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Comparison of usage of indocyanine green, 5-aminolevulinic acid, methylene blue and fluorescein sodium in oncology in human and veterinary medicine

Diploma Thesis

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Wien, den 09.10.2024 Sandra Bochsbichler

ZUSAMMENFASSUNG

Diese Diplomarbeit untersucht die Anwendung von fluoreszierenden Farbstoffen – Indocyaningrün, 5-Aminolevulinsäure, Methylenblau und Fluoreszein-Natrium – in der Onkologie der Veterinärmedizin der letzten zehn Jahre und vergleicht die Ergebnisse mit dem aktuellen Stand der Humanmedizin. Die Farbstoffe ermöglichen eine verbesserte Darstellung von Tumorgewebe, was die Erkennung von Tumoren, Tumorrändern, Metastasen und Lymphknoten während chirurgischer Eingriffe erleichtert. Der Fokus der Arbeit liegt auf der Nutzung dieser Farbstoffe bei Hunden und Katzen und vergleicht die erlangten Ergebnisse mit aktuellen Studien der Humanmedizin.

Eine systematische Literaturrecherche von 2014 bis 2024 zeigt, dass diese Farbstoffe in der Humanmedizin bereits umfassend verwendet werden, während ihr Einsatz in der Veterinärmedizin bisher nur begrenzt untersucht wurde. Indocyaningrün und Methylenblau zeigen in der Veterinärmedizin großes Potential, etwa bei der Färbung von Lymphknoten und der Abgrenzung von Tumorrändern, jedoch kommt es besonders bei der Verwendung von Indocyaningrün häufig zu falsch positiven Ergebnissen. 5-Aminolevulinsäure ist durch seine höhere Tumorspezifität vielversprechend, die bisherigen Studien umfassen jedoch nur sehr kleine Stichproben. Die aktuelle Forschungslage zu Fluoreszin-Natrium in der veterinärmedizinischen Onkologie ist stark begrenzt. Die Anzahl an Berichten von unerwünschten Nebenwirkungen im Zusammenhang mit der Verwendung der Farbstoffe bei Hund und Katze ist gering.

Die Arbeit identifiziert Herausforderungen wie falsch-positive Ergebnisse aufgrund von Entzündungen und sehr kleine Versuchsgruppen, und zeigt den dringenden Bedarf an weiteren Studien, um die Techniken für den Einsatz in der Veterinärmedizin zu optimieren. Insgesamt zeigen die Ergebnisse, dass der Einsatz von Fluorophoren die chirurgischen Ergebnisse in der Onkologie der Tiermedizin verbessern und die Rezidivraten von Tumoren verringern kann. Weitere Forschung ist jedoch notwendig, um die klinische Anwendbarkeit und Sicherheit dieser Methoden bei Tieren weiter zu validieren.

ABSTRACT

This diploma thesis investigates the use of four fluorescent dyes—indocyanine green, 5-aminolevulinic acid, methylene blue, and fluorescein sodium — in oncology veterinary medicine and compares the findings with current state in human medicine. Fluorescent dyes play a crucial role in enhancing the visualization of neoplastic tissues, aiding in the precise detection of tumors, tumor margins, metastases and lymph nodes during surgical procedures. This thesis focuses on the application of the fluorophores in dogs and cats, comparing the findings with recent studies from human medicine.

A systematic review of literature from 2014 to 2024 revealed that while all four dyes are already utilized in human oncology, their application in veterinary medicine remains underexplored. Indocyanine green and methylene blue show the most promise for sentinel lymph node mapping and tumor margin delineation, nevertheless, false positive results are commonly observed with the use of indocyanine green. 5-aminolevulinic acid is notable for its greater tumor specificity, however, the existing studies are limited by small sample sizes. The current research situation on fluorescein sodium in veterinary oncology is very limited as well. The number of reported adverse effects associated with the fluorophores in dogs and cats was low.

The study identifies challenges such as false-positive results due to inflammation and very small sample sizes, highlighting the urgent need for further research to optimize these techniques for the clinical use in veterinary medicine. Overall, the findings suggest that the use of fluorophores can improve surgical outcomes in veterinary oncology and reduce tumor recurrence rates. However, additional research is needed to further validate the clinical applicability and safety of these methods in dogs and cats.

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1. list of abbreviations

ICG – indocyanine green

ALA – 5-aminolevulinic acid

PPIX - protoporphyrin IX

MB – methylene blue

FS – fluorescein sodium

NIR – near-infrared

SLN – sentinel lymph node

SLNM – sentinel lymph node mapping

FDA - food and drug administration

HGG – high grade glioma

NADPH - nicotinamide adenine dinucleotide phosphate

tech99 – technetium-99

PRISMA - preferred reporting items for systematic reviews and meta-analyses

2. Introduction and Research Questions

Diagnostic and therapy in oncology develop further every day. Fluorescent dyes are playing an increasingly important role in the detection of neoplasia, as well as tumor margins, metastases and lymph nodes. The mechanism of action of these dyes is based on the accumulation in neoplastic tissue, due to increased vascular permeability, metabolism or existence of receptors or enzymes. The fluorophores are then made visible by the application of light with a specific wavelength, leading to emission of light with a different wavelength. The emitted fluorescence enables the surgeon to differentiate between healthy and tumorous altered tissue, which improves complete removal of the neoplasm without residual tumorous tissue and therefore reduces the likelihood of recurrence. In human oncology, substances as indocyanine green (ICG) or methylene blue (MB) are already utilised daily. In veterinary medicine, these methods seem promising to improve oncological surgery as well, but until now, there is little research regarding the efficacy, safety and optimal application of fluorescent dyes on dogs and cats. This thesis aims to provide an overview of the current state of research on the use of ICG, 5-aminolevulinic acid (5-ALA), MB and fluorescein sodium (FS) in veterinary oncology and compares the findings with the established applications in human medicine.

Hypothesis:

All four methods, indocyanine green, 5-aminolevulinic acid, methylene blue and fluorescein sodium, are equally qualified for identifying neoplasia in liver, spleen, lymph nodes and gastrointestinal tract in humans, dogs and cats.

The following research questions are addressed in this thesis.

- For which purposes are ICG, 5-ALA, MB and FS used in oncology in veterinary medicine?
- Which complications occur in the usage of ICG, 5-ALA, MB and FS in neoplasia in liver, spleen, gastrointestinal tract and lymph nodes?
- Does one of the methods (ICG, 5-ALA, MB, FS) have a better outcome compared to the others when used in veterinary oncology?

3. Literature Overview

3.1. Fluorophores

The purpose of fluorescent dyes is to visualize structures that are otherwise not distinguishable from their surroundings. This is particularly useful in oncology for the complete removal of tumors without residual margins, as well as for the detection of lymph nodes or metastases. Fluorophores can be applied *in vitro, in vivo*, and *ex vivo*. (van Keulen et al. 2023) Simplified, the mechanism of action of fluorophores involves their emission of light at a different wavelength when exposed to light of a specific wavelength. Using specialized filters, the emitted light is isolated by subtracting the illuminating light, allowing only the emitted light to be perceived by the surgeon. Some fluorophores can even be detected with the naked eye. Depending on where the fluorophore accumulates, the tissue can be made visible and differentiated from the surrounding tissue. One of the major advantages of this technique is the real-time information provided to the surgeon during the operation. However, the images are only visible in two dimensions, and in most cases, the fluorescence can only be subjectively perceived by the surgeon, without quantitative measurement. The current fluorophores are limited in their penetration depth due to the properties of light, which is generally not an obstacle for most surgeries, but it can be a limitation for lymph node mapping. (Stummer and Suero Molina 2017)

There are various types of fluorophores. The most precise results are obtained with fluorophores that specifically accumulate in certain tumors, for example, through molecular markers or antibodies. Research on such dyes is ongoing, but clinical application has not yet been established. Another mechanism of action is seen in fluorophores that are metabolized by neoplastic tissues, such as 5-ALA. ICG, MB and FS diffuse non-specifically into altered tissues for various reasons, such as increased permeability of vessels, causing them to accumulate in neoplasia. This type of fluorophores carries the highest risk of incorrectly identifying altered tissue, like inflammation, as neoplastic, resulting in false positive results. Another option is the measurement of tissue autofluorescence, which is the natural fluorescence emitted by the tissue itself without the use of specific dyes. (Stummer and Suero Molina 2017) However, autofluorescence can also interfere with the effect of fluorophores, as the emitted fluorescence may blend in with the one of the surrounding background. (van Keulen et al. 2023) The signal-to-background ratio, meaning the ratio of fluorescence intensity between the tumor and the surrounding tissue, should be as high as possible to ensure optimal visibility. (Stummer and Suero Molina 2017)

Fluorophores operating in the near-infrared (NIR) range (between 650 and 900nm) have the advantage, that tissue exhibits minimal autofluorescence under NIR light. This enhances the visibility of the fluorophore. Additionally, NIR light penetrates deeper into tissue compared to white light, reaching depths of up to 12 mm. (Bray et al. 2023)

The different fluorophores vary in the time required from administration to maximum effect, ranging from immediate effect upon injection up to 8 hours after administration. (van Keulen et al. 2023),

3.1.1. Indocyanine green

ICG is a water-soluble, amphiphilic tricarbocyanine fluorophore. It can be administered intravenously, intra-arterially, or directly into tissues. In the blood, 98 % of ICG binds to protein, primarily albumin, which helps retain ICG within the vasculature and prevents extravasation. ICG is processed by the liver and excreted in the bile within 15-20 minutes, with a half-life of 3-4 minutes. (Fransvea et al. 2024) ICG is considered a safe drug, with no side effects observed at doses up to 2 mg/kg. However, it should not be used in patients with iodine allergies due to the risk of allergic reactions. (Reinhart et al. 2016)

ICG is not photostable for a long period of time, which is why it is sold in powder form and should be used within 6-8 hours after dissolved in distilled water. ICG can be administered at concentrations up to 80 µg/ml, beyond which aggregation occurs, leading to a reduction in fluorescence. (Reinhart et al. 2016) Doses between 0.02 and 5 mg/kg are currently used in the veterinary field. (Thomson 2024) The lethal dose ranges between 50 and 80 mg/kg. (Alander et al. 2012) The clearance of ICG from the blood is determined by hepatic blood flow, the liver's ability to absorb the dye, and its excretion through bile. (Hoekstra et al. 2013) In healthy humans, the ICG clearance rate is > 500 ml/min/m². (Sakka 2018) The maximal removal rate for ICG in healthy beagle dogs is 0.24 +/- 0.09 mg/kg/min in male beagles and 0.23 +/- 0.06 mg/kg/min in female beagles. In mongrels, the rate in male dogs amounts 0.21 +/- 0.10 mg/kg/min and in bitches 0.20 +/- 0.07 mg/kg/min. (Furuhama et al. 1996) Within 6 hours after injection, 97 % of the fluorophore is eliminated from the canine body. Due to the rapid metabolism of ICG by the liver, multiple boli or a continuous infusion can be administered to ensure prolonged fluorescence. (Thomson 2024) Unlike radioactive substances, such as Technetium-99 (tech99), ICG can be used without concerns regarding radiation safety or highly specialized equipment.

