



Review

# Fluorescence-Guided Surgery in Head and Neck Squamous Cell Carcinoma (HNSCC)

Albrecht Blossse <sup>1</sup>, Markus Pirlich <sup>2,3</sup> , Andreas Dietz <sup>2,3</sup>, Christin Möser <sup>4</sup>, Katrin Arnold <sup>4</sup>, Jessica Freitag <sup>4</sup>, Thomas Neumuth <sup>1</sup> , David M. Smith <sup>3,4,5</sup> , Hans Kubitschke <sup>5</sup> and Maximilian Gaenzle <sup>2,3,\*</sup>

<sup>1</sup> Innovation Center Computer Assisted Surgery, Faculty of Medicine, University of Leipzig, 04103 Leipzig, Germany; albrecht.blosse@medizin.uni-leipzig.de (A.B.)

<sup>2</sup> Department of Otorhinolaryngology, Head and Neck Surgery, University Hospital of Leipzig, 04103 Leipzig, Germany

<sup>3</sup> Comprehensive Cancer Center Central Germany (CCCG), Partner Site Leipzig, 04103 Leipzig, Germany; david.smith@izi.fraunhofer.de

<sup>4</sup> DNA Nanodevices Unit, Department of Medical Bioinformatics, Fraunhofer Institute for Cell Therapy and Immunology, 04103 Leipzig, Germany

<sup>5</sup> Peter Debye Institute for Soft Matter Physics, Faculty of Physics and Earth Sciences, University of Leipzig, 04103 Leipzig, Germany

\* Correspondence: maximilian.gaenzle@medizin.uni-leipzig.de; Tel.: +49-341-97-21936

## Abstract

Head and neck squamous cell carcinomas (HNSCCs) are the seventh most common form of cancer worldwide, typically characterized by high mortality and significant morbidity, including pain and speech and swallowing disorders. Complete tumor tissue resection, the common first line of therapy, remains a surgical challenge with room for improvements. Because tumor cells express highly specific surface molecules serving as receptors for ligands, specific targeting ligands can be conjugated to fluorescent molecules in order to better visualize tumor borders. Targeted fluorescence-guided surgery (T-FGS) as well as tumor-targeted and near-infrared (NIR) fluorescence imaging are emerging techniques for real-time intraoperative cancer imaging. Targeting agents include nanodots or fluorophores, which have been conjugated to specific ligands like antibodies, peptides, or other synthetic moieties. This article surveys tumor-targeted ligands in recent and current preclinical studies and clinical trials related to HNSCC, highlighting common NIRF dyes used for molecular imaging and their physical properties, working concentrations, and associated risks. Smaller ligands, nanodots, dual-modality NIR dyes, and activatable agents can enhance tumor-targeting processes, resulting in faster, more penetrable, and clearer imaging, which could lead to improved clinical applications and better tumor removal rates in the future.

**Keywords:** fluorescence-guided surgery (FGS); head and neck squamous cell carcinoma (HNSCC); DNA constructs; antibodies; fluorophores



Academic Editor: Pier Paolo Claudio

Received: 17 June 2025

Revised: 14 August 2025

Accepted: 14 August 2025

Published: 22 August 2025

**Citation:** Blossse, A.; Pirlich, M.; Dietz, A.; Möser, C.; Arnold, K.; Freitag, J.; Neumuth, T.; Smith, D.M.; Kubitschke, H.; Gaenzle, M. Fluorescence-Guided Surgery in Head and Neck Squamous Cell Carcinoma (HNSCC). *Int. J. Transl. Med.* **2025**, *5*, 40. <https://doi.org/10.3390/ijtm5030040>

**Copyright:** © 2025 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Worldwide, around 600,000 cases of head and neck squamous cell carcinoma (HNSCC) are diagnosed yearly. In Germany, there are 14,000 new HNSCC cases per year, making it the sixth most common malignant tumor [1,2]. Typically, HNSCC is characterized by a high mortality rate and considerable morbidity, including pain as well as speech and swallowing disorders. In many cases, surgical intervention is used as the first line of treatment, in both early and some advanced cases [3].

For HNSCC, the primary objective of tumor surgery is to achieve tumor-free survival, while also prioritizing postoperative quality of life and functional preservation [4,5]. The residue-free resection of tumor tissue is a particular challenge in the oncological surgery of HNSCC. The clear visualization of surgical margins is crucial in ensuring a successful treatment, as residual tumor cells can lead to the recurrence of the malignancy. While postoperative radiotherapy reduces local recurrence, it is still mandatory to achieve clear margins during the initial resection [6–8]. A clear margin (or negative margin) is defined as a tumor-free circumference of the pathological specimen with at least 5 mm of healthy tissue surrounding the tumor. By contrast, a positive margin indicates the presence of cancer cells at the edge of the resected tissue, while close margins refer to a resection distance of less than 5 mm from the tumor. In cases of positive or close margins, current guidelines recommend either re-resection or postoperative chemoradiation. An exception is made for vocal cord cancer, where control microlaryngoscopy is preferred over re-resection in the presence of close margins [5].

The current gold standard for intraoperative margin evaluation is a combination of the surgeon's visual and tactile identification of malignant tissue, along with frozen section analysis (FSA). The effectiveness of FSA heavily depends on the surgeon's ability to anticipate the areas closest to the margin or likely involved with the tumor based on inspection and palpation. However, this method suffers from low sensitivity, reportedly around 36% [4,9]. In laryngeal carcinomas specifically, the sensitivity of frozen section analysis can be even lower—as low as 22% [10]. A study by DiNardo et al. revealed that 40% (8 out of 20) of patients with positive final margins and 100% (15 of 15) with close (<5 mm) margins were not detected by FSA. The overall accuracy of FSA in identifying close or positive final margins was 71.3%, with a sensitivity of 34.3% and a specificity of 100% [11].

Frozen section sampling can be performed in two distinct ways: sampling from the tumor bed or sampling from the edge of the resected specimen [12]. Several studies have demonstrated that tumor bed sampling is more effective for accurate margin assessment [13]. There are two primary sources of error in FSA for margin analysis: sampling errors occur when diagnostic tumor cells are absent in frozen sections but are present in deeper, permanent sections, whereas interpretation errors occur when tumor cells are present in frozen sections but not recognized by the pathologist. Sampling errors are generally attributed to the surgeon, while interpretation errors are typically the responsibility of the pathologist. False-negative results due to interpretation errors are typically more frequent than false positives [12,14,15]. Efficient use of FSA is crucial due to the processing time per section, which ranges from 20 to 60 min [10]. Woolgar et al. conducted a study on positive resection margins in head and neck cancer surgeries, analyzing 301 specimens. They found that 23% ( $n = 70$ ) of all tumor resections—limited to oral cavity and oropharyngeal carcinomas, excluding hypopharyngeal and laryngeal carcinomas—resulted in positive margins. Among these, 16% involved the superficial mucosal layer, whereas 87% affected the deep resection margins; some specimens contained both [16].

In most cases (except for some locations in the oral cavity), poorer outcomes due to residual tumor cells at positive surgical margins can be improved by re-excision of the positive margin several days following the initial surgery [17,18]. Re-resection is still difficult, with a major factor being the challenge in identifying residual cancer tissue during repeat surgery. Further research on local spread patterns has prompted some experts to recommend using wider margins for the primary tumor surgery than the conventional 0.5 cm macroscopic clearance [19]. However, wider surgical margins can subsequently lead to greater functional impairment in patients following surgery. Additionally, induction protocols offering chemo- or immunotherapy before and after surgery will change

the pattern of the remnant tumor borders, which enhances the difficulties for achieving clear margins [20–23].

The anatomical structures in the ENT (Ear, Nose, and Throat) region have close relationships with critical risk structures such as the carotid artery, jugular vein, and cervical nerves. This proximity underscores the importance of clearly defining tumor boundaries and ensuring precise resection margins during surgical procedures. Although there have been significant advancements in surgical techniques, including endoscopic, transoral laser, and robotic technologies designed to reduce patient morbidity, these procedures still primarily rely on the surgeon's anatomical visualization using white-light reflectance. Different tissues within the body present various shades; bone, nerves, cartilage, fat, connective tissue, and muscle range from white to pink, whereas blood vessels exhibit shades from red to deep red. This color similarity complicates the clear visual distinction of tissue boundaries. Moreover, the use of cameras and instruments inserted through narrow conduits during minimally invasive surgeries reduces the tactile feedback that surgeons traditionally depend on, increasing their reliance on visual cues.

Enhancing the visual differentiation between healthy and cancerous tissues by employing fluorescent probes that target specific structures or disease states in real time could significantly improve surgical precision. This approach, referred to as fluorescence-guided surgery (FGS), can be linked to visually color-coding the surgical field, thereby aiding in the more accurate identification and dissection of tissue types. Particularly in the context of deep surgical margins, *in vivo* fluorescence imaging has demonstrated high sensitivity for detecting residual disease in the wound bed [24]. Enhanced visual clarity during surgery could lead to the more precise excision of tumors, reducing the need for postoperative chemotherapy while concurrently reducing long-term negative effects on the surrounding healthy tissues. This, in turn, would minimize long-term toxicity for patients and lower overall healthcare costs [25].

