing with.

Step two is surgery. Remember that a chance to cut is a chance to cure. If you go for carcinologic surgery, first intention meaning you didn't mess around with a small surgery before because that's the worst thing to do. And so, you've going for an aggressive surgery, we are looking at Eiffelvet at three centimeter around CT margins. One click deep layer underneath. I just recall you that it's

impossible to see looking at the literature, this is the way to do, this is the number of centimeters you want. So, we decided to try to go for this. And the surgeon, when he goes for carcinologic surgery, he has his CT, doesn't think he cuts. He cuts. And then he starts thinking of, "How am I going to control and to reconstruct the cat?" And so, you need expertise as well. And you go for looking at any type of lymph node that you've seen during the CT, you go for it during the surgery.

So, remember that if you take your surgical margins from a palpation, you're going to underestimate the size of the tumor sometimes up to 50%. In this case, this is the palpable tumor, cytology, fibrosarcoma. You do the CT, look at this. You have another lesion here.

So, if you go for surgery here without the CT, it's going to turn catastrophic because you're going to cut right there within the tumor and then you're going to create immunologic chaos and then the tumor cells will grow. So, we do the CT scan and we try to reach our three centimeters around in one clean deep. And remember that getting incomplete margins, it's nearly an assurance that you're going to get recurrence.

So, the steps three is the histological analysis, but the pathologist needs you to do work. So, you need to have an adapted formal content depending on the side of the tumor. If you go for a surgery on a spleen, you need to put all the spleen in the bucket, but you need the bucket. And then you need to identify your tissue. Don't mess with the tissue, don't cut anything. Just put it in the right formal size content and then try to tell the pathologist, "Well, this is in front, this is in back, this is right, this is left," because if you go for radiation, if you have infiltrative margins, you need to know where you want to focus your attention.

So, forgive me, these are French report, but basically this is a French report with clean margins, with French report with closed margin, two millimeters, so less than five. And this is another report with infiltrated margins.

So, the step four, once you've done your surgery with your soft tissue sarcoma is whether or not you're going to go for radiation therapy. Well, the first question you need to ask yourself if you don't want surgery. And when we get referral from dogs or cats that have done surgery before, the first thing we do, we do the CT and we want to make sure we cannot go back for surgery and removing the scar tissue to make sure providing the extension assessment is negative, providing that maybe we're going to be able to cure this patient with surgery. Because remember that sarcomas are not very radiosensitive. So, margins, again, are important because it has a direct influence on the recurrence. And again, if you want to go for surgery, you look for the mitotic index and you decide, depending on the health of your patient, depending on the owner philosophy, ability to come regularly or not, or money availability, then you go for hyper or hypofractionated protocol and you start when the scar, of course, is healed.

The last question you may ask yourself is whether I go for systemic treatment for soft tissue sarcomas. Just forget about chemotherapy cats and dogs. The question about immunotherapy might be question. The problem is that the soft tissue sarcomas, whoever they are, whatever the breed, even in human, they're very often cold tumor, C-O-L-D, meaning that they don't contain tumor-infiltrative lymphocytes or we know that tumor-infiltrative lymphocytes is the relay you need within the tumor for your immunotherapy or sometimes tyrosine kinase inhibitor to be efficient. But still now we're sequencing our soft tissue sarcoma cases in dogs because we can do it with FidoCure. We've done eight of them. In four of them, we managed to find actionable mutation with possible tyrosine kinase inhibitor treatment behind. And there's nothing else you can propose once you've done your surgery and your radiation therapy and you know that you probably have a few cells going on in the pet. So, we try to go for sequencing in dogs. Do we recommend on a systematic basis in cats to add after treatment metronomic chemo, masitinib, toceranib, imatinib? No.