When exposed to light with a wavelength of 750-800 nm, ICG emits NIR light at a wavelength of 832 nm, although the exact value may vary depending on the chemical environment and physical state of the molecule. To visualize fluorescence, a light source within this wavelength range, a camera, and filters that prevent the mixing of fluorescence and excitation light are required. (Alander et al. 2012) Still, the interpretation of the fluorescence is qualitative and subjective to the surgeon.

Originally developed for photography, ICG is now used in various medical fields. It plays a significant role in angiography of the heart and brain, liver function tests, and the detection of sentinel lymph nodes (SLN). For the latter, the fluorophore is injected subcutaneously around the neoplasia. The visualization of lymphatic vessels is effective due to the high protein content of the lymph, to which ICG binds, allowing the surgeon to trace lymphatic vessels in real-time and sample or remove the lymph node closest to the tumor. (Thomson 2024) Additionally, ICG is used to quantitatively assess blood flow in various applications, such as flap surgery following breast operations and in anastomoses concerning the gastrointestinal tract. In oncological surgery, ICG is employed to identify tumors, although the fluorophore is not tumor-specific, leading to ongoing research into tumor-specific nanoparticles. (Reinhart et al. 2016; Dai et al. 2023) Shortly after the development of ICG, the dye was approved by the food and drug administration (FDA) for various applications. However, its use as a fluorescent dye for real-time imaging in surgery remains off-label. (Dai et al. 2023) In veterinary medicine, ICG has primarily been used for detection of lymphatic structures and mammary tumor resection. However, studies have also employed the dye for detection of other tumor kinds as well as angiography and cholangiography.

The mechanism of action underlying ICG-Fluorescence for oncologic surgery is believed to be the "enhanced permeability and retention effect." In neoplasms as well as in inflammatory conditions, there is increased vascular permeability. This results in a greater accumulation of ICG in these areas, facilitating the detection of tumors and their margins. However, false-positive results also occur due to accumulation in inflammatory tissues. (Thomson 2024) In addition, tumors with poor vascularisation appear less fluorescent. (Favril et al. 2018) ICG can alter pulse oximetry results for up to 10 minutes, depending on the dose. (Sidi et al. 1987)

3.1.2. 5-Aminolevulinic acid

5-ALA is a water-soluble fluorophore which physiologically occurs as an amino acid in the body. It is synthesized within mitochondria, metabolized into protoporphyrin IX (PpIX) and further converted into heme through the incorporation of iron molecules. While 5-ALA itself does not fluoresce, PpIX emits fluorescence when exposed to blue light. Due to altered enzymatic activity in neoplastic cells, the final step of conversion from PpIX to hem is often incomplete, leading to an accumulation of PpIX within tumors and therefore making it tumor-specific compared to other fluorophores. (Stummer and Suero Molina 2017)

When exposed to blue light with a wavelength of 375-440 nm, PpIX emits red light at 635 nm, enabling the visualization of tumors that might otherwise remain undetected or indistinct under white light. (Harada et al. 2022) The fluorescent effect of 5-ALA becomes visible approximately 3 hours after administration and reaches its peak at 6-8 hours. (Stummer and Suero Molina 2017)

5-ALA can be administered orally, intravenous or topically, with the recommended dose being a single administration of 20 mg/kg. (Ishizuka et al. 2011) Oral administration is preferred over intravenous administration due to the lower incidence of side effects. (Casas 2020) It is typically mixed with 50 ml of water and given 3-4 hours prior to anaesthesia. (Stummer and Suero Molina 2017) The fluorophore is absorbed within 2 hours and reaches its peak plasma concentration after 4 hours. The half-life of 5-ALA in a healthy individual is approximately 0.76 hours, and PpIX levels in the blood drop to the prior state within 48 hours after administration. (Harada et al. 2022) 5-ALA is excreted via the urine. It is commercially available in powder form, the costs remain relatively high. (Favril et al. 2018) Trace amounts of 5-ALA can also be found in some foods such as garlic. (Ishizuka et al. 2011)

One side effect of 5-ALA is photosensitivity within the first 24 hours after administration due to the accumulation of PpIX in the skin, which can result in redness similar to sunburn or rashes. Therefore, patients should be protected from direct light exposure during this time. However, no cases of drug-induced porphyria have been reported. Additionally, temporary elevations in liver enzyme levels may occur following 5-ALA administration. (Harada et al. 2022; Stummer and Suero Molina 2017) Other possible side effects include hypertension and nausea. (Ishizuka et al. 2011)

Some types of tumors exhibit low fluorescence due to reduced PpIX content. The visibility of these neoplasias can be significantly compromised by the autofluorescence of collagen and

flavin adenine dinucleotide, leading to a lower tumor-to-background ratio. (Favril et al. 2018) In addition, PpIX can accumulate in inflamed tissues, complicating the distinction between tumors and surrounding inflammation. In necrotic regions of neoplasms, 5-ALA may be converted into uroporphyrinogen I instead of PpIX, which fluoresces at 620 nm, thus aiding in the visualization of necrotic areas within neoplasms. (Harada et al. 2022)

In human medicine, 5-ALA is routinely used for the diagnosis of gliomas and bladder cancer. Currently, numerous studies are in progress to explore its application on other types of neoplasms. (Harada et al. 2022) In oncology, 5-ALA holds promise as a tool for photodynamic therapy (PDT) due to its role as a photosensitizer. When photosensitive tumors are exposed to light, reactive oxygen species are generated, leading to the destruction of tumor cells. (Casas 2020) This application is currently limited to superficial neoplasms, as even light with a wavelength of up to 800 nm penetrates tissue only to a depth of 1-2 cm. (Alexiades-Armenakas 2006) Additionally, 5-ALA is used in low doses in dermatology, for instance, as an anti-aging supplement or in the treatment of alopecia. (Ishizuka et al. 2011)

3.1.3. Methylene blue

MB, also known as methylthioninium chloride, is a dye developed approximately 150 years ago for staining textiles. Since its discovery, it has been used in a variety of fields. The dye is lipophilic and, as the name suggests, exhibits a greenish-dark blue colour. MB can be administered orally, intravenously, intraarterially, subcutaneously or directly into the tissue. When administered intravenously, it predominantly distributes to the whole blood, brain, and parathyroid glands. The underlying mechanism for the latter is not known. Intravenous administration should be performed over 3-10 minutes. (Cwalinski et al. 2020) Following oral intake, MB accumulates mainly in the intestinal walls and the liver. (Peter et al. 2000) MB primarily binds to haemoglobin in the blood. (Smith and Thron 1972) Peak plasma concentrations are reached 30-60 minutes after administration. (Bužga et al. 2022) The terminal half-life is approximately 5.5-6 hours. 65-85 % of MB is metabolized into non-fluorescent leucomethylene blue. (Cwalinski et al. 2020) This process occurs with the aid of the enzyme nicotinamide adenine dinucleotide phosphate (NADPH) -dependent reductase in erythrocytes. (Jaffey et al. 2017) Most of the dye is excreted through the kidneys and urine within 4-24 hours which can lead to a temporary green discoloration of the urine.

Under NIR light with a wavelength of 668-700 nm, MB emits light with a wavelength of 400-700 nm, some of which is visible to the naked eye. This fluorescent property is utilized, among other applications, in sentinel lymph node mapping (SLNM). Due to its hydrophobic properties, MB does not penetrate deeply into tissues and exhibits increased autofluorescence compared to other fluorophores. (Cwalinski et al. 2020)

Administration of MB is contraindicated in patients with renal disease, because in case of impaired renal function the excretion of MB is not properly managed, leading to elevated concentrations in the body. A small part of MB is excreted via bile. (Bužga et al. 2022; Cwalinski et al. 2020) Another contraindication for administration of MB is the presence of glucose-6-phosphate dehydrogenase deficiency, as this enzyme is responsible for the production of NADPH and therefore for the conversion of MB to leucomethylene blue. In the absence of this process, the dye cannot be metabolized and excreted and therefore accumulates in the body. (Cwalinski et al. 2020) There is no antidote for an overdose of MB. (Bistas and Sanghavi 2024) Additionally, MB should not be administered to pregnant patients as it may induce foetal hypoxia. (Bužga et al. 2022) Deficiency of essential enzymes like cytochrome b5 reductase prevents the conversion of MB to leucomethylene blue in the bodies of foetuses and neonates. This results in the accumulation of MB, leading to a lack of its reducing effect on methaemoglobin, and even further paradoxical methemoglobinemia. (McDonagh et al. 2013)

In human medicine, MB is used in doses ranging from 1-300 mg/kg/day, depending on the indication. (Bužga et al. 2022) At lower doses, under 2 mg/kg, side effects from MB administration are rare, aside from mild burning pain during intravenous administration. However, at doses of 7 mg/kg or higher, the likelihood of side effects significantly increases. (Bistas and Sanghavi 2024) The higher the dosage, the more probable the occurrence of adverse effects such as nausea, vomiting, hemolysis, paradoxical methemoglobinemia, chest pain, abdominal pain, hypertension, and temporary skin discoloration. Additionally, transient neurological deficits such as confusion have been reported following MB administration, which may be explained by accumulation of the dye in the brain after intravenous administration. Another adverse effect is the serotonin syndrome. MB, especially in combination with serotonergic agents, can lead to an accumulation of serotonin in synapses in the brain, resulting in neuromuscular hyperactivity and even death. (Bužga et al. 2022) Allergic reactions to the dye are more common at doses starting at 5 mg/kg and higher. Intravenous administration of MB can cause a transient drop in pulse oximetry readings, down to 65 %, as MB interferes with pulse oximeter function. This effect typically resolves after about 30

seconds. (Cwalinski et al. 2020) In the veterinary use of intravenous MB, the following side effects are mentioned: “Heinz body anemia, pseudocyanosis, increased serum alkaline phosphatase activity and kidney failure.” (Rossanese et al. 2021) Sidi et al. (1987) observed an increase in arterial blood pressure in 31.2 % (n=5/16) of dogs after injection of 1 to 5mg/kg ICG intravenously. In addition, methaemoglobin concentration increased by 1.4 % 1 minute after administration of 5mg/kg ICG. (Sidi et al. 1987) However, in the studies on MB for SLNM in veterinary medicine from 2014 to 2024, none of these side effects were observed, likely since MB was never administered intravenously in these studies.