This review paper will address the question of how FGS is already being used in preclinical and clinical studies and which physical principles underlie FGS technology, with an emphasis on approaches within or relevant to HNSCC surgery. There have been studies into the use of FGS *ex vivo* to support the process of pathological imaging. While there are distinct similarities between *ex vivo* examination and real-time, *in vivo* application due to the underlying technology, there still are significant differences in terms of toxicity, the control of external light sources (for example, surgical lamps interfering with the fluorescence), and the stability of the environment itself [4].

## 2. History of Fluorescence-Guided Surgery (FGS)

The origins of fluorescence-guided surgery can be traced back to the 19th century, when Sir George G. Stokes first described fluorescence in 1852 [26]. In 1929, Copeman et al. introduced the use of “activated” fluorescein in the systemic treatment of cancer, a form of early chemotherapy that showed significant success in reducing tumor size. However, due to severe side effects, the treatment was ultimately discontinued [27]. Its potential in surgical applications was not explored until the mid-20th century, when Moore et al. first described the use of fluorescein in the detection of different tissue types [28]. In the 1960s, Goldhahn investigated the use of tetracycline fluorescence for detecting brain tumor margins [29], though this did not achieve clinical implementation. Subsequently, various dyes have been employed for endoscopic diagnostics [30].

Efforts to utilize the natural autofluorescence of the endogenous molecules prevalent in tumors followed [31], yet progress in translating these findings from animal models to the human oral cavity has been limited. A pivotal study by Stummer et al. in the late 1990s highlighted the use of 5-aminolevulinic acid (5-ALA), which induces fluorescent porphyrin

production in glioma cells. Their study demonstrated that 5-ALA significantly improved glioma visualization and resection during surgery, improving patient outcomes. In parallel, the use of contrast agents such as indocyanine green (ICG) became more prevalent and was first described by Sheridan et al. in the evaluation of burns [32].

The use of the non-specific dye ICG in tumor surgery, in this case, in sentinel lymph node detection, was first described by Kitai et al. in 2005 [33]. Presently, FGS is widely utilized in several surgical specialties, including oncology, neurosurgery, and urology, showing promising results [34]. In the 21st century, significant advancements in fluorescence imaging technology have included targeted FGS (T-FGS), which targets fluorophores toward tumor-specific receptors in various tissues, such as the folate receptor- $\alpha$  in ovarian cancer [35]. A more detailed overview of T-FGS approaches in relation to HNSCC surgeries will be given in the subsequent section. Innovations such as Fluorescence-Assisted Resection and Exploration (FLARE<sup>TM</sup>) imaging systems in the field of near-infrared fluorescence imaging, combined with conventional fluorophores like methylene blue, ICG, or nanoparticles, represent potential advancements in this field [36], however, are yet to be integrated into standard clinical practice for tumor detection.

### 3. The Technology

FGS is an innovative technology that enhances the precision of surgical procedures by using fluorescent dyes and specialized imaging systems to visualize and distinguish tissues. By enabling a real-time, visual delineation of borders between cancerous and healthy tissues, it has the potential to revolutionize surgery and improve surgical outcomes by enhancing the visualization of tissues for either resection, as in the case of tumors, or preservation, as in the case of nerves or vasculature. Utilizing compact and relatively low-cost imaging systems, FGS can be readily implemented into many procedures to bridge the gap between preoperative imaging using methods such as magnetic resonance imaging (MRI) and computed tomography (CT) and the current intraoperative reality [37]. In the following sections, we describe several key molecular and technological components of FGS, emphasizing specific aspects relevant for HNSCC resections when applicable.

#### 3.1. Fluorescent Dye Selection and Administration

**Dye Selection:** Different dyes can be used depending upon the type of tissue or condition being targeted. Common examples include ICG, which is used for vascular imaging and perfusion assessment, and 5-ALA, which is metabolized into a fluorescent compound in certain cancer cells. More specific agents include intravenously administered Pafolacianine (Cytalux<sup>®</sup>), which has been shown to selectively target the folate receptor- $\alpha$  (FR- $\alpha$ ) in epithelial ovarian cancer [38]. Additionally, hexaminolevulinate (Hexvix<sup>®</sup>) is utilized for the diagnosis of urinary bladder carcinoma, enabling enhanced visualization of malignant cells through hexaminolevulinate fluorescence cystoscopy [39]. Methylene blue has also been investigated in clinical trials for intraoperative tumor staining, particularly in abdominal surgery. However, its efficacy in selective tumor labeling has yielded variable and sometimes contradictory results, preventing the establishment of a standardized protocol to date [40]. ICG still is the most used agent in untargeted FGS as well as in the testing and standardization of newly approved FGS imaging systems.

**Administration to Patients:** The dye can be injected intravenously or administered orally, depending on its properties and the clinical application. For instance, 5-ALA is often taken orally by patients undergoing brain tumor surgery. The time of conjugation differs between agents, as shown in Table 1, but most clinical agents were typically conjugated 24 h prior to the tumor resection. The peak fluorescence signal depends strongly on the conjugation time and also on the concentration of ICG that has been administered (Figure 1).

**Table 1.** Summary of clinical fluorescence imaging agents, detailing fluorophores, dose regimens with cohort distribution (mg/kg or mg/m<sup>2</sup>), and preoperative administration timing (conjugation intervals) across multiple recruiting or finished studies.

| Agent                  | Target | Fluorescence | Clinical Trials<br>Registered Number<br>( <a href="https://ClinicalTrials.gov">ClinicalTrials.gov</a><br>(accessed on 1 July 2025),<br><a href="https://Clinicaltrialsregister.eu">Clinicaltrialsregister.eu</a><br>(accessed on 1 July 2025),<br><a href="https://chictr.org.cn">chictr.org.cn</a> (accessed<br>on 1 July 2025)) | Dose (mg/kg<br>or mg/m <sup>2</sup> ; Cohort<br>Distribution) or Total<br>Mass Administered<br>(mg)                                                    | Conjugation<br>(Time Before<br>Operation in h) |
|------------------------|--------|--------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------|
| ONM-100                |        | ICG          | NCT03735680 [41]                                                                                                                                                                                                                                                                                                                  | 1.2 mg/kg (7/13)<br>0.1 mg/kg (3/13)<br>0.5 mg/kg (2/13)<br>0.8 mg/kg (1/13)                                                                           | 24 ± 8                                         |
|                        |        |              |                                                                                                                                                                                                                                                                                                                                   | 1 mg/kg (3/30)<br>3 mg/kg (3/30)<br>2 mg/kg (3/30)<br>1 mg/kg (6/30)<br>1 mg/kg (11/30)                                                                | 3 ± 2<br>3 ± 2<br>6 ± 3<br>16–80<br>24 ± 8     |
|                        |        |              |                                                                                                                                                                                                                                                                                                                                   |                                                                                                                                                        |                                                |
|                        |        |              |                                                                                                                                                                                                                                                                                                                                   |                                                                                                                                                        |                                                |
|                        |        |              |                                                                                                                                                                                                                                                                                                                                   |                                                                                                                                                        |                                                |
|                        |        |              |                                                                                                                                                                                                                                                                                                                                   |                                                                                                                                                        |                                                |
| Cetuximab-<br>IRDye800 | EGFR   | IRDye800CW   | NCT03733210 [42]                                                                                                                                                                                                                                                                                                                  | 2.5 mg/m <sup>2</sup> (3/12);<br>25 mg/m <sup>2</sup> (6/12);<br>62.5 mg/m <sup>2</sup> (3/12)<br>(NCT01987375)<br>and 30 mg/m <sup>2</sup><br>(14/14) | 24 ± 8                                         |
|                        |        |              | NCT03134846 [43]                                                                                                                                                                                                                                                                                                                  | 10 mg/m <sup>2</sup> (3/9)<br>25 mg/m <sup>2</sup> (3/9)<br>50 mg/m <sup>2</sup> (3/9)                                                                 | 24 ± 8                                         |
| Pegsitacianine         |        | ICG          | NCT05576974 [44]                                                                                                                                                                                                                                                                                                                  | 1 mg/kg                                                                                                                                                | 6–300                                          |
| FG001                  | uPAR   | ICG          | FG001-CT-003 [45]                                                                                                                                                                                                                                                                                                                 | 4 mg (4/16)<br>16 mg (8/16)<br>36 mg (4/16)                                                                                                            | 12 ± 4                                         |
| PARPi-FL               | PAR1   |              | NCT03085147 [46]                                                                                                                                                                                                                                                                                                                  | 15 mL<br>100 nM (3/12)<br>250 nM (3/12)<br>500 nM (3/12)<br>1000 nM (3/12)                                                                             | 0.1                                            |
| cMBP-ICG               | c-MET  | ICG          | ChiCTR2200058058 [47]                                                                                                                                                                                                                                                                                                             | 2.5 µM (5/10)<br>5.0 µM (5/10)                                                                                                                         | 16 ± 8                                         |

There have also been efforts to apply fluorescent dyes directly to the tissue of interest, either in vivo as a spray or ex vivo on resected specimens. For example, cMBP-ICG, a c-MET-targeted fluorescent probe, demonstrated a significant 11% increase in tumor detection accuracy when applied topically to resected head and neck cancer tissue [47,48]. In another approach using superficial (topical) application, the activatable enzymatic probe gGlu-HMRG provided rapid and high-contrast fluorescence; however, it also produced false-positive signals in areas affected by electrosurgical cutting, due to auto fluorescent artifacts [49].