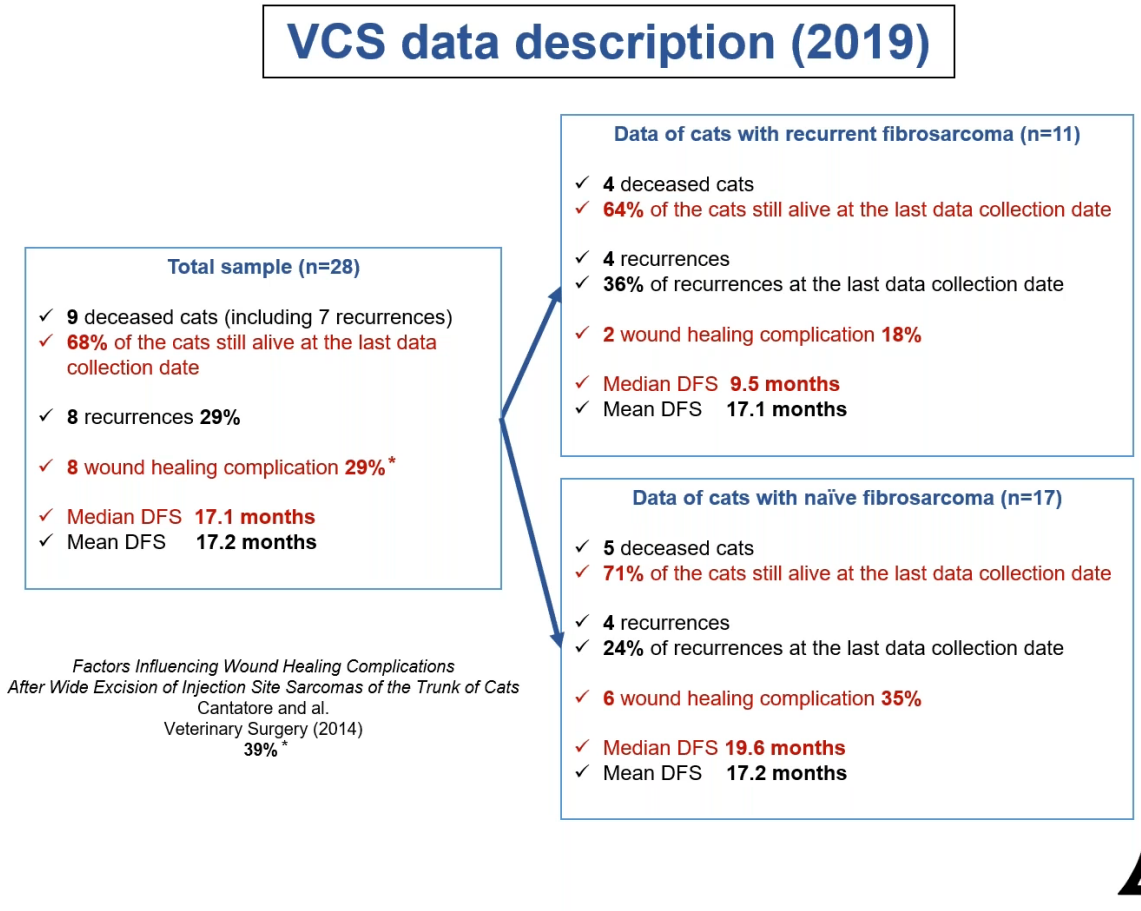
So, now we've done our five steps for managing our soft tissue sarcomas. What if we add another step, which is intraop radiation therapy during the surgery? Because when you look on PubMed, you have 2,540 results. That was very recently I've done this for you. And that was back in 2010 Elekta and very quickly Varian came back with a solution like this one. And you have a little bubble. In this bubble here you have an anode and a cathode and you are giving energy and you're creating photons and you irradiate locally.

So, I've really considered using this machine in the vet field at Eiffelvet. But the way the design, the applicators were not adapted to our pets. And right now it's nearly a standard of care for small breast cancer tumor without metastases. And once they've done surgery, they have this round applicator, they deliver radiation, you don't need shielding and it's photons you're delivering locally and it's looked at for colon, pancreatic, gastric, glioma, thymoma. So, interop radiation therapy is something to be considered in veterinary medicine. And not only because we have superficial tumor, we can do it with orthovoltage, but because it's a solution that is a growing solution in the human field.

So, now if we were to use it in the vet field where we need to prove its efficacy and it's innocuity, of course, and as I told you, there's no data with orthovoltage in the vet literature from recently. So, that's the standard of care for fibrosarcoma in cats. CT scan radical surgery followed by hyperfractionated megavoltage radiation either with photons or electrons. So, that's what we've done at Eiffelvet. Same thing, but we add interop orthovoltage radiation therapy and if the margins were infiltrated or closed, we recommend hyperfractionated adjuvant post-surgical radiation therapy. So, that's what we do.