MB is used in a wide range of medical applications. Its most common use is as a stain in microscopy. Due to its antioxidant properties, it is also employed as a neuroprotective agent. In cases of methemoglobinemia, MB promotes the conversion of methaemoglobin to haemoglobin through donation of electrons, thereby improving tissue oxygenation. MB can be used to treat hypotension by inhibiting cyclic guanosine monophosphate, which leads to vasoconstriction. Additionally, it serves as an antidote for cyanide poisoning and phosphamide-induced encephalopathy. (Bužga et al. 2022) The fluorescent properties of MB are utilized for visualizing various structures, such as lymph nodes or ureters, as well as neuroendocrine tumors, although the underlying mechanism remains unclear. (Cwalinski et al. 2020)

In veterinary oncology, MB is currently only used for SLNM.

3.1.4. Fluorescein sodium

FS, the salt form of fluorescein, has been applied across various fields since the mid-19th century. FS is a water-soluble dye. (Acerbi et al. 2014) In its powdered form, it appears orange. When dissolved in water, the colour shifts to yellow. FS can be administered intravenously, orally, intrathecally as well as subcutaneously. Following intravenous administration, peak plasma levels are achieved rapidly. In the body, 80-90 % of FS binds to proteins while the remainder circulates freely and rapidly diffuses into surrounding tissues. In the liver, the majority of FS undergoes glucuronidation and is primarily excreted through the kidneys within 24 hours, leading to yellow discoloration of the urine. FS can be detected in urine for up to one week after administration. (O'goshi and Serup 2006) In clinical practice, FS is typically used at doses ranging from 3 to 10 mg/kg. (Acerbi et al. 2014) When exposed to light with a wavelength of 460-500 nm, FS emits a yellow-green fluorescence in the range of 540-690 nm. (Acerbi 2016) Maximum fluorescence is observed at a pH of 8-9. (O'goshi and Serup 2006)

Within seconds of FS injection, yellow discoloration of the skin and mucous membranes occurs, which subsides after approximately 6 hours. (O'goshi and Serup 2006) Other side effects may include nausea and vomiting. Anaphylactic reactions or more severe adverse effects are rare. As with other fluorophores, the likelihood of side effects increases with the doses of FS. (Acerbi et al. 2014) Systemic adverse effects are most commonly observed with intravenous administration. (O'goshi and Serup 2006) The use of FS should be avoided in patients with liver or kidney disease, allergic reactions to contrast agents, and pulmonary spasm. (Schebesch et al. 2016)

In human medicine, FS is primarily used in ophthalmology for vascular imaging of the retina via intravenous injection and topically for staining corneal ulcers. (O'goshi and Serup 2006) In oncology, FS is particularly employed in the detection of brain tumors. Malignant tumors, such as high-grade gliomas (HGG), disrupt the blood-brain barrier due to their invasive growth, allowing FS to accumulate and thus render neoplastic regions visible. In tumors where the blood-brain barrier remains intact, no FS accumulation is observed, as it does not accumulate within tumor cells but in extracellular space. (Acerbi 2016; Schebesch et al. 2016) The applications are similar to those of 5-ALA, but the costs of FS are significantly lower. (Acerbi et al. 2014) A filter that selectively transmits wavelengths between 540-690 nm enables the visualization of surrounding tissue while simultaneously displaying tumor fluorescence and therefore eliminates the need for constant switching between white and blue light. Additionally, the use of this filter allows for a significant reduction in the required FS dose, down to 2-4 mg/kg, while improving visibility. (Acerbi 2016; Schebesch et al. 2016) Under white light, FS accumulation can still be detected, though this requires higher doses of up to 20 mg/kg, which increases the risk of adverse effects. (Schebesch et al. 2016)

4. Material and methods

From February to April 2024, the databases PubMed, ScienceDirect and SpringerLink were searched for potential papers concerning fluorescence-based resection of neoplasia in veterinary medicine. A systemic literature search was implemented using a combination of the keywords "indocyanine green", "ICG", "5-aminolevulinic acid", "ALA", "methylene blue", "MB", "fluorescein", "neoplasia", "dog", "canine", "cat", "feline", "liver", "spleen", "gastrointestinal tract", "gut", "lymph nodes", “glioma”, “meningioma”, “skin”. The search protocol can be found in the appendix.

Exclusion criteria were: papers published before 2014, concerning other species than dogs and cats, languages other than German and English, fluorescent dyes other than ICG, ALA, MB and FS, *in vitro* studies, no full text available, other applications beyond oncological surgery

Papers that were published after 2013 and are referring to feline or canine patients and intraoperative usage of fluorescent dyes for oncologic surgery were incorporated. Following additional usages for the named fluorophores were identified and added in the process: papers on the use of ICG for breast tumors, lung neoplasms, and superficial tumors, as well as on the use of 5-ALA for breast tumors, lung neoplasms, mesotheliomas, sarcomas, and carcinomas. No papers concerning the use of fluorophores for tumors in spleen or gastrointestinal tract were found between 2014 and 2024.

2190 papers were identified including 169 duplicates. After removing duplicate records, studies were evaluated for suitability based on their title and abstract. The majority of the papers either originated from human medicine or focused on *in vitro* research and was therefore eliminated. The full text of 41 suitable papers was analysed, of which 10 were excluded due to missing data, other languages than German and English und different fluorophores. 31 papers were included into the study. The process of paper selection is displayed in the flow chart in figure 1. Due to the limited number of papers, case reports and studies with a small patient sample size were incorporated. A detailed analysis of the references in the original studies was conducted to identify potential additional articles.

To identify comparable publications in human medicine, the database PubMed was searched for articles analogous to those selected concerning veterinary medicine from July to August 2024. The search commenced with publications released in 2024. In some cases, no suitable papers from 2024 were found. Therefore, a retrospective search was conducted, going back

year by year until appropriate papers were found. One to two publications, most like the chosen veterinary medicine topics, were selected to ensure the best possible comparison.

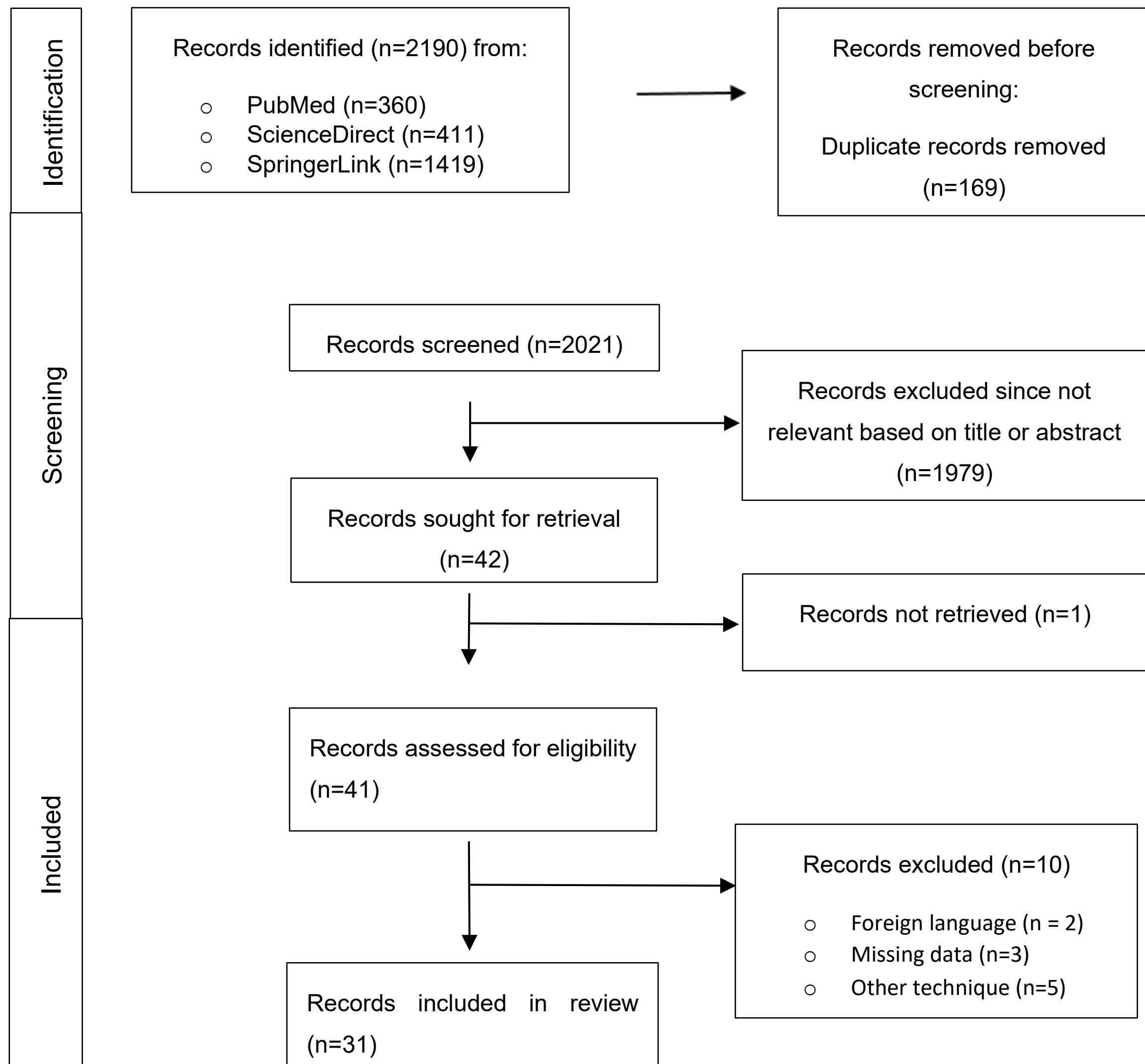

Figure 1: Flowchart

The found studies were classified concerning their scientific evidence by the following preferred reporting items for systematic reviews and meta-analyses (PRISMA) classification listed in table 1.

Tab. 1 PRISMA classification system (Follin and Charland 1997)	
Ia	At least one systematic review based on methodical, high-grade controlled, randomized studies
Ib	At least one large enough, methodical high-grade randomized study
IIa	At least one high-grade study without randomization
IIb	At least one high-grade study of another type, as an experimental study
III	More than one methodical high-grade nonexperimental study
IV	Opinions and convictions from notable authorities (with clinical experience); commission of experts; descriptive studies

5. Results

At the time this diploma thesis was written, the number of papers on the application of fluorophores in veterinary oncology was limited. The studies conducted this far have yielded diverse results, but overall, they offer promising prospects for the use of fluorophores in veterinary medicine. Only four papers included cats in their sample group, all other studies focused exclusively on dogs.