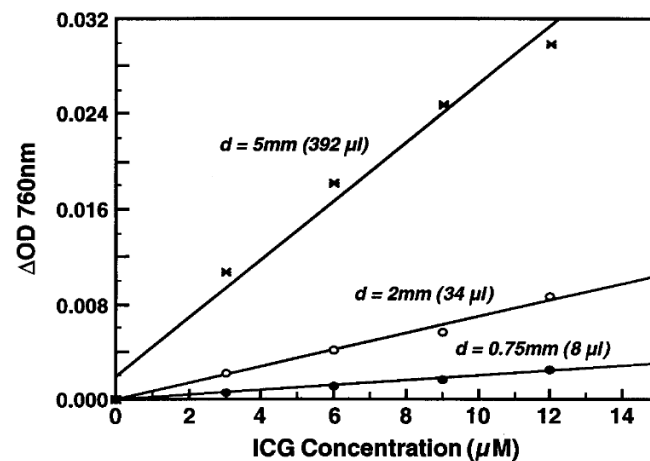


**Figure 1.** Detection of different concentrations of ICG at the NIR wavelength 760 nm in model systems of 8  $\mu$ L, 34  $\mu$ L, and 392  $\mu$ L. Reprinted with permission from Ref. [42]. Copyright 2006, John Wiley and Sons.

### 3.2. Fluorescence Emission

**Light Activation:** During the procedure, the targeted tissue labeled with the specific dye is exposed to a certain wavelength of light (Figure 2), which stimulates fluorescence by photon absorption. The subsequent emission of a photon with lower energy resets the dye molecule to its ground state.

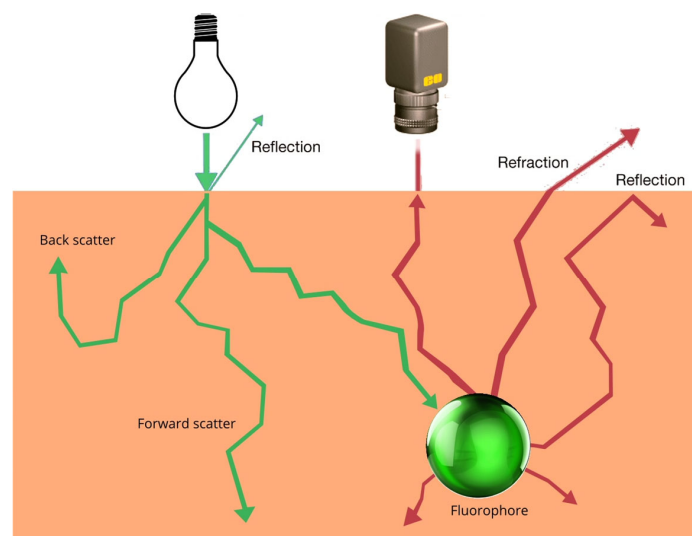


**Figure 2.** Conceptual description of interactions and photon paths within tissues during FGS. Within the tissue, photons experience various types of scattering interactions with cells and other biological materials, with some eventually exciting the embedded fluorophore. Those emitted by the excited fluorophore as it returns to its ground state also experience scattering interactions within the tissue, as well as reflection and refraction interactions at the tissue–air interface, before a subset reach the detector.

**Emission:** The dye in the targeted tissue emits fluorescence at a different wavelength than the applied excitation, red-shifted due to the lower energy (and associated longer wavelength) of the emitted photons. This fluorescence is detected by specialized cameras equipped with filters to block out the excitation light and allow only the emitted fluorescence to pass through. The optimal window for fluorescence imaging in the NIR light region lies between 650 and 900 nm [50,51] (Figure 3). The source emission density lies between 10 and 25 mW/cm<sup>2</sup> in available commercial systems, which makes fluorophore excitation possible without significant photo-bleaching, which might harm the patients' healthy tissue [52].

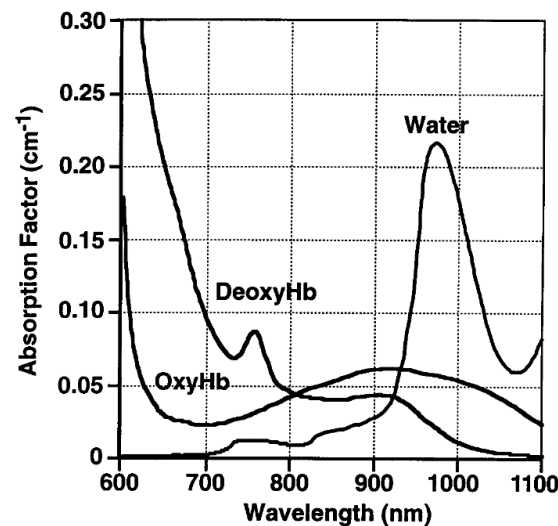


**Figure 3.** The “NIR window” based on absorption of water and blood. Differences in vasculature and oxygenation levels between normal and tumorous tissues can enable a delineation of tumor boundaries. Reprinted with permission from Ref. [42]. Copyright 2006, John Wiley and Sons.

There are two different physical properties that reduce the visibility of fluorescence emission within tissues: On the one hand, absorption plays a role; the most relevant absorbers of photons are water, lipids, oxyhemoglobin, and deoxyhemoglobin (Figure 2) [50]. Blood absorbs most of the photons in the visible region, with the highest absorption in the blue–green region. The large absorption of hemoglobin and myoglobin under 600 nm and water above 900 nm leaves a small “therapeutic window” where tissue information can be obtained deeper than 1 mm below the surface [53].

The pathlength of penetrating light ranges between 0.1 mm and 10 cm and can be described as follows:

$$\log \frac{I}{I_0} = -\mu_a L$$

where  $I$  is the transmitted intensity,  $I_0$  the emitted light intensity,  $\mu$  the absorption coefficient, and  $L$  the traveled pathlength [53].

On the other hand, scattering can hinder visualization by changing the trajectory of the excitation source or emitted light. Particularly in the aforementioned “therapeutic window” of light frequencies optimal for FGS, scattering is more prevalent than absorption. Elastic tissue scattering depends on the heterogenic refractive indices of extracellular, cellular, and subcellular components [53]. Light transmission through tissue increases the degree of randomization of the propagation direction [50]. This diffusion hinders the exact determination of the signal strength and signal location. The diffusion and reflections inside the tissue help to trigger the excitation of the fluorescence. The fluorescence signal depends on the optical properties and the penetration depth of photons inside the tissue.

### 3.3. Physical Interactions with Cells

**Dye Interactions with Cells:** The administered dye circulates through the bloodstream and accumulates in the target tissues. Tumors, for example, may uptake more dye due to their vascular properties, enhanced permeability and retention (EPR) effect, and higher number of fibroblasts or due to metabolic characteristics (e.g., tumor acidosis). Cancer cells often exhibit altered surface charge properties compared to normal cells due to changes in their membrane composition, including altered levels of sialic acid, glycoproteins, and glycolipids [54,55]. These changes often lead to a different net surface charge, typically more negative, which can be exploited for detection, e.g., through electrophoresis and Zeta potential measurements [56]. Thus, fluorescent probes having a positive (surface) charge help in preferentially binding to

negatively charged (cancer) cell membranes [57,58]. Furthermore, a hydrophobic domain on the probe molecule assists in its integration into lipid-rich environments, namely, the cell membrane, thus enhancing its ability to effectively label certain types of tissues or tumors. A large hydrophobic domain facilitates integration into the cell membrane; however, the associated drawback is a significant decrease in water solubility. Progression in cancer treatment and nanotechnology might overcome this physical hurdle by using self-assembling micelles, i.e., lipid monolayers with a hydrophobic core and a hydrophilic shell, which can engulf a hydrophobic agent and thereby provide a hydrophilic shell [59,60]. These probe-carrying micelles can then fuse with cell membranes, releasing the probes into their interior, e.g., via membrane fusion proteins like the SNARE family [61].

The intra- and extracellular environment of many tumors is more acidic compared to that of normal tissues due to the abnormal anaerobic metabolism of cancerous cells, rendering pH an effective, albeit unspecific, method for marking and targeting cancers [62–64]. Logically, pH-activatable NIR probes have been developed, such as NIR dyes BODO-3 [65] and PH08 [66], a dye for the green fluorescence channel CS-1 [67], and many more [68].

### 3.4. Tissue Targeting

**Target Selection:** Some ligands are designed to target specific molecular receptors and markers on the surface of cancer cells or within the tumor microenvironment. In T-FGS, fluorescent dyes are chemically conjugated to a monoclonal antibody or some other targeting moiety to achieve enhanced accumulation on the desired tissues [69]. The selected receptors should either be overexpressed or exclusively expressed on the surfaces of cells present in target cancerous tissues. In HNSCC, proteins such as the epidermal growth factor receptor (EGFR), which is directly overexpressed on HNSCC tumor cells [70], are commonly selected. Several studies have also identified other potential protein targets on HNSCC tumor samples or representative cell lines through either proteomic, histological, or other immunohistochemical means [71]. Beyond EGFR, several other tyrosine kinase receptors, in particular, ephrin receptors EphA2 [72] and EphB4 [73,74] have been detected in moderate or high amounts on tumor cells. Other potential targets include the Mucin-1 receptor (MUC-1) [75] or the family of CD44 variants [76,77], which have recently been used as molecular targets in preclinical CAR-NK-based therapies [78], and the urokinase plasminogen activator receptor (uPAR) [79,80]. The  $\alpha V\beta 6$  integrin, a relatively recent target, is highly expressed in 80–100% of HNSCCs but weakly or not at all in healthy epithelium [81–83]. Its expression appears to increase with disease stage [84] and is higher than that of EGFR in HNSCC [81,83].