If we have metastasis, it's palliative. If we have no metastasis, we go for the radical surgery. If we have clean margins, we consider it secure. If it's closed or incomplete, first question, can we go back for surgery? If our surgeon has done the first surgery, we're not going to be able to go back. And then, so, if we have

this situation, we recommend additional hyperfractionated radiation because it's part of the gold standard, but most of the time, the owner will decline because the cat is too old, because the cat has renal insufficiency, because of a money issue, because of an ability to come here on a regular basis. So, that's the way we deal with this prospective trial.

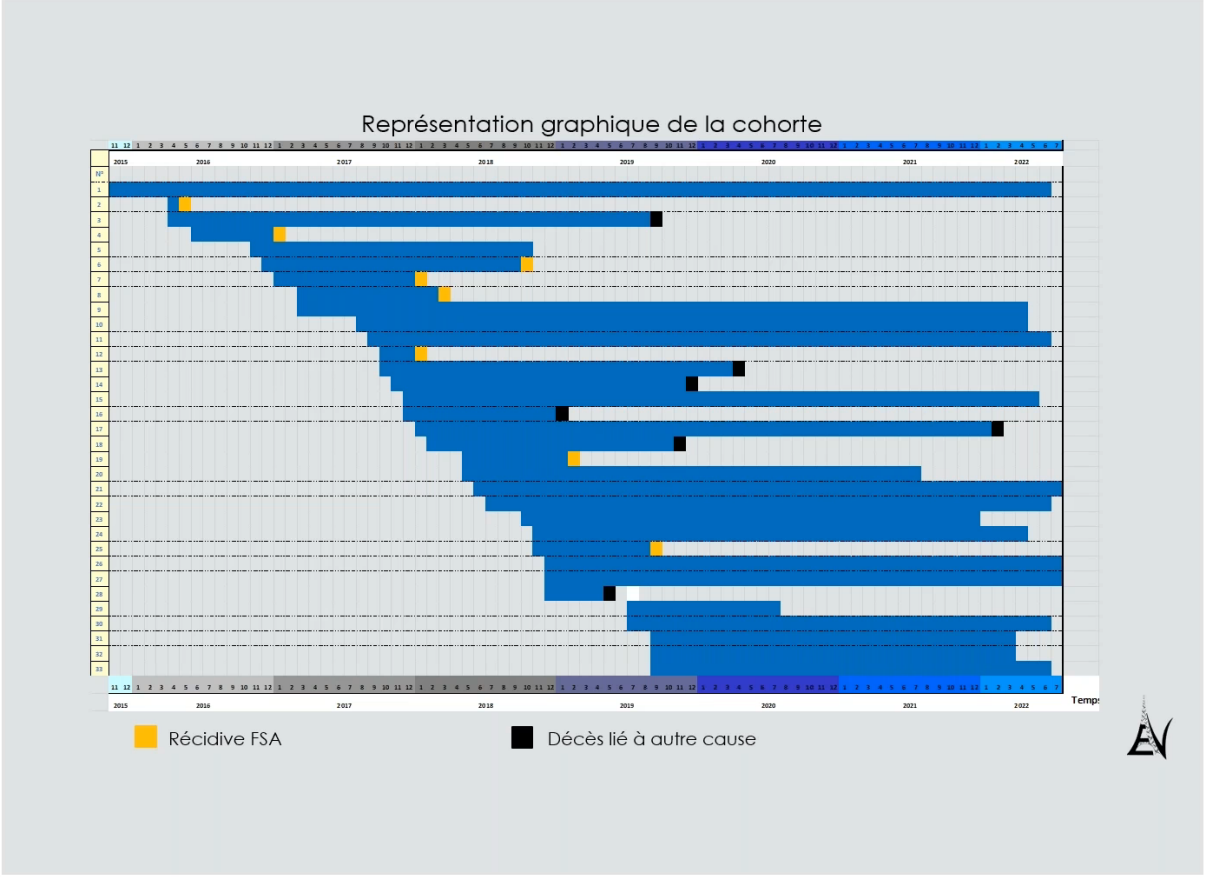


So, this is the abstract that I presented orally at VCS just before COVID in 2019 in Houston. And these were the data I get and 68% of our 28 cats were alive at the data collection when I presented them. And we still had a fairly good median disease for survival. Not that many healing complication compared to what you get when you are only doing carcinologic surgery of cats.