5.1. Fluorescein sodium

Only one paper was found regarding the use of FS in veterinary medicine, categorized as IIb in the PRISMA classification. In this study, FS was used for the diagnosis of intracranial lesions in dogs. The study by Nakano et al. (2018) reported an 81.8 % (n=18/22) success rate in fluorescing of the lesions, with 68.1 % (n=15/22) of lesions showing strong fluorescence and 13.6 % (n=3/22) of the lesions showing mild fluorescence. Two lesions that did not show staining were found to be normal brain tissue. The third unstained lesion was suspected to be a glioma. The side effects of yellow discoloration of the skin, mucous membranes, urine, and faces known from human medicine, were observed in all 22 cases. Two dogs experienced recurrent vomiting for 48 hours post-surgery. Prior to the intravenous administration of the final dose of 20 mg/kg FS, 2 mg/kg of the dye were injected to pre-emptively rule out anaphylactic reactions, none of which were observed in any of the patients. The timing of the injection depended on the location of the lesion. For intra-axial lesions, the dye was administered after the dura mater was opened, whereas for extra-axial lesions, it was administered before the durotomy. (Nakano et al. 2018)

In human medicine, FS is primarily used in the treatment of HGGs. Xiao et al. (2024) employed a dosage of 3-5 mg/kg FS for their study on HGGs, which was administered intravenously after the induction of anaesthesia. To enhance visibility, the YELLOW 500 fluorescence filter (KINEVO 900, Carl Zeiss, Germany) for light with a wavelength of 560 nm was used. Nevertheless, there was frequent switching between white and fluorescent light intraoperatively. In 84.4 % (n=27/32) of the cases, complete tumor resection without residual margins was achieved with the help of FS. In 6.3 % (n=2/32) of cases, less than 97 % of the tumor was removed. No further adverse side effects, except for temporary urine discoloration, were observed,

5.2. Methylene blue

For the use of MB in veterinary oncology, only papers addressing lymph node mapping were found in the period from 2014-2024. One study (Rossanese et al. 2021) is assigned to category III in accordance with the PRISMA classification system, while the remaining studies are classified under category IIb. (Gariboldi et al. 2023; Manfredi et al. 2021; Chiti et al. 2021; Ferrari et al. 2020; Randall et al. 2020; Brissot and Edery 2017; Annoni et al. 2023; Soultani et al. 2017; Worley 2014) In 9 out of 10 records (n=379/446), MB was merely employed as an adjunct tool to facilitate the localization of the SLN for surgical removal or biopsy intraoperatively. In these cases, the actual identification of the lymph node draining the affected area was achieved using tech99 (n=226), iomeprole (n=80), iopamidol (n=33), iohexol (n=15) or iodized oil (n=25). Only one study (n=67) used MB as the sole technique for identifying the SLN. (Rossanese et al. 2021) In all cases, MB was injected either peritumorally or directly into the tumor. The doses used ranged between 1 and 10 mg/tumor, with the exact dosage not specified in two studies (n=136/446). The most common dosage, used in 25.8 % (n=115/446) of the cases, was 2 mg/tumor. In 8 out of the 10 studies, MB was divided across four quadrants, with the injected volume ranging from 0.1 to 1 ml. The timing of the injection varied between 15 minutes before start of surgery or before aseptic preparation, and intraoperatively. The success rate for SLN detection ranged from 79.4 % to 99.4 %, although 53.4 % (n=238/446) did not distinguish between the success rate of MB alone and in combination with other methods. The study with the lowest success rate administered 0.4 ml of a 10 mg/ml MB solution 10-15 minutes prior to incision, whereas the study with the highest success rate used 0.5-1 ml of a 10 mg/ml MB solution 5 minutes before incision. In both cases, ICG was injected peritumorally in four quadrants. This suggests that higher dosages administered closer to the start of surgery may lead to improved outcomes. However, Rossanese et al. (2021) also used 0.1-0.5 ml of a 10 mg/ml solution and achieved a success rate of 87%. No clear correlation between dosage and success rate can be identified across the studies. However, different preceding techniques were employed, which may have influenced the outcomes. It appears that studies administering injections closer to the start of surgery tend to report better results. Further studies are required to determine the most effective dosage by comparing various doses under consistent conditions. In one study, leakage into the surrounding subcutaneous tissue was observed in 9 % (n=6) of the cases. (Rossanese et al. 2021) Manfredi et al. (2021) reported MB uptake in inflamed tissue in one case, as well as mild bleeding after injection in 5.9 % (n=3) of the cases. No side effects of the fluorophore were observed in the remaining 8

studies. None of the papers mentioned the use of specific wavelengths or filters, suggesting that the effect of MB was observed with the naked eye in all cases. Only one study (n=23) stated sensitivity and specificity for a combination of MB and tech99, being 88.9 % and 100 %. (Chiti et al. 2021) All papers on SLNM using MB are found in table 2.

Tab. 2 Methylene blue for sentinel lymph node mapping in veterinary medicine between 2014 and 2024

	sensitivity	specificity	Success rate for SLN	Complications/side effects	Dosage	Time of administration	Number of patients	PRISMA
Evaluation of Surgical Aid of Methylene Blue in Addition to Intraoperative Gamma Probe for Sentinel Lymph Node Extirpation in 116 Canine Mast Cell Tumors (2017-2022) (Gariboldi et al. 2023)	-	-	95% (n=186/196 nodes) MB + tech99	9%, mild; likely from MCT-reaction to puncture	0.4ml of sterile MB peritumoral in 4 quadrants; 0.2ml and a single injection site for MCTs smaller than 0.5cm	-	103 dogs	IIb
Ultrasound-guided placement of an anchor wire or injection of methylene blue to aid in the intraoperative localization and excision of peripheral lymph nodes in dogs and cats (Rossanese et al. 2021)	-	-	87% (n=58/67)	9% Intraoperative leakage in subcutaneous tissue	0.1 to 0.5 mL of 1% MB, in node or perinodal	15 min before start of surgery	67 patients (62 dogs, 5 cats)	III
Preoperative planar lymphoscintigraphy allows for sentinel lymph node detection in 51 dogs improving staging accuracy: Feasibility and pitfalls (Manfredi et al. 2021)	-	-	90.2% (n=46/51)	5.9% minimal bleeding after injection 1 case of MB uptake of inflamed tissue	0.4ml of 5mg/ml sterile methylene blue peritumorally	Prior to aseptic preparation	51 dogs	IIb
To map or not to map the cN0 neck: Impact of sentinel lymph node biopsy in canine head and neck tumours (Chiti et al. 2021)	88,9% in combination with tech99	100% in combination with tech99	79.4% (n=27/34 nodes) MB 83% (n=19/23) MB + tech99	-	0.4ml of 1% sterile methylene blue peritumorally in four quadrants	10 - 15 min before incision	23 dogs	IIb
Biopsy of sentinel lymph nodes after injection of methylene blue and lymphoscintigraphic guidance in 30 dogs with mast cell tumors (Ferrari et al. 2020)	-	-	91,1% (n=31/34 nodes) MB + tech99	-	0.4ml of sterile 5mg/ml methylene blue peritumorally in four sites	Injection after aseptically preparation	30 dogs	IIb
The development of an indirect computed tomography lymphography protocol for sentinel lymph node detection in head and neck cancer and comparison to other sentinel lymph node mapping techniques (Randall et al. 2020)	-	-	93.3% (n=14/15)	-	0.4ml of sterile 5mg/ml methylene blue dilution, 4 quadrants, each 0.1ml	5 min before surgical incision	15 dogs	IIb
Use of indirect lymphography to identify sentinel lymph node in dogs: a pilot study in 30 tumours (Brissot and Edery 2017)	-	-	88% (n=22/25) 80% (n=20/25) good MB uptake 8% (n=2/25) fair MB uptake	-	0.5-1ml of 5mg/ml methylene blue; animals <10kg solution was 1:1 diluted with dextrose 5%; In 4 quadrants peritumoral 0.5-1cm away from the border of the mass or intratumoral	15 min before surgical incision	25 dogs	IIb
Incorporation of sentinel lymph node mapping in dogs with mast cell tumours: 20 consecutive procedures (Worley 2014)	-	-	93.1% (n=27/29 nodes) 94.7% (n=18/19)	No acute reaction	0.4ml of 5mg/ml methylene blue diluted with sterile saline peritumoral in 4 quadrants	5 min before surgery	19 dogs	IIb
Assessment of sentinel lymph node metastasis in canine mammary gland tumors using computed tomographic indirect lymphography (Soultani et al. 2017)	-	-	-	-	0.5ml of methylene blue in four quadrants in the periareolar skin	intraoperatively	33 dogs	IIb
Sentinel lymph node mapping in canine mast cell tumours using a preoperative radiographic indirect lymphography: Technique description and results in 138 cases (Annoni et al. 2023)	-	-	99.4% (n=167/168 nodes)	-	0.5-1ml of 10mg/1ml methylene blue subcutaneous peritumorally in four quadrants	5 min before skin incision	80 dogs	IIb

In the study from human medicine from 2024, which is used for comparison, 40 mg/tumor MB were injected peritumorally into 4 quadrants after induction of anaesthesia. (Vemula Venkata et al. 2024) The success rate in this paper was 90 % (n=18/20), with a sensitivity and specificity of 100 % and 93.75 %, respectively. The only mentioned side effect of MB was temporary discoloration of urine in 30 % (n=6/20) of cases, lasting 1-5 days. Another study, focused on SLNM in colorectal cancer, differs from the use of MB in veterinary medicine due to the intra-arterial administration. (Carvalho et al. 2024) The exact dosage as well as time of injection is not specified, but 15-20 ml of a MB solution (50 mg diluted 1:3 with 0.9 % NaCl solution) were injected directly into the main artery of the concerned area. In 91.8 % (n=67/73) of cases, the SLNM results were satisfactory. In 6 cases, an insufficient number of lymph nodes were stained, but in no case were no lymph nodes stained at all. There are no veterinary publications on the intra-arterial administration of MB in cats or dogs published between 2014 and 2024.

5.3. Indocyanine green

In the literature published between 2014 and 2024, various applications of ICG in veterinary oncology can be found. ICG is used for tumors of the lung, liver, skin and mammary glands, as well as for SLNM.