Tumor-adjacent targets such as the folate receptor- $\beta$  (FR- $\beta$ ) [85] and the fibroblast activation protein (FAP) [86] are found in HNSCC to be expressed on tumor-associated macrophages (TAMs) and on cancer-associated fibroblasts (CAFs), respectively. It is important to note that heterogeneity in expression patterns among patients, resulting from severity, staging, or other factors, limits a one-size-fits-all strategy for T-FGS, potentially creating the need for a combination of methods to delineate boundaries in some patients.

**Multimodal Targeting and Imaging:** For many dyes and conjugations to biomolecules used for targeting specific tissues, it is vital to have a hydrophobic and hydrophilic domain for good solubility, to enable the efficient distribution and uptake of the dye in bodily fluids, and to reduce non-specific accumulation. Non-specific uptake is a key antagonist of FGS, which can, for instance, be remedied to a certain extent via paired-agent imaging (PAI) like in the case of targeting EGFR in HNSCC [87]. Therein, a non-targeting fluorescent dye is paired with a targeting moiety, providing a path for signal normalization [87], effectively enhancing the signal-to-noise ratio. As a modern development, targeting CD44v6 in HNSCC via a fluorescent tracer is currently under investigation and displays promising results, with an accuracy of over

90% for detecting invasion regions [76,77]. In FGS, it is common to combine an NIR imaging agent with a second, radioactive labeling technique, termed dual-modality imaging, like in FAP targeting, by combining an NIR dye with a PET marker [88].

**Ligand Selection:** Small targeting ligands exhibit faster pharmacokinetic properties and, thus, achieve sufficient contrast more quickly than large molecules, which enables imaging sooner after administration [69]. On the other hand, small targeting ligands are less amenable to conjugation and chemical modifications than large molecules, and small structural changes can drastically affect their targeting and pharmacokinetic properties [89]. Antibodies are large (>10 nm, 150 kDa), and, therefore, fluorophore–antibody conjugates exhibit long circulation times and non-specific tumor uptake through the EPR effect, the phenomenon of the passive accumulation of macromolecules, liposomes, or nanoparticles in tumor tissues due to their leaky vasculature [90]. Nevertheless, for HNSCC, several ready-to-use options with cetuximab conjugates exist [91,92] and have shown promising results in the head and neck region, overcoming both surgical and pathological assessment [93].

**Synthetic Targeting Components:** Apart from monoclonal antibodies and fragments thereof (e.g., nanobodies), which currently make up the bulk of agents in regular use or in clinical trials for T-FGS, the generation of targeted imaging agents through chemical or biosynthesis offers the means to create designer labels through the modular assembly of chemically defined building blocks. While still largely in the conceptual or early preclinical stage, their eventual implementation could ease some hurdles related to the manufacturing of biologics, as well as support better performance due to the enhanced biodistribution of smaller agents and integration of multiple components for targeting, protection, and signal generation. The aforementioned dual-modality probe targeting FAP in HNSCC tumors for both NIR and PET imaging is one such example [88], utilizing a seven-amino-acid residue peptide, FAP-2286, chemically conjugated to ICG to achieve a high degree of localization to xenograft tumors from several HNSCC cell lines (FaDu, CAL27, CNE2) in mouse models. High-affinity small-molecule inhibitors can also be repurposed for visualization, as was done with the FAP-inhibitor FTL, which was conjugated to the NIR cyanine dye CI-S0456, as was demonstrated by Mukkamala et al. to localize to FaDu xenografts in mice [94]. Exploiting the overexpression of DNA repair enzymes in the nuclei of tumor cells resulting from genomic instability, PARPi-FL is a molecularly specific, small-molecule, fluorescent agent that targets poly ADP-ribose polymerase 1 (PARP1) [46]. C-MET-binding peptides (cMBP-ICG) in combination with ICG could also be topically applied [47].

In addition to peptides and small molecules, aptamers composed of DNA or RNA strands [95,96] are also attractive options for targeting structures on the tumor surface due to their small size, the ease of nucleotide synthesis even under good manufacturing practice (GMP) requirements, their straightforward chemical conjugation methods to dyes, and the optimization of their binding properties via selection. Several aptamers have been developed to specifically bind tyrosine kinase receptors [97], with HNSCC-relevant targets such as EphA2, EphB4 [98,99], EGFR [100–102], and Glucose transporter 1 [103] being prominent examples, although most were originally selected for direct therapeutic intervention. Aptamers chemically conjugated to dye molecules or gold nanoparticles have been used for the *in vivo* fluorescent imaging of other xenografted tumor entities, such as glioma [104,105], ovarian [106], or breast [107] cancer, which indicates their potential applicability to T-FGS in HNSCC.

More complex nanoparticle-based agents can be rationally constructed from the chemical conjugation and/or self-assembly of multiple biocompatible components on an underlying scaffold, such as those formed through the self-assembly of DNA strands (Figure 4). This strategy can, for example, exploit the multivalent presentation of small ligands [108] to amplify binding properties. It should be noted that all the following examples are more

conceptual in nature and were not developed with T-FGS in mind; however, all addressed targets were found to be associated with HNSCC tumors in prior literature. Using a minimalistic DNA–peptide structure consisting of three partially complementary oligonucleotides conjugated to a 12-amino-acid-long EphA2-binding peptide and a fluorescent dye, Möser et al. [109] were able to show significantly enhanced binding and pharmacological action on a prostate cancer cell line overexpressing the protein on its surface. A similar approach using a small DNA tetrahedron functionalized with aptamers against the MUC-1 surface protein successfully targeted breast cancer cell lines [110,111], with the integration of a Cy5 dye giving a convenient route toward in vivo labeling. EGFR was addressed using significantly larger DNA origami structures [112], typically in the 4–5 MDa range, which were conjugated to EGFR-binding aptamers or monoclonal antibodies along with the red-spectrum ATTO647N dye and used to track the diffusive motion of receptor clusters on breast cancer cells. Finally, EGFR and FR- $\beta$  on xenograft tumors in mice were targeted by functionalized DNA tetrahedron nanostructures in vivo; in the former, a chemotherapeutic payload was delivered to localized tumors, while in the latter case, the NIR dye Delight 755 enabled their imaging through the labeling of tumor-associated macrophages.

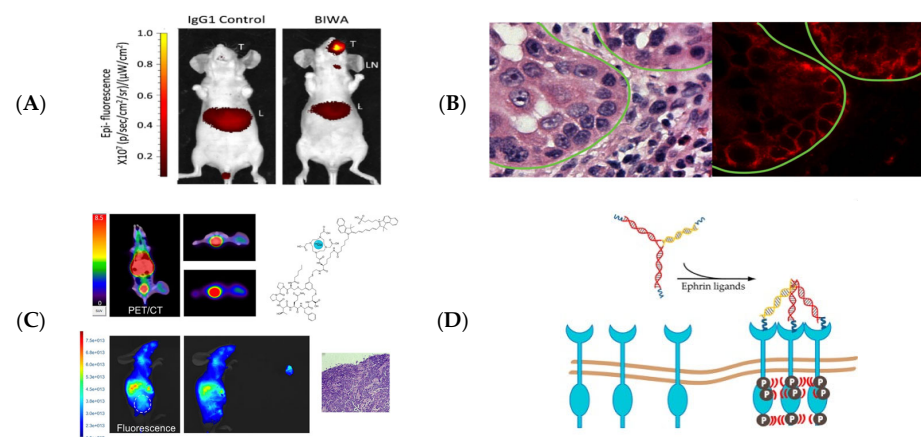


**Figure 4.** Specific tissues and features on impacted cells in the tumor can be targeted with various types of nanoagents. (A) The NIR dye IRDye800CW was conjugated to monoclonal anti-CD44v6 antibody along with radioactive label to produce the BIWA label. This was able to localize to human HNSCC xenografts in mice. Reprinted with permission from Ref. [76]. Copyright 2018, Springer Nature. (B) The EGFR-targeting antibody cetuximab was also conjugated to IRDye800CW and successfully labeled tumor cells in HNSCC surgical resections, with a comparable performance to standard haematoxylin and eosin staining. Reprinted with permission from Ref. [93]. Copyright 2016, John Wiley and Sons. (C) HNSCC xenografts in mice were also positively identified with a small labeling construct consisting of a short peptide fragment conjugated to NIR dye ICG. Reprinted with permission from Ref. [88]. Copyright 2023, Frontiers Media SA. (D) DNA-based nanoconstructs can be used to enhance the binding properties of small EphA2-targeting peptides through cooperative multivalence. Reprinted with permission from Ref. [109].

Further details about various agents and markers that have been recently used in surgical procedures for HNSCC or tested in clinical studies will be provided in Section 4 (*Applications*).