So, for us, for somehow a proof-of-concept that interop radiation for feline fibrosarcoma with orthovoltage could be efficient and without increasing the rate of local complication of the surgery. So, we followed our cases and these are the data we now want to publish and we are under writing the paper. So, this is the last data was last day of September 2022. So, the three years down the road for all of the cases. So, we managed to get 33 cases where we have three years down the road, all of these 33 cases. So, 55% of our cats are still alive. When you look at the rate of complications for the [inaudible 00:48:35]

after surgery, well, it's not that bad. It's the same. So, basically delivering a Gray in the tumor bed doesn't complicate your healing process.

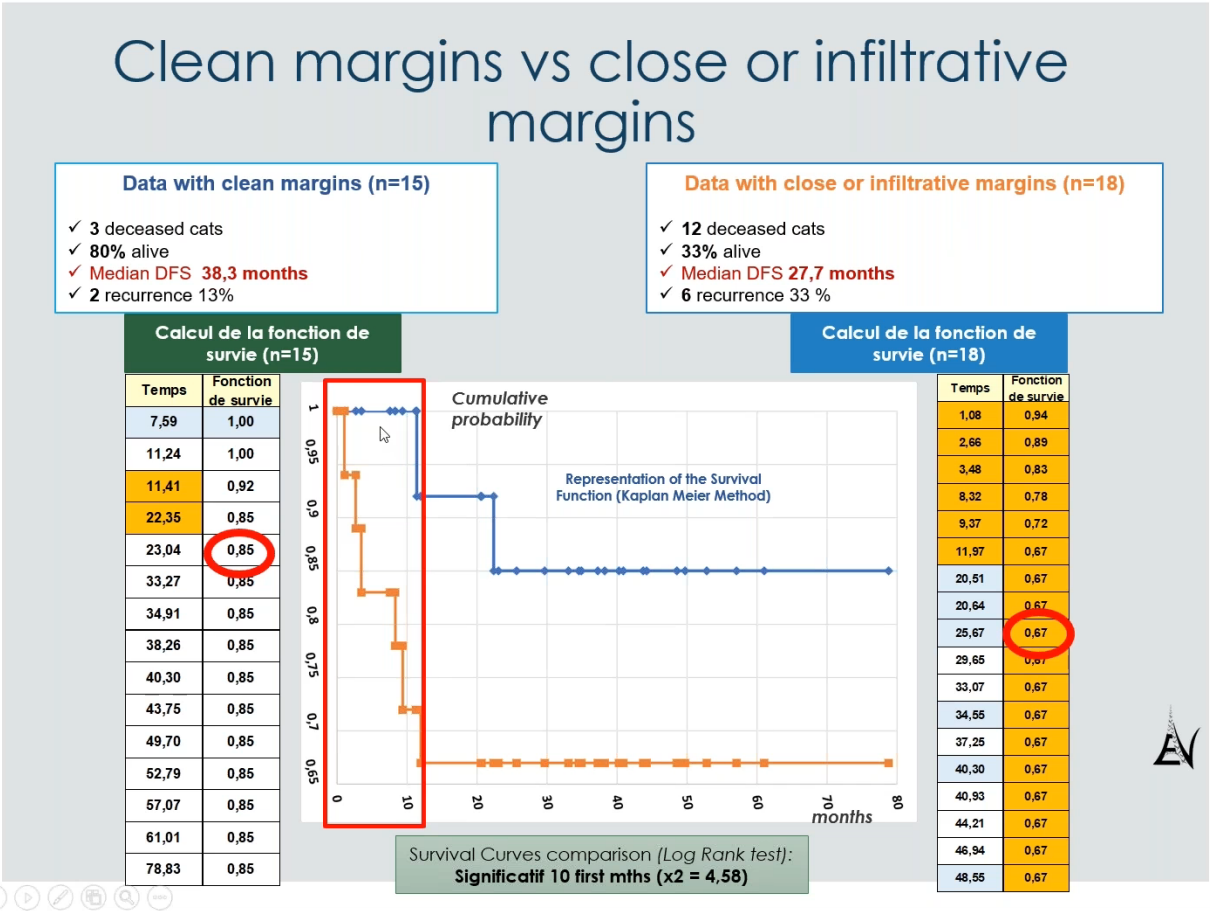
And look at the disease-free survival. Now it's 33.3 months. But this is excellent when you compare to the rest of the literature. There is somehow a difference between the cats with recurrent fibrosarcoma and the cats with naive fibrosarcoma. But it's opposite of what you thought because it seems to be better for recurrent than naive. And even though you will see that it's not very statistically significant. I think the reason why is that when vets are sending us fibrosarcoma as a naive case, it's because the sarcoma is already a big fibrosarcoma. So, difficult to go for surgery first line.