5.3.1. ICG for lung neoplasia

Two publications were identified that focused on the diagnosis of lung tumors using ICG (n=48). Sakurai et al. (2023), categorized IIb for PRISMA classification, administered 2 mg/kg of a 5 mg/ml ICG solution intravenously 12-24 hours prior to surgery. No adverse reactions were observed. The success rate for staining neoplasia was 100 % (n=40/40). Sensitivity and specificity regarding complete resection using ICG fluorescence were 67.7 % and 60 %, respectively, while for the detection of lymph node metastases, they were 100 % and 75 %. The lung surface was examined using a NIR camera system, HyperEye Medical System (Mizuho Medical Co. Ltd, Tokyo, Japan), before the start of resection. The study reported a high false-positive rate, the exact number was not mentioned. The publication by Holt et al. (2014) investigated the ability of ICG to differentiate lung tumors from inflamed areas, placed under III in the PRISMA classification. For this purpose, 5 mg/kg ICG was administered intravenously 24 hours before surgery. 100 % of the nodules were stained with ICG, but in 37.5 % (n=3/8) of cases, surrounding inflamed and oedematous tissue without tumor cells also

absorbed ICG. No adverse side effects were observed. An self-developed NIR device with a 740 nm LED emission and a 780 nm filter was used for optimal fluorescence visualization, as well as a handheld spectrometer for quantification.

Comparatively, a 2024 study in human medicine by Han et al. (2024) involved the peritumoral injection of ICG for the detection of ground glass nodes in the lung (n=48). ICG was injected within 10 mm distance to the tumor, 3 hours prior to resection. A dose of 0.75 mg in 0.3 ml per nodule was used. The fluorescence of the dye was visualized using PINPOINT (Novadaq, Mississauga, Canada), an endoscopic fluorescence imaging system. The study reported a 100 % success rate in localizing the nodule using ICG. No adverse effects of the fluorophore were observed. (Han et al. 2024) Abdelhafeez et al. (2023) administered 1.5 mg/kg ICG via continuous drip infusion over 15 minutes, one day prior to surgery. The success rate for nodule detection was 73.4 % (n=58/79), with a false-negative rate of 26.6 % (n=21/79). However, tumors were visualized in only 7 out of 12 patients (58.3 %). Inflammatory myofibroblastic tumor, atypical cartilaginous tumor, neuroblastoma, adrenocortical carcinoma, and papillary thyroid carcinoma did not show ICG staining. Fluorescence visualization was achieved using an Iridium NIR System (Visionsense Corp, Philadelphia, PA, USA). No adverse side effects were reported.

ICG can also be administered via inhalation, requiring a lower dose than intravenous administration. Healthy lung tissue absorbs the fluorophore, while the tumor does not, resulting in a negative contrast visualization. Wang et al. (2023) administered 0.2-0.25 mg/kg ICG with 4-6 mL/min of oxygen over an average of 13.7 minutes. Inhalation occurred 50-90 minutes prior to the start of surgery. The success rate was 87.1 % (n=27/31 nodules). The remaining four nodules were already identifiable using white light or palpation, so NIR-imaging was not needed. A total of 16.1 % (n=5/31) of the nodules were detectable only with ICG, and not by white light or palpation. A NIR thoracoscopic system (DPM-III-01, Zhuhai Dipu Medical Technology Co., Ltd, Zhuhai, China) was used for fluorescence visualization. No side effects of ICG were observed, however, the distribution of the fluorophore was less efficient in the lungs of smokers due to black deposits. Nodule visualization was still achievable in smokers, but harder to differentiate. The time to locate small nodules was reduced by 45.5 % with the assistance of ICG inhalation.

5.3.2. ICG for liver neoplasia

One study on the use of ICG for the detection of liver neoplasms in veterinary medicine was published between 2014 and 2024, categorized IIb in the PRISMA system. Sakurai et al. (2022) administered 0.5 mg/kg of a 5 mg/ml ICG solution intravenously 12 to 24 hours prior to tumor removal. This resulted in a success rate of 86.8 % (n=90/104) for tumor fluorescence compared to the surrounding tissue. Sensitivity and specificity regarding tumor margin evaluation were 100 % and 77.2 %, respectively, while for complete tumor resection using ICG, sensitivity and specificity were 100 % and 82.1 %. In 9.6 % (n=10/104) of cases, false-positive fluorescence of the wound bed was observed after complete resection. An infrared camera system (HyperEye Medical System, Mizuho Medical Co. Ltd, Tokyo, Japan), positioned 30-50 cm from the liver surface, was used to visualize the fluorescence. No systemic side effects of ICG were observed.

For comparison with human medicine, a 2024 paper on the use of ICG for liver tumor detection was utilized. (She et al. 2024) In the study, patients (n=22) were administered 0.25 mg/kg of ICG one day prior to surgery, the route of administration was not mentioned. Sensitivity and specificity for tumor detection were 70.6 % and 100 %, respectively. A fluorescence imaging system (NIR/ICG System using the OPAL1 technology with the modular IMAGE1 S system, KARL STORZ, Germany), was used, which allowed switching between white and fluorescent light and had the capability to filter wavelengths below 810 nm and above 800 nm. Both yellow (77.3 %, n=17/22) and green (54.5 %, n=12/22) fluorescence were observed. No adverse reactions were mentioned. Another study from 2024 utilized ICG for the visualization of 40 liver tumors in 27 patients. (Hu et al. 2024) ICG was administered intravenously at a dose of 0.5 mg/kg two days before surgery. The overall success rate was 82.5 % (n=33/40). The sensitivity for superficial lesions was 96.4 %, while the sensitivity for deep lesions was 50 %. ICG enabled the visualization of 8 lesions that were not detected by ultrasound. For fluorescence imaging, the OptoMedic System (OPTO-CAM2100) or the Mindray fluorescence imaging system was used, and the tissue was irradiated with 805 nm light.

5.3.3. ICG for superficial neoplasia

For the visualisation of superficial tumors with ICG, two studies were published between 2014 and 2024, assigned to Level III (Favril and Abma et al. 2020) and IIb (Holt et al. 2015) according to the PRISMA classification. Dosages of 3 and 5 mg/kg were utilised, with the fluorophore being administered intravenously 24 hours prior to surgery in both instances (n=24). The

success rates regarding the staining of skin tumors were 93.3 % (n=14/15) and 66.6 % (n=2/3), examined sort of tumors being soft tissue sarcomas and mast cell tumors. Favril and Abma et al. (2020) reported a sensitivity and specificity for differentiating between tumor and surrounding tissue of 72 % and 80 %, respectively, although these values also included five mammary tumors and a melanoma. Holt et al. (2015) reported a sensitivity and specificity for detecting residual neoplastic tissue at surgical margins of 100 % and 90.9 %, respectively. In 100 % of cases with negative tumor margins, no tumor was detectable in the tissue. However, Holt et al. (2015) reported a high incidence of false-positive wound bed fluorescence at 9.09 %, where inflammation tissue was stained in the wound bed without residual tumor tissue. A special instrument (BioVision Technologies, Exton, Pennsylvania) was developed for the study by Holt et al. (2015) to aid in the visualization of fluorescence, consisting of a 742 nm LED, a camera, and a filter with a wavelength of 832 nm. Additionally, a NIR fluorescence spectroscopy device (Spectropen, InPhotonics, Norwood Massachusetts) was employed, which captured light in the 800–930 nm range using a long-pass filter with an 800 nm edge. This tool quantified the fluorescence during the resection. Favril and Abma et al. (2020) used the Fluobeam 800 (Fluoptics, Grenoble, France), a NIR clinical imaging system, which takes brightfield images as well as NIR images, with different exposure durations. The tool was positioned at a distance of 17 cm from the body surface. No adverse side effects of ICG were reported in both studies. In the study concerning mast cell tumors, the dogs were prophylactically administered an antihistamine intramuscularly to mitigate a degranulation of the tumor in response to the ICG injection.

In comparison, a paper from 2023 was selected that studied the use of ICG for visualizing soft tissue sarcomas in humans. In this study, 2–2.5 mg/kg of ICG were administered intravenously approximately 3 hours before surgery over a period of 45 minutes. 55.5 % (n=10/18) of patients received 2.5 mg/kg. In these subjects, oversaturation of the tumor was observed which made adequate tumor visualization impossible. For subsequent cases, the dosage was reduced to 2 mg/kg, improving tumor visibility. In 55.5 % (n=10/18) of cases, the ICG-demarcated margin matched the histologically confirmed tumor margin. The study reported a sensitivity and specificity of 22 % and 89 %, respectively. The Stryker SPY-PHI (Stryker Endoscopy, Kalamazoo, MI, USA) NIR camera system was used to visualize the fluorescence. No side effects were reported. (Gong et al. 2023)

5.3.4. ICG for mammary gland tumors

Three veterinary papers were found that focus on the use of ICG for visualizing mammary tumors, rated Ia (Pop et al. 2024), IIb (Newton et al. 2020) and III (Favril and Abma et al. 2020) for PRISMA classification. The dosages range between 1-5 mg/kg of ICG, administered intravenously either as a bolus (n=29) or over 3 minutes (n=16), from within 24 hours before surgery to intraoperatively. The success rate was 40-100 %. Sensitivity ranges from 72 % to 93.3 %, and specificity from 30 % to 80 %. In 40 % (n=2/5) and 18.8 % (n=3/16) of cases, false-positive fluorescence of the wound beds was observed. (Favril and Abma et al. 2020; Newton et al. 2020) The results show that studies using the highest dosage of 5 mg/kg administered 24 hours prior to surgery yielded the poorest outcomes, with a success rate of only 40 % (n=4/10). In contrast, a dosage of 3 mg/kg administered 20 hours before surgery achieved success rates of 68.3 %(n=11/16) and 80 % (n=16/20). Surprisingly, the study with the lowest dosage of 1 mg/kg, administered approximately 30 minutes before surgery, demonstrated the highest success rate of 100 % (n=2/2). These findings might indicate that the timing of the injection is more influential on fluorescence outcomes than the dosage, with injections administered closer to the start of surgery proving to be more effective. Various NIR fluorescence camera systems were utilized. Newton et al. (2020) used a prototype imaging system (Solaris, Perkin Elmer), Favril and Abma et al. (2020) utilized the Fluobeam 800 (Fluoptics, Grenoble, France) NIR system, while in the systematic reviews from Pop et al. (2024) two locally developed systems as well as the Fluobeam 800 (Fluoptics, Grenoble, France) and a system from Solaris (Perkin Elmer) were employed. No adverse effects from the fluorophore injection were reported in any study.