### 3.5. Adverse Effects

In the use of fluorescent labels to label tissues in humans, different risks are feasible, but recent studies discussing risks are rare: in a 1971 study involving 54 patients who received intravenous fluorescein, Parker and Stein reported that 67.3% experienced allergic reactions, of which 29.1% were severe in nature [113]. Generally, most case studies are 20–30 years old and have shown low differences in blood glucose measurements up to 12 h after injection [114]. Within the aforementioned study using a cetuximab-IRDye800

conjugated [93], 13 adverse events occurred in the study amongst 12 patients. Two patients were excluded from the study based on allergic reactions. Monoclonal antibodies still need further studies to assess the risks within the context of T-FGS to determine whether common side effects such as skin irritations occur. The recent studies of PARPi-FL [46] and FG001 [79] showed no adverse events in treating OSCC.

### 3.6. Imaging and Visualization

**Real-Time Imaging:** The emitted fluorescence photon is captured by a camera in real time (Figure 5) and displayed on monitors. There are different NIR imaging systems on the market, including the ‘Spy Elite’ from Stryker, the ‘Luna Imaging System’ from Novadaq, the ‘Explorer Air’ from Surgvision, the Fluobeam “LX”, and Quest Spectrum. The US FDA has approved 30 (as of November, 2023) fluorescence-guided clinical imaging systems for FGS [115]. A rigorous performance evaluation of these systems has not yet been carried out, even though there are physical test methods with composite phantoms aiming for the standardization and benchmarking of FMI imaging systems [116–119]. Surgical microscopes such as the Leica FL400 and Zeiss BLUE 400 have different configurations for ICG imaging. The quality of the imaging is mostly determined by contrast metrics or signal-to-noise ratio (SNR). This visual feedback allows the surgeon to see the fluorescently labeled tissues clearly against the non-fluorescent background [118]. There are various NIR fluorescent materials that vary in wavelength and penetration depth [120]. A new aggregation-induced-emission (AIE) dye called PH molecule, which was assembled into nanoparticles, emitted wavelengths up to 1550 nm and could penetrate up to 6 mm into chicken breast, as demonstrated in vitro [121]. The use of artificial neural networks to enable a machine learning approach for laser-scanning imaging generated letter patterns on fluorescent panels visible through image processing up to a depth of 10 mm in pork skin [122].

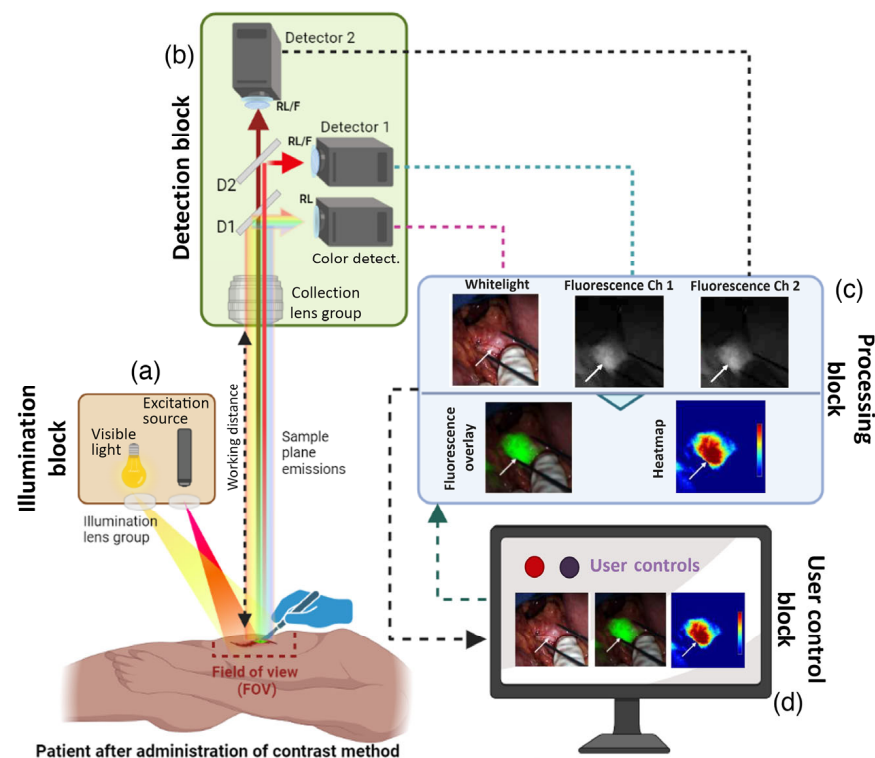


**Figure 5.** Core components of a generic fluorescence-guided surgery (FGS) system. (a) The illumination module, which provides excitation and visual aid lighting. (b) The detection module, exemplified

with three detectors—two for fluorescence and one for white light—along with relay lenses (RL), optical filters (F), and dichroic mirrors (D1 and D2). (c) The processing unit, responsible for image analysis and rendering, with example images courtesy of Lu et al. [123]. (d) User interface displaying options for manipulating image display settings. Reprinted with permission from Ref. [114]. Copyright 2023, International Society for Optical Engineering (SPIE).

### 3.7. Surgical Precision

**Guidance:** The real-time fluorescence imaging guides the surgeon in identifying and delineating the target tissues from the surrounding healthy tissues. However, even after nearly four decades of extensive research, the fundamentals for optical detection still leave room for errors. Next-generation approaches using machine learning to differentiate the signals between autofluorescence and target-specific fluorescence as well as analyzing the lifetime of fluorescence will bring new innovations [124–126].

The previously used blue dye and radioactive tracers for illuminating the lymphatic activities within the resection area had the problems of contrast visibility within the blue spectrum (Figure 2) and storage, as well as patient exposure to radioactive materials [127]. The currently standard method of palpation combined with the interpretation of the visual aspects of the tumor allows detailed border lineation only for experienced surgeons. Therefore, systemic non-targeted fluorophores demonstrated in animal models as well as in standardization phantoms that tumor boundaries can be clearly demarcated by optical imaging [116–119]. Fluorescence can show a picture of the local lymphatic network and its activity within the tumor region [127]; however, images containing the lesion still depend on the observer's knowledge in order to be labeled malignant or benign [113]. Within the scattering ratio and the halo, the margin of resected tissue can be reduced to millimeters. This leads to improved postoperative functionality, improving quality of life and decreasing the recurrence rate [128].

Despite the fact that the penetration depth of FGS is limited (as shown in detail in the section Imaging and Visualization), due to current surgical practice, the surgeon will systematically expose the tumorous area throughout resection. However, the problem of missing information about the deep margin below the cancer lesion still remains. By contrast, the mucosal margin is better displayed due to the lesser penetration depth. The limitation of penetration depth is determined by the Rose criterion:

$$SNR = |S_A - S_B| / \sigma_0$$

where the SNR should be  $>5$  ( $S_A$  is signal intensity in the foreground,  $S_B$  is signal intensity in the background, and  $\sigma_0$  is the standard deviation of the background) [116]. Perineural or perivascular growth can still be detected when the SNR is above 5, and signaling photons can be differentiated from the autofluorescence. Therefore, Steinkamp et al. determined that a tumor-to-background ratio (TBR) of 1.5 or higher should be necessary for real-time FGS (Figure 6) [114]. Even after optimizing surface properties, tissue properties can still cause unknown optical artifacts, but, in the words of Keereweer et al., “we should not forget that a very advanced system is available that has the capability to identify distinct tissue components (e.g., blood vessels) during the surgical process: the surgeon him or herself” [128].

If the SNR is close to 5, there are also new approaches for including perfusion analysis into the diagnostics procedure using hyperspectral imaging devices. This can help by providing more information about perfusion, which is part of FGS's absorption spectrum, and can indicate highly active tumor areas, as well as lowly perfused necrotic tumor parts. However, these devices still need to be validated by clinical studies and formally approved for clinical use [129,130].

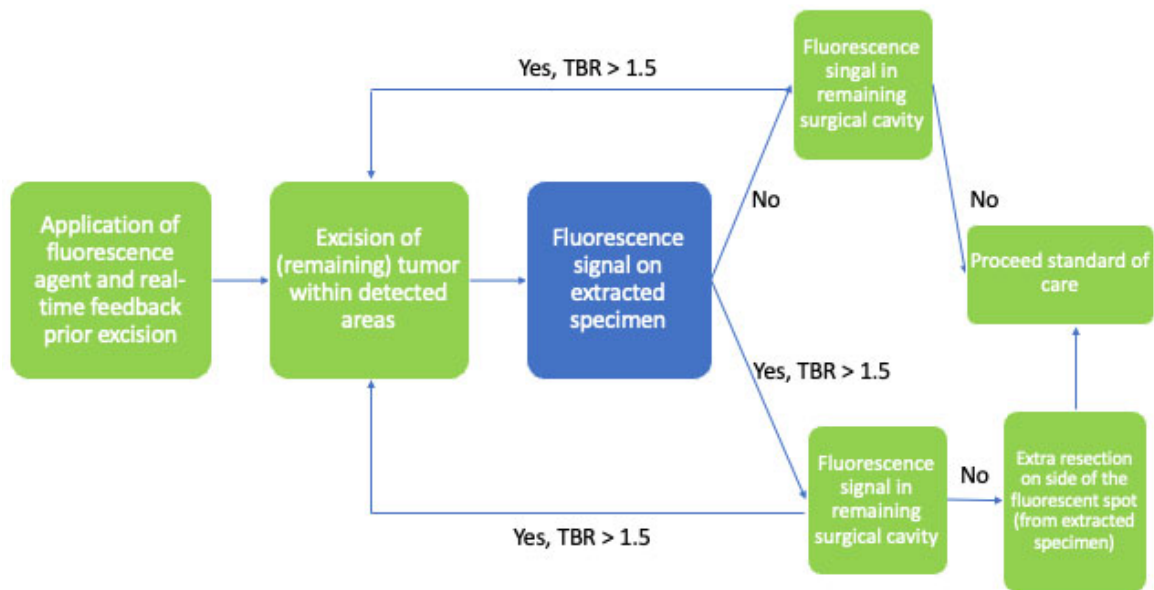


**Figure 6.** Flow chart for real-time clinical decision-making. This proposed flowchart outlines the use of fluorescence for guiding surgery and making intraoperative decisions with a tumor-specific fluorescence imaging agent. In cases where fluorescent spots with a tumor-to-background ratio of 1.5 are identified on the surgical specimen or within the cavity, additional resection may be indicated (green = in vivo and blue = extracted tissue). (Adapted from [114]).