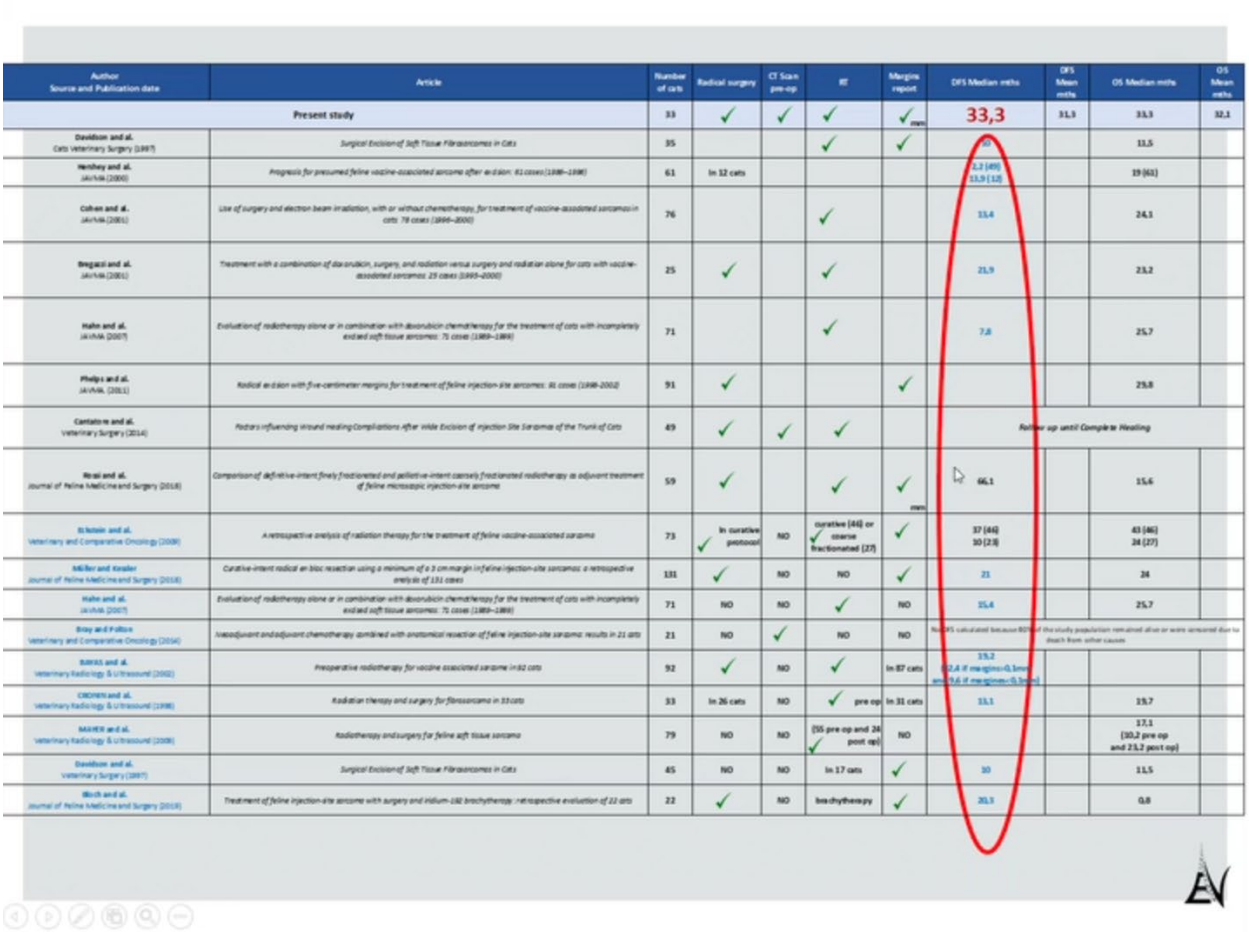
So, this is our cohort over the years, by the other years, and this is when the cat has a recurrence. This is the event we're looking at statistically because disease-free survival for soft tissue sarcoma is the most important data to look at. And this is when the cat dies from any other cause other than fibrosarcoma.