Pop et al. (2024) examined seven studies on the use of ICG for breast cancer in human medicine. The studies utilised dosages ranging from 0.25 to 5 mg/kg, with one study administering a fixed dose of 12 mg per patient regardless of body weight (n=10). All studies injected the fluorophore intravenously. The timing of administration varied from 24 hours to less than five minutes before the start of surgery. The success rate for tumor detection ranged from 40 % to 100 % (n=145), with higher dosages generally associated with better success rates. Sensitivity and specificity ranged from 40 % to 100 % and 31.7 % to 98 %, respectively. Three studies reported false-positive rates of 40 % (n=14/35), 50 % (n=6/12) and 68 % (n not stated) while the remaining four did not provide data on this metric. Two studies utilized locally developed devices, while the others used commercially available fluorescence imaging systems (Fluobeam 800 (Fluoptics, Grenoble, France, n=2), Real-ICS (Nuoyuan Medical Equipment, China, n=1), PDA (Hamamatsu Photonics, Japan, n=1), FloCam (BioVision1,

Exeter, PA and BioMedicon1, Moorestown, NJ), the Artemis Fluorescence Imaging system (Quest1 Medical Imaging, Middenmeer, the Netherlands), or the Iridium (Visionsense1, New York, NY, n=1). No adverse side effects were reported. A systematic review from 2023 investigated the use of indocyanine green (ICG) for non-palpable breast tumors. (Jansen et al. 2023) In 9 out of 11 studies (n=338/393), doses ranging from 0.2 to 2 ml and 0.5 to 10 mg were injected intratumorally (n=214) or peritumorally (n=124). Two studies (n=55) administered the fluorophore intravenously at a dose of 5 mg/kg, either 2 hours or 24 hours prior to surgery. The success rate for tumor visualization was 100 % (n=363/393) in all but one study. One study (n=30) used an ICG-hyaluronic acid mixture, with 0.1 to 0.2 ml injected intratumorally within 3 days prior to surgery. The success rate in this study was 86.7 % (n=13/15) for a 0.1 ml dose and 93 % (n=14/15) for a 0.2 ml dose. The second study using 0.2 ml of an ICG-hyaluronic acid mixture reported a 100 % success rate (n=51/51), with injections administered between 1 day and 1 hour before surgery. Mild nausea was reported in one case (n=1/393, 0.25 %). Various systems were used for fluorescence visualization. In both hyaluronic acid studies, the NIR system was not mentioned. The remaining papers used the Photodynamic Eye (PDE; Hamamatsu Japan, n=3), IC-View (Pulsion Medical Systems AG, n=1), Europrobe 3 (Eurorad SA, n=1), FloCam (BioVision1, Exeter, PA and BioMedicon1, Moorestown, NJ), the Artemis Fluorescence Imaging system (Quest1 Medical Imaging, Middenmeer, the Netherlands), or the Iridium (Visionsense1, New York, NY, n=1), Visual Navigator (SH system, Gwangju, Korea, n=1), Hypereye Medical System (Mizuho Medical Co. Ltd, Tokyo, Japan, n=1), and Real-ICS (Nuoyuan Medical Equipment, China, n=1).

5.3.5. ICG for sentinel lymph node mapping

Between 2014 and 2024, six papers were identified that investigated the use of ICG for SLNM. Two studies were classified as IIb (Wan et al. 2021; Alvarez-Sanchez et al. 2023) and 4 studies as III (Beer et al. 2022; Kim et al. 2015; Favril et al. 2019; Townsend et al. 2018) within the PRISMA classification. The dosages used in the studies varied between 0.05 and 5 mg of ICG per tumor, which were injected into three to four sites (n=77/89) or one site (n=12/89). Kim et al. (2015) did not specify the dosage of their emulsion. The timing of injection ranged from 3-4 hours before surgery to intraoperative administration. Three studies were conducted on healthy dogs (n=20). (Kim et al. 2015; Townsend et al. 2018; Favril et al. 2019) The success rate for SLN visualization ranged from 80 % (n=16/20) to 100 % (n=6/6, n=8/8). In two studies, cases of extravasation or staining of surrounding tissue were reported. (Kim et al. 2015; Beer

et al. 2022) Two studies (n=34/89) performed computed tomography (CT) prior to fluorophore administration to compare the outcomes with ICG. (Alvarez-Sanchez et al. 2023; Wan et al. 2021) Alvarez-Sanchez et al. (2023) reported a significantly higher SLN detection success with CT compared to ICG (95 % (n=19/20) vs. 80 % (n=16/20) detection rate). In contrast, Wan et al. (2021)'s results showed the opposite effect, with a CT-SLN success rate of 42.1 % (24/57 SLN) and an ICG-SLN success rate of 91 % (52/57 SLN). Various NIR imaging systems from different manufacturers were employed, being Fluobeam 800 (Fluoptics, Grenoble, France, n=2), Fluobeam 700 (Fluoptics, Grenoble, France, n=1), VITOMII (KARL STORZ, Germany, n=1), IC-Flow (Diagnostic Green, Germany, n=1). Kim et al. (2015) used the 1288 HD system (Stryker Endoscopy, Kalamazoo, MI, USA) without NIR device for laparoscopic surgery, for robotic surgery da Vinci Si Surgical System (Intuitive Surgical, California, USA) with NIR optical device was utilized. In one case, a small nodule developed at the injection site, which resolved spontaneously within a few days. (Favril et al. 2019) No further adverse effects were reported. All papers on SLNM using ICG are summarized in table 3.

Tab. 3 Indocyanine green for sentinel lymph node mapping in veterinary medicine between 2014 and 2024

	Success rate	Complications	Time of administration	dosage	equipment	Number of patients	PRISMA	notes
Determining agreement between preoperative computed tomography lymphography and indocyanine green near infrared fluorescence intraoperative imaging for sentinel lymph node mapping in dogs with oral tumours (Wan et al. 2021)	91% (n=52/57 SLN)	-	Prior to draping	1ml of 2.5mg/ml ICG Solution into three to four equal parts over 30 seconds in each site	VITOMII Karl Storz Endoscopy NIR exoscope	14 dogs	IIb	
Near-infrared fluorescent image-guided lymph node dissection compared with locoregional lymphadenectomies in dogs with mast cell tumours (Beer et al. 2022)	82.9% (n=58/70 SLN)	Extravasation of ICG in individual cases	intraoperative	0.5 to 1 ml of 2.5 to 5 mg/ml ICG solution injected peritumoral in four parts	IC-Flow imaging system, hand-held near infrared fluorescent imaging camera	35 dogs	III	
Comparison of indirect computed tomographic lymphography and near-infrared fluorescence sentinel lymph node mapping for integumentary canine mast cell tumors (Alvarez-Sanchez et al. 2023)	80% (n=16/20)	-	intraoperative	1ml 0.5mg/ml ICG mixture (1ml of 2.5mg/ml mixed with 4ml of dextrose 5%) peritumoral in four quadrants	Fluobeam 700 NIR system	20 dogs	IIb	
Fluorescent iodized emulsion for pre- and intraoperative sentinel lymph node imaging: validation in a preclinical model (Kim et al. 2015)	100% (n=2/2) for robotic surgery	Injection site was visible from peritoneal surface of stomach	3 - 4 h after injection	Endoscopically into gastric submucosa, 0.3 ml in 3 areas; Emulsion made of ICG + surfactants + iodized oil + ethanol	For laparoscopic surgery 1288 HD; Stryker Medical without NIR device; for robotic surgery da Vinci Si Surgical System with NIR optical device	8 dogs	III	Healthy dogs
Feasibility of near-infrared fluorescence imaging for sentinel lymph node evaluation of the oral cavity in healthy dogs (Townsend et al. 2018)	100% (n=6/6)	No clinical reactions	-	1ml bolus of 0.5mg/ml ICG-Solution (2.5mg/ml ICG Solution diluted with dextrose 5%) into gingival mucosa	Fluobeam 800 NIR system	6 dogs	III	Healthy dogs
Sentinel lymph node mapping by near-infrared fluorescence imaging and contrast-enhanced ultrasound in healthy dogs (Favril et al. 2019)	94.4% (n=17/18 SLN)	one case a hard nodule appeared at injection site, but resolved by its own in a few days	5 min between injection and identification of SLN	0.1ml of 0.5mg/ml ICG solution (4.5ml aqua ad iniectabilia mixed with 0.5ml 5mg/ml ICG solution) ubdermally injected; injection site was gently massaged for 1 min	Fluobeam 800 NIR system	6 dogs	III	Healthy dogs

To compare with the current state of human medicine, a 2024 paper on the use of ICG for SLNM in melanoma was used. (Wölffer et al. 2024) In 89.2 % (n=839/941) of the cases, a concentration of 2.5 mg/ml of ICG was used, though the exact dosage per kg or tumor was not specified. In 10.8 % (n= 102/941), no dosage was mentioned at all. The overall success rate for SLN staining was 89 % (2296/2588 SLNs). 5 out of 7 studies utilized the SPY system (Stryker Endoscopy, Kalamazoo, MI, USA) for fluorescence imaging, one study used Fluobeam (Fluoptics, Grenoble, France), and another one used an unspecified PDE system. No adverse effects were reported. A different 2024 study on ICG-SLNM employed a dosage of 2-4 mg, which was injected into four peritumoral quadrants 10-20 minutes before surgery. (Campwala et al. 2024) A 100 % success rate was reported. The SPY system (Stryker Endoscopy, Kalamazoo, MI, USA) was used for fluorescence visualization. No adverse effects of the dye were observed (n=9).

5.4. 5-Aminolevulinic acid

In veterinary medicine, there are only a limited number of studies on the use of 5-ALA in oncology to be found. Between 2014 and 2024, six papers were published, including three case reports. The limited data on 5-ALA in veterinary medicine may be due to the high cost of the fluorophore.

5.4.1. 5-ALA for intracranial lesions

There are two case reports on the use of 5-ALA for the diagnosis of intracranial lesions, both being assigned to IV in accordance with the PRISMA classification system. One case report describes the application of 5-ALA in a case of glioblastoma in a dog (Yamashita et al. 2020) while the second case report details its use on a feline meningioma. (Osaki et al. 2020) In both cases, 40 mg/kg of 5-ALA were administered orally 4 hours prior to cranial opening. In the case of the glioblastoma, the tumor demonstrated complete fluorescence, allowing differentiation from the surrounding tissue, with the strongest fluorescence observed in the centre. It should be noted that the dog was euthanized 3 hours after drug administration before craniotomy. In the case of the meningioma, the tumor also exhibited good fluorescence, which was helpful during resection, although complete removal could not be achieved. To visualize and quantify fluorescence, in both cases the HDR-CX180 (SONY, Tokyo, Japan) video camera as well as three spectrometers (LED405-SMA-TI, R600-8-UV-VIS-SR, Black-Comet CXR-50

TEC; StellarNet, Tampa, USA) were employed. The highest measured fluorescence value was 635 nm in both cases.