#### 4. Clinical Applications of FGS in HNSCC

Published studies were identified using the keywords ‘HNSCC’ and ‘Fluorescence’ via the PubMed Database. Of the 314 results within the last five years, only 232 were human-based, and 137 included imaging procedures. Out of these, 67 studies were selected to be included in this analysis. The review process for selecting relevant preclinical and clinical studies is shown in Figure 7.

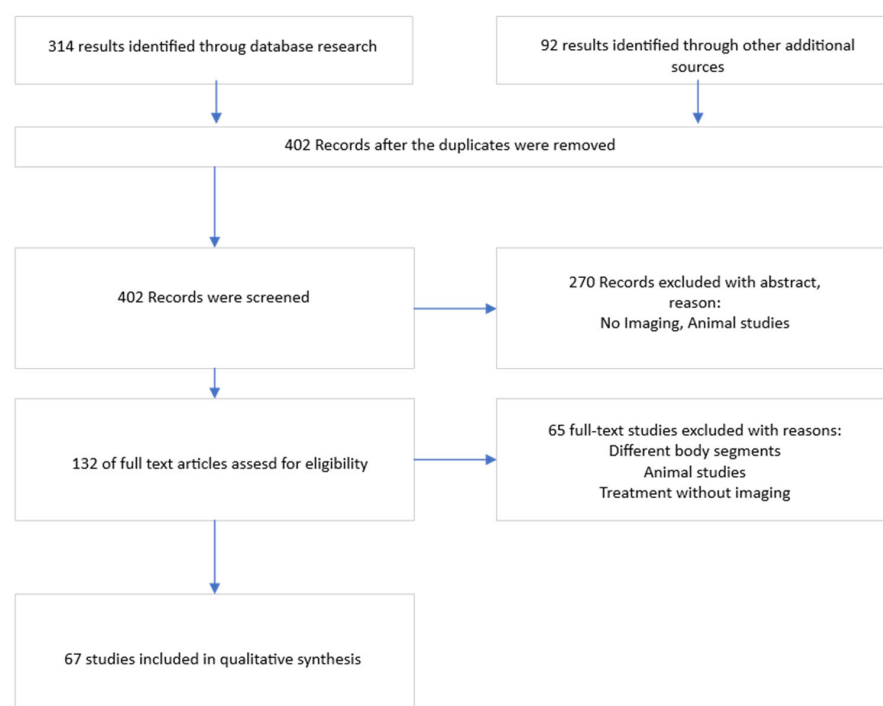


**Figure 7.** Review process for identification of relevant recent and ongoing studies covering the use of FGS in HNSCC resections.

In cancer diseases, particularly HNSCC, the resection of tumor tissue is often the first treatment. The delineation of malignant and healthy tissue is generally based on visible characteristics such as morphology and is confirmed by subsequent histopathological analysis [16]. While this is fundamentally effective, it still leads to the frequent recurrence of malignant tumors, repeated surgical interventions, the impairment of quality of life due to the removal of healthy tissue, and, often, significantly reduced survival rates [131]. Although the idea that tumors can absorb macromolecules more intensively compared to healthy tissue due to the EPR effect, thus enabling the use of dyes to create better visualization for distinguishing between tumor and healthy tissue based on the vascular network [34,132], is compelling, FGS for HNSCC still remains investigational and has not yet been integrated into routine clinical practice. Zhu et al. demonstrated the potential of locally administered indocyanine green (ICG) to enhance the detection of regional lymph node metastases in the oral cavity while minimizing the risk of anaphylactic reactions [133]. Additionally, Stone et al. employed the targeted FGS (T-FGS) approach using panitumumab-IRDye800CW to assist transoral robotic surgery, achieving favorable outcomes [134].

In oncologic surgery in general, ICG has been investigated more intensively and has shown promising results in securing the completeness of lymph node dissection, facilitating surgical dissection, and visualizing anastomotic perfusion and, thus, might be implemented in further areas of application in the future. However, ICG fluorescence lymphography failed to show good selectivity for metastatic lymph nodes [135]. It should also be considered that recent studies observed a decline in sensitivity for detecting all metastatic stations in advanced tumor stages [136,137]. A similar conclusion was drawn in a systematic review by Zweedijk et al., which found that fluorescence guidance primarily enhances the accuracy and efficiency of postoperative pathological processing but does not provide convincing results for intraoperative tumor targeting [138].

In liver surgery and in the detection of micrometastases, the use of ICG has been shown to be superior to conventional visual and palpable detection of the malignant tissue [139,140]. Its use for visualizing tissue perfusion has already been shown [141–144]. ICG accumulates in tumor regions a few hours after infusion and is considered safe, but its overall effectiveness is limited by its lack of specificity for structures on tumor cells and poor tissue penetration, similar to other recently studied dyes (5-ALA, FITC, methylene blue) [69,145,146].

Since the 2020s, 39 FGS agents have been used in clinical trials, covering 85 separate clinical trials; however, none of these include testing and development for head and neck cancers specifically. Out of these, ONM-100 (Figures 8 and 9) stands out for already showing promising results in finding all tumor-positive margins ex vivo up to 5.7 TBR; nevertheless, it localized 25% false-positive results in surgical-cavity-driven margins and one out of six in ex vivo specimen-driven margins [114]. New contrast agents SGM-101, BLZ-100, LUM015, and OTL38 were tested up to phase II trials, with mean tumor-to-background ratios up to 1.7 and cancer lesion sensitivity up to 97.97% (OTL38 in phase II study) [37,147]. Nevertheless, none of these new agents have been used so far for HNSCC surgery.

In T-FGS, fluorescently labeled antibodies, proteins, or other molecules are used to specifically bind to a known surface marker of the tumor, ideally without binding to healthy cells that do not express this specific marker. T-FGS with monoclonal antibodies (mAbs) is the most common strategy for many tumor types [148]. uPar and  $\alpha v\beta 6$  integrin have been promising in older clinical studies but show an “on/off” phenomenon when it comes to HNSCC and stromal cells [70]. As described in earlier sections, preclinical studies focus on antibody–dye conjugates, targeting receptors such as vascular endothelial growth factor (VEGF), epidermal growth factor receptor (EGFR), prostate-specific membrane antigen (PSMA), and others [149]. In 2017, a phase I study for the PARP1 agent PARPi-FL showed

for OSCC a TBR above 3 for the highest dose (1000 nM) after 60 s of gargling and 60 s of clearing solution. The specificity was confirmed *ex vivo*, and a phase II study for the *in vivo* diagnosis of PARPi-FL is planned and could shorten the administration procedure from 12 h to 2 min [46]. Another gargling solution is cMBP-ICG, which targets c-MET receptors. Gargling this solution two times for 30 s and clearing it two times for 30 s 24 h prior to the surgery showed a promising TBR of 4.12 for primary OSCC tumors, while intraoperative imaging showed a TBR of 2.71 and 100% sensitivity for the 5  $\mu$ M group and 3.11 TBR and 46% sensitivity in the 2.5  $\mu$ M group [47].

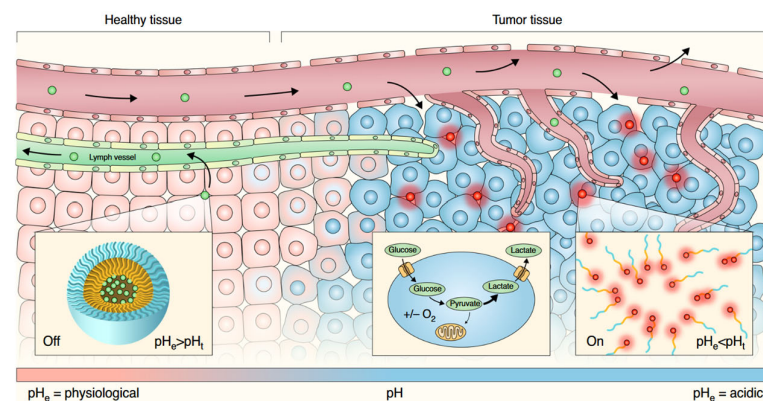


**Figure 8.** During the transition from premalignant to invasive cancerous tissue, the tumor microenvironment becomes acidic. ONM-100 nanoparticles extravasate through the permeable tumor vasculature and accumulate in the tumor due to poor lymphatic drainage. This accumulation in the acidic extracellular matrix triggers a pH-dependent fluorescence change, switching from an ‘off’ (green) to an ‘on’ (red) state.  $pH_e$ —extracellular pH;  $pH_t$ —threshold pH. Reprinted with permission from Ref. [146]. Copyright 2020, Springer Nature.