When you look at the Kaplan-Meier, why, it's not that bad. When you go at 23 months, you have a 78% chance that the cat did not experience the recurrence event that you're looking at statistically. What you can say more simply is that

you nearly have eight chance out of 10 to be free of reoccurrence after two years. So, in our mind, even though it's a small cohort, and that's often the problem in veterinarian literature, but somehow the efficacy is validated for us.



When you look at naive versus recurrence, as I told you, there's no statistical difference. When you look at clean margin versus close or infiltrative margins. Well, just look at one thing. Among all of our cases 18 have closed or infiltrative margins. Despite our city, despite the experience of our surgeon, it's difficult to get the margins on a sarcoma in a cat.



Author Source and Publication date	Article	Number of cats	Radical surgery	CT Scan pre-op	RT	Margins report	DFS Median mths	DFS Mean mths	OS Median mths	OS Mean mths
Present study		33	✓	✓	✓	✓ mm	33,3	31,3	33,3	32,1
Davidson and al. Cats Veterinary Surgery (1997)	*Surgical Excision of Soft Tissue Fibrosarcomas in Cats*	35			✓	✓	10		11,5	
Hershey and al. JAVMA (2000)	*Prognosis for presumed feline vaccine-associated sarcoma after excision: 61 cases (1988–1998)*	61	In 12 cats				2,3 (49) 11,9 (12)		19 (61)	
Cohen and al. JAVMA (2001)	*Use of surgery and electron beam irradiation, with or without chemotherapy, for treatment of vaccine-associated sarcomas in cats: 78 cases (1999–2000)*	76			✓		13,4		24,1	
Bregazzi and al. JAVMA (2001)	*Treatment with a combination of doxorubicin, surgery, and radiation versus surgery and radiation alone for cats with vaccine-associated sarcomas: 25 cases (1993–2000)*	25	✓		✓		21,9		23,2	
Hahn and al. JAVMA (2007)	*Evaluation of radiotherapy alone or in combination with doxorubicin chemotherapy for the treatment of cats with incompletely excised soft tissue sarcomas: 71 cases (1989–1999)*	71			✓		7,8		25,7	
Phelps and al. JAVMA (2011)	*Radical excision with five-centimeter margins for treatment of feline injection-site sarcomas: 91 cases (1998-2002)*	91	✓			✓			29,8	
Cantatore and al. Veterinary Surgery (2014)	*Factors Influencing Wound Healing Complications After Wide Excision of Injection Site Sarcoma of the Trunk of Cats*	49	✓	✓	✓		*Follow up until Complete Healing*			
Rossi and al. Journal of Feline Medicine and Surgery (2018)	*Comparison of definitive-intent finely fractionated and palliative-intent coarsely fractionated radiotherapy as adjuvant treatment of feline microscopic injection-site sarcoma*	59	✓		✓	✓ mm	66,1		15,6	
El Banan and al. Veterinary and Comparative Oncology (2009)	*A retrospective analysis of radiation therapy for the treatment of feline vaccine-associated sarcoma*	73	✓ In curative protocol	NO	✓ curative (46) or coarse fractionated (27)	✓	37 (46) 10 (23)		43 (46) 24 (27)	
Miller and Kessler Journal of Feline Medicine and Surgery (2018)	*Curative-intent radical en bloc resection using a minimum of a 3 cm margin in feline injection-site sarcomas: a retrospective analysis of 131 cases*	131	✓	NO	NO	✓	21		24	
Hahn and al. JAVMA (2007)	*Evaluation of radiotherapy alone or in combination with doxorubicin chemotherapy for the treatment of cats with incompletely excised soft tissue sarcomas: 71 cases (1989–1999)*	71	NO	NO	✓	NO	15,4		25,7	
Bray and Polton Veterinary and Comparative Oncology (2014)	*Neoadjuvant and adjuvant chemotherapy combined with anatomical resection of feline injection-site sarcoma: results in 21 cats*	21	NO	✓	NO	NO	*No DFS calculated because NO of the study population remained alive or were censored due to death from other causes*			
BIRKS and al. Veterinary Radiology & Ultrasound (2002)	*Preoperative radiotherapy for vaccine associated sarcoma in 92 cats*	92	✓	NO	✓	In 87 cats	19,2 (12,4 if margins<0,1cm and 9,6 if margins<0,1cm)			
CRONIN and al. Veterinary Radiology & Ultrasound (1998)	*Radiation therapy and surgery for fibrosarcoma in 33 cats*	33	In 26 cats	NO	✓ pre op	In 31 cats	13,1		19,7	
MAYER and al. Veterinary Radiology & Ultrasound (2009)	*Radiotherapy and surgery for feline soft tissue sarcoma*	79	NO	NO	(55 pre op and 24 post op) ✓	NO			17,1 (30,2 pre op and 23,2 post op)	
Davidson and al. Veterinary Surgery (2007)	*Surgical Excision of Soft Tissue Fibrosarcomas in Cats*	45	NO	NO	In 17 cats	✓	10		11,5	
Bisch and al. Journal of Feline Medicine and Surgery (2019)	*Treatment of feline injection-site sarcoma with surgery and iridium-192 brachytherapy: retrospective evaluation of 22 cats*	22	✓	NO	brachytherapy	✓	20,3		0,8	