In human medicine, 5-ALA is routinely used as an adjunct for the delineation of gliomas and other intracranial tumors from surrounding brain tissue. In a recent 2024 study, participants were administered 20 mg/kg of 5-ALA orally, four hours prior to the induction of anaesthesia. (Da Silva et al. 2024) To enhance intraoperative fluorescence visualization, a surgical microscope (Pentero 900, Zeiss, Vienna, Austria) with BLUE400 filter (375–440 nm wavelength) was employed. In this study, 94.8 % (n=163/172) of meningiomas successfully demonstrated fluorescence, with 7 % (n=12/172) showing heterogeneous fluorescence, likely due to calcification. The success rate for fluorescence in glioblastomas was 97.8 % (n=227/232), with 3 % (n=7/232) exhibiting heterogeneous staining, presumably due to necrotic tissue. Across the entire cohort, which included various other types of intracranial tumors as well, a success rate of 79.3 % (n=561/707) was observed. In 2.69 % (n=19/707) of all cases, a transient, mild photoreaction was noted within the first 24 hours postoperatively. In 0.28 % (n=2/707) of participants, more severe photoreactions including itching were reported, which resolved within five days following treatment with antihistamines. In one participant, the rash persisted for up to 10 days. In three cases, vomiting occurred shortly after the administration of 5-ALA due to increased intracranial pressure, resulting in the absence of fluorescence.

5.4.2. 5-ALA for lung neoplasia

A study from 2019 on the application of 5-ALA for lung neoplasms was identified, assigned to IIb according to the PRISMA classification.. (Predina et al. 2019) 20 mg/kg of 5-ALA were used, administered 2–4 hours prior to surgery. The route of administration was not specified, though it is presumed that the fluorophore was given orally. Fluorescence of the tumor was observed in 58.3 % (n=7/12) of cases. In one case, the tumor only exhibited fluorescence after excision. The proportion of primary lung tumors that fluoresced was 85.7 % (n=6/7), though peripheral tumor margins were visualized in only 29 % (n=2/7) of these cases. Mild nausea was observed in one case following 5-ALA administration. Fluorescent imaging was performed using an endoscopy system (KARL STORZ, Germany) adapted for 5-ALA/PPIX utilisation.

Between 2014 and 2024, only one paper in human medicine was identified that investigated the use of 5-ALA for the detection of lung tumors. (Kitada et al. 2020) Twenty mg/kg of 5-ALA

were administered four hours prior to the start of surgery. Nausea was observed in 3.7 % (n=3/82) of cases following fluorophore administration. Fluorescence was visualized using a high-sensitivity HbCMOS high-vision camera (Flovel Co., Tokyo, Japan), attached to the thoracoscope and a 405nm LED. The sensitivity and specificity were reported as 81 % and 62.5 %, respectively. The sensitivity and specificity for lung adenocarcinomas were 93.9 % and 74.3 %, indicating that this type of tumor is more effectively visualized with 5-ALA compared to other lung tumors.

5.4.3. 5-ALA for mesothelioma

One case report on the use of 5-ALA for mesotheliomas in veterinary medicine was found between 2014 and 2024, PRISMA classification IV. (Osaki et al. 2023) Two cases were described: a cat with thoracic mesothelioma and a dog with peritoneal mesothelioma. In both cases, 5-ALA was administered orally at a dose of 40 mg/kg two hours prior to the start of surgery. The success rate for staining tumors was 100 %. No side effects of the fluorophore were observed. For the visualization of fluorescence, the nodes were examined under white and blue light. Neither false positive nor false negative lesions were detected.

The number of studies on the use of 5-ALA for mesotheliomas is also limited in human medicine. A paper from 2016 describes 5-ALA fluorescence detection of peritoneal masses, including mesotheliomas. (Yonemura et al. 2016) In the study, 20 mg/kg of 5-ALA was administered four hours before surgery. The detection rate for mesotheliomas using 5-ALA was 67 % (n=4/6). Tumors were first evaluated under white light and then under blue light with a wavelength of 375-445 nm. In the entire cohort, which included various other tumor types as well, nausea was observed in 0.87 % (n=1/115) of cases and vomiting in 0.87 % (n=1/115) of cases as well. Direct sunlight or other intense light sources were avoided for up to 24 hours after administration. For mesotheliomas, a sensitivity of 66 % and a specificity of 100 % were determined.

5.4.4. 5-ALA for mammary gland tumors

One paper on the use of 5-ALA for mammary tumors was published between 2014 and 2024, categorized as IIb under the PRISMA classification. (Osaki et al. 2017) The study used 40 mg/kg of 5-ALA, which were administered orally four hours prior to surgery. The success rate was 100 % (n=16/16), although in one case, the centre of the tumor did not stain due to

necrosis. Fluorescence was observed using white and blue light with a wavelength of 405 nm, and the HDR-CX180 (SONY, Tokyo, Japan) video camera. Additionally, three spectrometers (LED405-SMA-TI, R600-8-UV-VIS-SR, Black-Comet CXR-50 TEC; StellarNet, Tampa, USA) were employed to quantify the fluorescence, with a peak value of 635 nm.

A comparable paper from 2021 in human medicine compared dosages of 15 and 30 mg/kg, which were administered orally two to four hours before surgery. (Ottolino-Perry et al. 2021) The lower dose recorded a sensitivity and specificity of 65 % and 84.6 %, respectively, while the higher dose stated a sensitivity and specificity of 68.2 % and 80 %, respectively. A custom-built device for imaging was used, which emitted light at a wavelength of 405 nm. Ottolino-Perry et al. (2021) reported no adverse side effects.

5.4.5. 5-ALA for various tumors

A 2019 study utilized 5-ALA for the detection of various tumors (n=124, including sarcomas, carcinomas, lymphomas, mast cell tumors, benign tumors, and non-neoplastic lesions) in dogs and cats, assigned to IIb according to the PRISMA classification. (Osaki et al. 2019) A dosage of 40 mg/kg of 5-ALA was administered orally four hours before surgery. For malignant tumors, this approach showed a success rate of 88.8 % (n=103/116), with 89 % (n=57/64) success rate for carcinomas, 88.8 % (n=32/36) for sarcomas, 84.6 % (n=11/13) for mast cell tumors and 100 % (n=3/3) for lymphomas. 57.1 % (n=8/14) of benign and 42.9 % (n=6/14) of non-neoplastic lesions turned fluorescent. The sensitivity and specificity of this study were 89.5 % and 50 %, respectively. Multiple false positives as well as some false negatives were identified. No 5-ALA-induced fluorescence was observed in hemangiosarcomas. For imaging, a 405 nm LED light source, the HDR-CX180 (SONY, Tokyo, Japan) video camera as well as three spectrometers (LED405-SMA-TI, R600-8-UV-VIS-SR, Black-Comet CXR-50 TEC; StellarNet, Tampa, USA) were employed to quantify the fluorescence. The peak fluorescence value of PpIX was at 635 nm. No adverse side effects were reported (n=144).

In comparison, a 2024 study in human medicine used 5-ALA for the detection of squamous cell carcinoma. (Filip et al. 2024) A dose of 20 mg/kg of 5-ALA was administered orally 3-5 hours before the initiation of anaesthesia. Six out of seven tumors fluoresced in situ, resulting in a success rate of 85.7 %. In one case, the surgery was delayed to 8.5 hours after the administration of the fluorophore, which likely resulted in the absence of fluorescence. In one instance, a lymph node fluoresced, which was later confirmed to be metastatic. Fluorescence

was triggered using a light source with a wavelength of 405 nm. A camera system with an external filter was used for imaging. The operating room lights were equipped with a filter blocking wavelengths below 470 nm. In 42.9 % (n=3/7) of cases, side effects such as photosensitivity in the form of a rash, elevated liver enzymes, and nausea were observed.

6. Discussion

This review shows that the use of ICG, 5-ALA, MB and FS in veterinary oncology is not yet widespread and has only been investigated in a limited number of studies. However, the results of the existing literature are promising, showing potential for reducing tumor recurrence and improving prognosis in dogs and cats.

FS is the least studied fluorophore, with only one published paper. Due to the small sample size, a comparison with human medicine is not truly feasible. Notably, the dosage in veterinary medicine is four to seven times higher than in human medicine (20 mg/kg in veterinary medicine versus 3–5 mg/kg in human medicine). This may explain why yellow discoloration in humans is limited to urine, whereas in dogs, it also affects faeces, skin, and mucous membranes.

MB has so far only been used for SLNM in veterinary medicine. The body of research on its use is relatively large compared to other applications, with 10 papers and 446 dogs included. However, in 90 % of these studies, other techniques, such as scintigraphy with tech99, were employed as well, which limits the significance of the reported success rates of 79.4–99.4 %. In human medicine, MB is used at doses four to forty times higher than in veterinary medicine (1–10 mg/tumor in veterinary medicine, 40 mg/tumor in human medicine), though it should be noted that veterinary patients generally have significantly less body weight than human patients. The timing of administration in veterinary medicine varies from 15 minutes before surgery to intraoperative administration. No veterinary studies mentioned the side effect of temporary urine discoloration, but some reports on discoloration of surrounding tissue were found. To date, intra-arterial administration of MB for SLNM has only been described in human medicine, with promising results.

5-ALA has been used in veterinary medicine for various neoplasms (intracranial lesions, mesotheliomas, mammary and lung tumors, carcinomas, and sarcomas). However, the sample sizes for each tumor type are small (n = 126 across 6 papers, 3 of which are case reports), which reduces the significance of success rates ranging from 57.1 % to 100 %. Although the doses in veterinary and human medicine are quite similar (20–40 mg/kg in veterinary medicine, 15–30 mg/kg in human medicine), more side effects are reported in human medicine, such as photoreactions, rashes, vomiting, nausea, and temporarily elevated liver enzymes. In veterinary medicine, mild nausea has been reported as the only adverse effect. The absence of photosensitivity in animals may be explained by the presence of fur.

None of the veterinary papers mention liver enzyme controls after the administration of fluorophores, therefore no conclusions can be drawn. To prevent nausea and potential vomiting before adequate 5-ALA absorption, an antiemetic drug should be administered. The high costs of 5-ALA may prohibit its routine use in veterinary practice due to the lack of pet insurance and financial constraints.