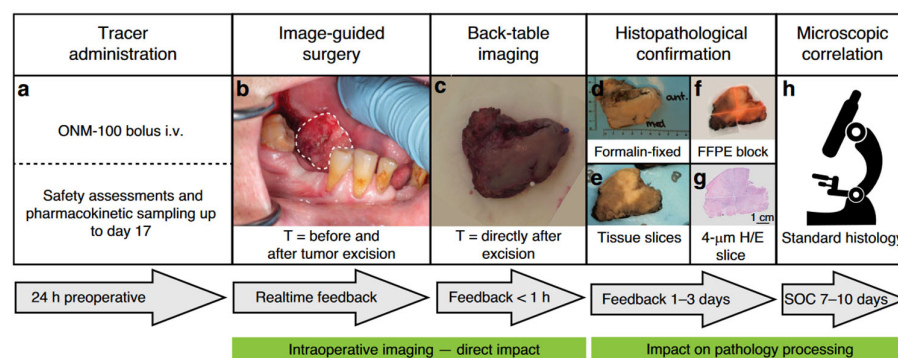


**Figure 9.** Study design. (a) ONM-100 was administered intravenously approximately 24 h ( $\pm$  8 h) before surgery. A 10-day safety assessment period followed, including laboratory tests, pharmacokinetics, and ECG monitoring; adverse events were tracked up to day 17. (b) Intraoperative imaging was performed before incision of the surgical cavity and after excision of the tumor. (c) Following excision, the specimen was imaged to assess the (positive) surgical margin. (d,e) Fluorescence imaging was conducted throughout all standard pathology processing steps, (f–h) with H/E slices correlated to standard histopathology sections. i.v.—intravenous; ECG—electrocardiogram; H/E—hematoxylin-eosin; SOC—standard of care. Reprinted with permission from Ref. [146]. Copyright 2020, Springer Nature.

For HNSCC, preclinical studies have confirmed the mAb-mediated targeting of the usual HNSCC markers Epithelial Cell Adhesion Molecule (EpCAM) and EGFR [150,151] as well as the HNSCC marker CD44v6 [76,152–155]. DTPA-BIWA-IRDye800CW showed a tumor-to-blood ratio of 10:1 when systemically applied to mouse models but also false positives in the cervical lymph nodes. It also detected 87% of the invasive tumor nests [76]. The results in other studies showed a promotion of the epithelial-to-mesenchymal process

(EMT) for CD44s or while isoforming CD44v to CD44s [156]. These studies were conducted on resected tumor tissue using confocal laser scanning endomicroscopy (CLE), with the EpCAM antibody showing 100% antigen specificity. Clinical phase I/II studies achieved a high contrast between tumor and healthy tissue with EGFR-targeted, fluorescence-labeled antibodies (panitumumab-IRDye800CW, cetuximab-IRDye800, cetuximab-800CW), with no adverse side effects observed [92,157,158]. Overall, in the search window of July 2019 through July 2024, there are eight clinical trials for FGS in HNSCC registered in clinicaltrials.gov (Table 2, website accessed on 1 July 2025), with six still in the planning phase, thus indicating a steadily growing interest.

**Table 2.** Registered clinical trials at clinicaltrials.gov for FGS and T-FGS in HNSCC.

| Description                                                                                                                         | Clinical Trial Phase | Clinical Trials Registered Number (ClinicalTrials.gov (accessed on 1 July 2025) or clinicaltrialsregister.eu (accessed on 1 July 2025)) | Patients | State          | Results                                                                                                                                                                                                                                                                                                                                                                                                                               |
|-------------------------------------------------------------------------------------------------------------------------------------|----------------------|-----------------------------------------------------------------------------------------------------------------------------------------|----------|----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| ASP-1929 Photoimmunotherapy (PIT) Study in Patients with Recurrent Head/Neck Cancer                                                 | Phase II             | NCT05182866 [159]                                                                                                                       | 22       | Recruiting     | No results published.                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Panitumumab-IRDye800 and 89Zr-Panitumumab in Identifying Metastatic Lymph Nodes in Patients with Squamous Cell Head and Neck Cancer | Phase I              | NCT03733210 [42]                                                                                                                        | 14       | Completed 2021 | From 19 lymph nodes that were histopathologic-labeled tumor-positive, 6 were false negatives using panitumumab-IRDye800: 30 mg administered intravenously (IV) and Zirconium Zr-89 panitumumab: 0.8 to 1.2 mCi (29 to 45 Mbq) administered intravenously (IV). Adverse events: Of 14 patients, 57 of the tumor-positive patients and 28.57% of the non-tumor patients were diagnosed with vascular hypertension after administration. |
| Fluorescence-guided Surgery Using cRGD-ZW800-1 in Oral Cancer                                                                       | Phase II             | NCT04191460 [160]                                                                                                                       | 28       | Recruiting     | No results published.                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Evaluating the Use of Dual Imaging Techniques for Detection of Disease in Patients with Head and Neck Cancer                        | Phase I              | NCT05945875 [161]                                                                                                                       | 40       | Recruiting     | No results published to date, but prior research showed in HNSCC patients infused with a molecularly targeted fluorescent tracer that endogenous expression of the target antigen can be used as a reference standard to detect LN metastasis. Additionally, the performance of the background in determining metastatic LN can be improved by utilizing patient-specific reference standards [162].                                  |

Table 2. Cont.

| Description                                                                                                                                                                                                                                                   | Clinical Trial Phase | Clinical Trials Registered Number (ClinicalTrials.gov (accessed on 1 July 2025) or clinicaltrialsregister.eu (accessed on 1 July 2025)) | Patients | State              | Results                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|-----------------------------------------------------------------------------------------------------------------------------------------|----------|--------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Real-time Margin Assessment in Head and Neck Cancer                                                                                                                                                                                                           | Phase II             | NCT05499065 [163]                                                                                                                       | 20       | Finished           | No results published to date, but University Medical Center Groningen already experimented with cetuximab-800CW tracer for the detection of EGFR, bevacizumab-800CW for VEGF, and others such as ONM-100 as pH-sensitive probes [164].                                                                                                                                                                                                                                                 |
| Fluorescence-guided Surgery in Laryngeal- and Hypopharyngeal Cancer: a Feasibility Trial                                                                                                                                                                      | Phase II             | NCT05752149 [165]                                                                                                                       | 27       | Not Yet Recruiting | No results published to date.                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| A Study to Evaluate ONM-100, an Intraoperative Fluorescence Imaging Agent for the Detection of Cancer                                                                                                                                                         | Phase II             | NCT03735680 [41]                                                                                                                        | 30       | Completed 2021     | 3 patients with administration of 3 mg/kg showed the best tumor-to-background ratio (TBR) of mean 4.022 compared to 1.098 for one patient with 1 mg/kg and 2.280 for 3 patients with 2 mg/kg [41]. 2/3 patients with 3 mg/kg experienced serious adverse events: cellulitis (33%), abscess neck (33%), and superficial vein thrombosis (33%). In all groups, a minimum of 66% had adverse events; in most groups, infusion-related reactions made up most of the other adverse events. |
| A Phase 2a, Single-dose, Open-label Study to Evaluate Diagnostic Performance and Safety of Pegsitacianine, an Intraoperative Fluorescence Imaging Agent for the Detection of Cancer, in Patients with Unknown Primary Head and Neck Cancer (ILLUMINATE STUDY) | Phase II             | NCT05576974 [44]                                                                                                                        | 40       | Recruiting         | No results published to date.                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| An open-label, non-randomized, single center, single dose, exploratory phase II trial of FG001 (an imaging agent) for localization of oral and oropharyngeal squamous cell carcinoma                                                                          | Phase II             | FG001-CT-003 [45]                                                                                                                       | 16       | Completed 2023     | 16 patients undergoing primary surgical resection were systemically administered 36 mg ( $n = 4$ ), 16 mg ( $n = 8$ ), or 4 mg ( $n = 4$ ) of FG001 the evening prior to surgery. Intraoperatively, using a near-infrared imaging system, real-time optical imaging successfully identified all 16 tumors (sensitivity: 100%, mean TBR: 2.99, range: 2.02–3.95), and tumor specificity was confirmed by histology.                                                                     |

## 5. Discussion

Clear visualization of tumor margins during surgeries may result in shorter operation times, better postoperative functionality, and a reduction in recurrence rates. Multiple visualization solutions already use the toolkit of fluorescence. One common method involves using unspecific albumin-binding dyes such as indocyanine green or methylene blue, thus highlighting areas of increased tissue perfusion. A specialized camera is required to capture and display the emitted color spectrum. This technique has been applied in colorectal surgery, adrenal surgery, neuroendocrine surgery, and liver surgery, although it is still in its nascency for HNSCC. A notable limitation is the necessity for systemic administration of the dye, because the dyes do not always bind specifically to the surface of tumor cells. This leads to delays and precludes localized application to the tumor.

Due to its unspecific binding, ICG has not yet proven to be useful in the HNSCC field, where precise discrimination between healthy and malignant tissue is essential within confined anatomical spaces. One exciting approach shown by Pal et al. was the idea to monitor the fluorescence lifetime of indocyanine green in tumor tissue, which is longer than the fluorescence lifetime of the same dye in non-cancerous tissue [166], and they proved its value with an accuracy of over 97%. Nevertheless, this approach is still limited to a well-perfused vascular network of the tumor for EPR effects, which is not the case for later-staged tumors that could have dysfunctional or disrupted vascular networks [167].