So, what you see here on the Kaplan-Meier is that there is a statistically different difference during the first 10 month. When you go further, there's no difference. What it means is that if you have infiltrative margins, if a recurrence happens, it happens quicker. That's probably what means this statistical difference right there during the first 10 month.

So, when you look at the literature, these are the disease-free survival when they're available. These two articles are, I have to be honest and present them, but I also have to tell you that I cannot understand these data. And even if I read the articles a bunch of time, how can you have a disease-free survival of 66 months with an overall survival of 15 month? And there are some discrepancy there. So, I have to present them because they have a better disease-free survivor than most of the other articles in literature and 33 months for when you compare to the rest of the articles. Well, it's very good. It's even better than every other articles and we're the only one to look at having radical surgery, CT scans, pre-op every time, radiation somehow every time, and margins we got.

And remember then in our 33 cases, only six of them had external beam adjuvant radiation therapy after surgery. Among the three, three had clean margins, so should not have had external beam radiation therapy. But the owner wanted to do it because it was standard of care, so we couldn't refuse it.

So, only three, really, of the 33 cases had external beam adjuvant radiation therapy. So, it's a very good disease-free survival, 33.3 month for just one procedure.

So, the question is why does it work? Because you don't delivering a lot of radiation therapy. Well, first of all, you direct at the tumor bed and remember that when you go for surgery, you create an immune chaos and it's going to be in favor of tumor cell proliferation in general and tumor cell specifically. So, when you look at different studies, somehow tumor irradiation will go for an inhibition of the growth factors. You will provoke immunogenic cell death and they're thinking in human as associating interop with checkpoints inhibitor, which are not available so far in various medicine. And when you look in the detail of these articles, it's interesting to see that photons seems to be better than electrons. And we're using photons of course with orthovoltage.

This is some study in people and you see that they go for up to 20 Gray. So, the question, do we need to increase our dose in veterinary medicine? Well, I don't know, but we're a little shy so far and we need to evaluate this protocol in other tumors and we using at Eiffelvet on all tumors, squamous cats, when surgery is physical or melanomas in dogs, mast cell tumors, mammary tumors, melanomas and people, they're thinking of trying to throw in some radiosensitizing nanoparticles with the tumor bed to increase the local control of the tumor.