The applications of FS and 5-ALA overlap in neurosurgery. While FS has a clear cost advantage, 5-ALA is more tumor-specific due to its metabolic mechanism. Costs and benefits should be carefully weighed in such cases.

5-ALA also has potential as a photosensitizer for photodynamic therapy in veterinary medicine but has been minimally explored to date. Osaki et al. (2019) administered 40 mg/kg of 5-ALA to fourteen animals (4 cats, 10 dogs, 15 tumors) for photodynamic therapy. The tumors were irradiated using LED (n=4) or diode laser light (n=10). Eleven of the fifteen tumors exhibited fluorescence (73.3 %). Each animal underwent between 1 and 19 treatments. Due to additional chemotherapy administered in 6 cases, the exact success rate and long-term prognosis of PDT with 5-ALA could not be determined. Disease progression was observed in 20 % (n=3/15) of cases. Stabilisation of the disease was noted in 20 % (n=3/15), partial response in 40 % (n=6/15), and a complete response in 20 % (n=3/15).

ICG has been the most researched fluorophore in veterinary medicine over the past decade (n = 310, 14 papers), although the sample sizes remain limited, with an average of n = 22. ICG was mostly used for SLNM but has also been applied in liver, lung, mammary, and superficial tumors. Similar intravenous doses were used in both veterinary and human medicine (0.5–5 mg/kg in veterinary medicine, 0.25–5 mg/kg in human medicine). For peritumoral injections, human medicine employed considerably higher doses (0.05–5 mg in veterinary medicine, 0.5–10 mg in human medicine). Given the typically higher body weight of humans to dogs or cats, the dosages cannot be compared adequately. No side effects were observed in humans. In veterinary medicine, only one case of temporary nodule formation at the injection site was reported. Success rates in studies ranged from 40 % to 100 %. The primary limitation of ICG is the frequent occurrence of false-positive results due to the staining of non-neoplastic tissue, such as inflammation. Further research with larger sample sizes is needed to assess the reliability of ICG.

ICG and FS have also been used for visualizing the vessels in the fundus in veterinary ophthalmology. Occelli et al. (2022) studied 8 dogs and 13 cats with a physiological fundus.

ICG was injected as a 1 mg/kg intravenous bolus, while FS was administered at a dose of 20 mg/kg, also intravenously. Fundus images were captured from the time of injection up to 5 minutes afterwards. A challenge with this technique is accurately capturing the various phases (arterial, arteriovenous, venous, late). If a phase is missed and images cannot be evaluated, one must wait until the fluorophore clears from the vascular system before attempting another try. Unlike FS, ICG allows visualization of the choroidal vessels as well, though this can result in poor visibility of retinal vessels. The laser used for FS fluorescence imaging caused eye movements even in anaesthetized animals. A benefit of these two methods is the ability to visualize blood flow, thereby identifying vascular leakage and poor perfusion.

ICG has also been used to visualize vessels and facilitate the creation of skin flap plasties. Eiger et al. (2024) injected 15 dogs with 0.5 mg/kg ICG intravenously. Immediately thereafter, three regions for flap plasties (caudal superficial epigastric flap, omocervical flap, thoracodorsal flap) were examined in each dog for fluorescence using a SPY-Portable Handheld Imaging System (SPY-PHI Elite, Stryker, Kalamazoo, Michigan) and images were taken. Five board-certified surgeons evaluated the quality of the fluorescence angiography images, scoring them from 0 to 4. The caudal superficial epigastric flap appeared most suitable for this method with a mean score of 3.97. The omocervical flap scored 1.27, and the thoracodorsal flap scored 1.59. ICG angiography thus seems to be a good method for visualizing the vessels of a caudal superficial epigastric flap before incision to ensure optimal perfusion.

ICG has also been used for liver function assessment in dogs. The ICG clearance of 10 healthy dogs was measured during and post-anaesthesia. ICG was administered intravenously at a dose of 0.5 mg/kg, and the ICG content in the blood was measured transcutaneously on the tail using an NIR spectrometer, expressed as a percentage. Additionally, blood samples were taken every 5 minutes up to 30 minutes to compare results. In awake animals, the two methods demonstrated a strong correlation (81% variance). However, results in anaesthetized animals differed more significantly (14% variance), suggesting that the transcutaneous measurement method should only be used for liver function assessment in awake animals. The study also reaffirmed that physiological clearance in dogs is significantly lower than in humans (3.7 ml/min/kg vs. 8.9 ml/min/kg). (Grobelna et al. 2016)

A 2016 study evaluated a different fluorophore for its ability to visualize residual cells in mast cell tumors and soft tissue sarcomas. (Bartholf DeWitt et al. 2016) LUM015 (Lumicell, Inc, Wellesley, MA) is a macromolecule categorized as a metabolized fluorophore, activated by

proteases. These are overexpressed in certain tumor types, leading to accumulation of LUM015 in these neoplasms and the production of far-red fluorescence. In physiological tissues with high protease levels, such as the liver, the signal-to-background ratio decreases. Bartholf DeWitt et al. (2016) injected LUM015 intravenously into 19 dogs 24–4 hours before surgery. After tumor removal, the wound bed was examined using a handheld imaging device, and biopsies were taken for histological analysis. The success rate in distinguishing neoplastic from healthy tissue was 92 % (n=93/101). LUM015 has shown a sensitivity and specificity of 92 % and 92 %. Five false-positive biopsies were obtained, which turned out to be fat (n=4) and collagenous tissue (n=1), and three false-negative samples, which did not fluoresce but contained tumor cells, were also noted. In 10 out of 19 dogs (53 %), erythema and facial swelling, with or without itching, were observed but resolved after administration of antihistaminic drugs.

Although the research on ICG, 5-ALA, MB and FS yields encouraging results, future research should focus on targeted fluorophores as well, due to the high number of false positives associated with passive mechanisms. These tumor-specific dyes can provide clearer visualization of neoplasms without the risk of staining inflammatory or other non-neoplastic tissue. In 2020, Favril and Brioschi et al. (2020) investigated the NIR fluorophore DA364, composed of cyanine dye and a peptidomimetic moiety, which targets integrins. DA364 was administered intravenously to 24 dogs with superficial tumors 24–48 hours before surgery. Only 50 % (n =15/30) of neoplasms were well-demarcated by the fluorophore. After tumor removal, the wound bed was examined for residual fluorescence with a handheld NIR device. 15 wound beds (50 %, n=15/30) showed no fluorescence and no residual tumor cells, while 4 biopsies (13.3 %, n=4/30) fluoresced and contained tumor cells. In 36.6 % (n=11/30), fluorescence was observed without evidence of residual tumor tissue. DA364 showed a success rate of 63.3 % (n=19/30) in correctly identifying residual neoplastic tissue. No side effects were observed. In 2017, Mery et al. (2017) also employed an integrin-targeting fluorophore, RAFT-(cRGD)4, called Angiostamp, which was administered to one cat and four dogs 15–18 hours before surgery. Of the eight known masses, six were stained and histopathologically identified as malignant neoplasms. The remaining two nodules did not fluoresce and were classified as non-neoplastic. Angiostamp thus achieved a 100% success rate in identifying neoplastic masses. Furthermore, five additional neoplasms were detected through fluorescence that would have otherwise been missed. It is important to note for integrin

targeting dyes, that only 25–50 % of tumors express αvβ3 integrins, so only these tumors will be stained by integrin-targeting methods.

A 2017 study used a folate receptor-targeted NIR fluorophore, OTL0038, to visualize primary canine lung tumors. (Keating et al. 2017) The dye was administered intravenously to 10 dogs 2–3 hours before surgery. OTL0038 enabled clear delineation of all tumors (100 %, n=10/10), and residual tumor tissue in one case, as well as three metastatic lymph nodes, were detected. NIR systems (Fluobeam, Fluoptics, Grenoble, France, and Karl Storz, Germany) were used for fluorescence imaging. No side effects were observed.

A completely different approach was taken by Martano et al. (2019), who used acridine orange, a fluorophore and photosensitizer, in seven cats with feline injection site sarcoma to combine photodynamic surgery and photodynamic therapy. Acridine orange accumulates in acidic environments. Due to increased glycolysis and other mechanisms, neoplastic tissue has a lower pH than healthy tissue, causing the dye to preferentially accumulate in tumors. In the study, the wound bed was covered with acridine orange intraoperatively after tumor removal. After 10 minutes in the dark, the fluorophore was removed, and the wound was examined for residual fluorescent tissue under blue light. Any fluorescing tissue was excised, and acridine orange was reapplied to the wound bed, followed by 10 minutes of white light irradiation to kill any overlooked residual cells through the photosensitizing effect. None of the seven cats experienced recurrence. In the control group of 30 cats with feline injection site sarcoma, 56.6 % (n=17/30) developed local recurrence and/or lung metastases.

Future research on ICG, 5-ALA, MB and FS should involve higher-quality studies with larger participant groups. Control groups or direct comparison between the fluorophores could provide valuable insights for integration of these dyes into clinical practice. Additionally, a largely overlooked variable that warrants further investigation is the long-term outcome and recurrence rate following surgery performed with fluorophores. Additionally, research should focus on the use of targeted fluorophores, as their tumor-specific action provides a safer method for visualizing neoplasms.

This systematic review was limited by the small number of available studies. Over the past 10 years, only 31 papers have been published in veterinary medicine. Moreover, the sample sizes in the published papers were small, with a total of 934 animals, averaging 30 animals per study. The largest included study investigated 124 animals. Furthermore, only four of the 31 papers included cats, with two being case reports. In total, only 22 feline patients were included (2.4 %,

n=22/934). This limits the conclusions about the efficacy and safety of fluorophores used on cats. Another limitation of the study is the mixed scientific quality of the available publications. Only one publication was classified as Ia, which had a small sample size as well. The remaining publications were classified as IIb (n = 19), III (n = 8), and IV (n = 3, case reports).

In conclusion, it can be stated, that ICG, 5-ALA, MB and FS have the potential to improve oncological surgery and its outcome. However, surgeons must be aware of the risk of false-positive results, especially when employing ICG. Further studies with larger subject groups, particularly including cats, are necessary. Additional research is especially needed on FS and 5-ALA, as the current body of research on these fluorophores is still limited. To reduce the frequent occurrence of false-positive results, research should also focus on tumor-specific targeted fluorophores.

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9. Appendix

Appendix 1: search protocol for the veterinary papers

https://docs.google.com/document/d/e/2PACX-1vQ8XqEKmGzNjdtQUcxce6Lo2Zxkm5c_FLRp2GduHiwEd9ObNYGvA0bT-ODo98I2R-GuRqHWw7FGkHSo/pub

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