So, interop radiation therapy in veterinary medicine, well, no significant side effects, no more postsurgical complication like plastic surgery. You're sparing the normal adjacent tissue and you have, in our theory, a median disease for survival if, well, better than most of the articles with the standard of care, even with linac and even with electrons.

And remember that avoiding post-surgical external beam radiation therapy, well, it limits the number of anesthesia and that's a very important matter for us veterinarians for our patient and client for the comorbidities, for the budget, for the proximity. Very interesting.

So, to conclude, I will take back the slides that I proposed you during the previous webinar. And vet oncology is aiming at quality of life above all. Ethics have to be very important to us, and that means providing realistic and affordable treatment modalities to as many patients as possible. Multimodal protocols where you include radiation therapy are the best, but they need to be well tolerated. So, you need to find an equilibrium between what you're doing as a treatment and the side effect, we need the patient to go through the treatment with hardly few side effects, otherwise the owner will stop.

So, in this regard, orthovoltage, either very interesting therapeutic modality answering these goals. And I really think, and I'm working for it, that we should consider with more accuracy the orthovoltage in our community because it will probably allow us to do many different things, very adapted to our patient. And

I hope in the future, while more of you will follow us in our experience to go for orthovoltage. Thank you.

Kristie Amobi: Thank you so much. That was a really excellent presentation. We are tight on time, but I do have a few questions here for you, Dr. Keravel. Can you describe more about the training requirements and how long it took you and your team to implement the system?

Dr. Olivier Ker...: Well, that's a good question. Well, theoretically in the vet field, if you want to do radiation therapy, you need to be boarded in radiation oncology, which I'm not. I've mainly studied radiation therapy in human medicine.

Then, now, if you talk about the time it took us to have a good appreciation of the machine and to optimize our ability of using it, I would say two to three years. But in France, there is no obligation for a vet to be boarded in radiation oncology to be authorized to use a radiotherapy device. But there's an auto limitation because there's no way you can go for orthovoltage without deep knowledge in radiation therapy.

Kristie Amobi: Okay, thank you. Can you also describe more about your case mix for curative versus palliative care?

Dr. Olivier Ker...: Oh, clearly we're doing more palliative and the majority of our indications are palliative interop because we do a lot of carcinologic surgery. So, I'd say 40% of our case are curative intent and 60% palliative for the external beam protocols. And then we have the intraop indication.

Kristie Amobi: Okay. How often do you need to refer your patients out to a provider with a linear accelerator?

Dr. Olivier Ker...: Anytime I consider that I cannot, with my machine, propose an adapted treatment that will control correctly the tumor and limit the side effect. So, anytime I have a consult on a brain tumor, anytime I have a consult on a nasal cavity tumor, these are the tumors where I will present all the different options, all the different machine, either palliative or curative intent. And so, even there's a facility in Italy, in Europe where they do conformational radiation therapy just like it's been done in human, where you can hypofractionate it with a megavoltage to increase the local dose to the tumor. So, I sometimes send my patients in Italy if they're willing to go there. So, this is about ethics and I could not give you a percentage, but it's anytime I know that, with my machine, I would not deliver an appropriate treatment. I recommend to go elsewhere.

Kristie Amobi: Okay. Yeah. Of course, that makes sense. And final question, can you talk more about the ongoing maintenance requirements for your system?

Dr. Olivier Ker...: Well, we have with Xstrahl, well we have the French distributor of Xstrahl, so we have a maintenance contract with them. So, they're coming twice a year to

check the machine. And then we have the physician that comes in every year just to make sure that, again, that's what I was telling you during the consult, that there's a term in French, but sorry, I don't have the translation in my mind in English, but it's just give you the, not a certainty, but a certainty that the dose you're giving at the skin is really the dose you want to give. So, the machine has to be evaluated at least every year by a physicist, probably is the good term.

Kristie Amobi: Okay. Correct. Yeah, that makes sense to make sure that the physics has been commissioned in it. So, right.

Dr. Olivier Ker...: Yes, yes, yes.

Kristie Amobi: Okay. All right. Well, that is our final question. Again, on behalf of Xstrahl, I'd like to thank you for today's session. I would also like to thank everyone on the line for your attention. Thank you so much. And with that, I will close today's session. Thank you.

Dr. Olivier Ker...: Thank you.