In order to overcome the limitation of the generally unspecific binding of FGS dyes, a promising approach is targeted fluorescence-guided surgery (T-FGS). Indeed, a strategy utilizing antibodies conjugated to fluorophores has been employed in this context. The anti-CD44v6 antibody Bivatuzumab could show high value as its pendant BIWA and had high sensitivity (eight times higher than EGFR) in mouse models. Furthermore, ONM-100 could be examined in regard to real-time surgical margin determination, as a result of the precise ex vivo detection results (Figure 8). The commonly used EGFR-targeting monoclonal antibody cetuximab is suboptimal, as it is not specific to HNSCC, which limits its effectiveness in imaging these tumors. This has resulted in false positives in several studies targeting head and neck cancers and also in some targeting the non-malignant salivary glands in the deep margins, which are known to be EGFR-positive tissues [167,168].

Current approaches to T-FGS are still at the level of experimental, preclinical studies for HNSCC and have not yet reached standard clinical practice. The basic idea in many published studies is the application of an antibody in combination with a drug conjugate, which is used for visualization after application. There are also recent non-drug-dependent approaches for the distinct differentiation of malignant and non-malignant tissue. These range from live imaging, in which the surgeon receives direct feedback on the distribution of the dye via an intraoperatively guided camera through intraoperative visualization using large technical instruments such as CT or SPECT, which require large technical equipment in the operating theater, to approaches in which freshly frozen sections saturated with the antibody are used to make statements about the extent of the tumor, in particular, to support conventional pathological diagnosis [169].

One of the most promising attempts demonstrated that the administration of FG001, which targeted uPAR in OSCC and HNSCC, showed great potential throughout different concentration doses (mean TBR 2.99), without serious adverse side effects or off-target labeling in healthy epithelial tissue. The outcome needs to be validated in a clinical trial with more than 16 patients reported [79], where the wide variations in expression (from low to overexpressed) can be further researched.

Other imaging modalities also show early promise as complementary methods or eventual replacements for standard FGS optical setups as described above. Hyperspectral imaging (HSI) shows promise in surgical applications like assessing tissue perfusion

and identifying tumor margins, despite facing challenges in clinical adoption due to its complexity and the need for further validation of its clinical utility [71]. It has its main strength in assessing tissue perfusion [129,130,170]; however, similar to FGS, it is not tumor-specific. Narrow Band Imaging and Optical Coherence Tomography primarily detect superficial lesions [171], while autofluorescence imaging may yield false positives and negatives caused by inflammatory reactions or scarring by hyperkeratosis, thus limiting its reliability [172–175]. This utilization of an (auto)fluorescence spectrum has a similar underlying physical basis as FGS, but due to autofluorescence limitations, it has a lower SNR and is restricted to the mucosa. High-Resolution Microendoscopy (HRME) offers detailed imaging but struggles with diagnostic challenges when it comes to keratinization and submucosal tumor spread [176]. FGS has the advantage of binding to albumin and, therefore, also showing subsurface tumors or regions of higher tissue perfusion. HRME can also be combined with FGS procedures.

Raman Spectroscopy (RS) analyzes molecular composition based on photon scattering, thus providing detailed biochemical information, although interpretation can be challenging due to weak autofluorescence and complex molecular profiles. The optical fiber technology enables the *in vivo* assessment of various body locations with handheld RS probes [177]; however, those fibers generate intense background emission and require complex optical filtering [178].

Current research approaches aim at the detection of Oral Squamous Cell Carcinoma with Raman Histology assisted by an AI algorithm [179,180]. It has been shown that RS can objectively discriminate between normal and malignant tissue [181,182] and enable the detailed identification of malignant tissues with high sensitivity and specificity [183]. However, it does not provide the surgeon with a wide-field image, as it typically relies on point-based measurements (i.e., of small tissue volumes). In an effort to combine RS with FGS to exploit FGS's high sensitivity and RS's superior specificity, Grimbergen et al. [184] demonstrated in bladder cancer that the use of 5-ALA affected the Raman spectra of bladder tissue. Consequently, the differentiation between benign and malignant tissues required a preliminary Principal-Component-fed Linear Discriminant Analysis algorithm. Another problem to address here is the fact that ICG is the most widely used fluorescent agent in clinical trials of FGS and has its peak fluorescence emission in the near-infrared I (NIR-I) region (650–900 nm) and a long emission tail that extends into the near-infrared II (NIR-II) region (1000–1700 nm) [185], which poses a significant challenge for integration with RS due to reduced Raman scattering at higher wavelengths. Nevertheless, many authors suggest that a combination of both modalities could benefit from the strengths of the two individual technologies [186].

The need for the systemic application of most FGS agents also holds the risk of anaphylactic reactions within patients. The small number of recent studies targeting the risks of systemic fluorescence admission shows a need to focus on further research into the side effects and pharmacovigilance of new dyes targeting DNA and antibodies.

The two new trials of gargling solutions cMBP-ICG and PARPi-FL showed administration times under 5 min and also had no adverse effects on the OSCC patients. Solutions like this could change the impact of FGS in short-term resection decisions and could also be used additionally in case of difficulties in the distinguishing of tumor boundaries. Up to now, it has been difficult to decisively judge the true additional value of FGS and T-FGS in comparison to classic tumor boundary identification, as the processes need further, widespread application in the clinical setting and a better physical understanding of the absorption and refraction of photons in the impacted tissues. A blood-flooded or necrotic region can lead to lower fluorescence signals due to higher absorbance or less clear fluorescence boundaries that cannot clearly exclude the presence of malignant tumor tissue. Currently, it is still up

to the surgeon to interpret the results of the suspicious region, knowing that suction of the surface area may lead to higher SNR as well as better discovery of tumor boundaries and necrotic tissue. Working with the physical understanding in mind can lead to a flexible process of injecting targeted fluorescent agents and the precise resulting distinguishment between malignant and healthy tissue [128].

The modest number of phase I and phase II trials for FGS and T-FGS in HNSCC (no phase III trials are currently registered on [clinicaltrials.gov](https://clinicaltrials.gov)) suggests that there is still much research to be done to find suitably strong binding receptors on the surface of tumor cells and to make a clear distinction between the intensity of the tumor accumulation and the accumulation in the background area. In the latest phase II study, TBR ratios of up to 5.7 were achieved, with an average peak value of 1.7, still only slightly above the 1.5 threshold required by the Standardized Framework [114] for live imaging in FGS surgery.

In the future, the aforementioned markers for HNSCC (ONM-100, EGFR, EpCAM, CD44v6) as well as other potentially relevant markers (e.g., fibroblast activation protein—FAP [187–189], various ephrin receptors) should be investigated. Additionally, previous studies on other tumor entities have shown that smaller ligands, such as antibody fragments like nanobodies (Nbs), or even smaller binding formats like affibodies can enhance performance by enabling faster imaging and quicker clearance of the agents post-surgery [149]. Antibody fragments such as Nbs or single-chain antibody fragments (ScFv) [190–194] are often used, typically resulting in a shorter time to imaging compared to full antibody variants. These smaller variants might potentially be applied topically [109,195], eliminating much of the risk of potential adverse reactions resulting from systemic application and giving more flexibility for real-time adjustments by surgeons.

## 6. Conclusions

The goal of FGS should be to achieve a low-risk, localized application of a highly specific tumor marker in the head and neck region, conjugated to a fluorophore that can be visualized with simple VR glasses or a monitor. This approach would eliminate the need for prolonged interpretation of recorded images, preventing delays in tumor surgery. Additionally, the clear demarcation of tumor margins is crucial to ensure safe resection. This could be achieved through a combination of HNSCC-specific surface molecules and corresponding antibodies linked to fluorophores on nanoparticle gels, allowing for local tissue diffusion without the necessity for systemic intravascular administration. However, this theoretical approach faces several practical challenges. Currently, there is no commonly used targeted marker for HNSCC on the market, and as evidenced by various clinical trials, a suitable solution with good SNR and TBR has yet to be identified. Additionally, surface markers alone are insufficient to provide a fully accurate visual delineation of the entire tumor. Achieving precise tumor identification necessitates a comprehensive understanding of factors such as the binding affinity of the marker, the extent of the tissue's vascularization, and the presence of necrotic regions. These aspects require careful interpretation by the clinician to ensure accurate diagnosis and treatment planning.

The advancement of FGS in modern surgical oncology is evident. This represents a paradigm shift in cancer surgery, enhancing patient outcomes while reducing both mortality and morbidity. By enabling more precise, targeted tumor resection, FGS contributes to shorter hospital stays, ultimately lowering overall healthcare costs. This general advancement and engagement also raises the authors' hope that in the near future, the missing link toward precise, targeted FGS markers for the head–neck region will be established.

**Author Contributions:** A.B. and M.G. made an equal contribution to the conceptualization, writing, review, and editing of this paper. H.K. and D.M.S. assisted in writing, editing, and carrying out background research for the manuscript. All authors contributed to the conceptualization, editing, and literature research for the manuscript. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## References

1. Jansen, L.; Moratin, J.; Waldmann, A.; Zaoui, K.; Holleczeck, B.; Nennecke, A.; Pritzkeleit, R.; Plinkert, P.K.; Hoffmann, J.; Arndt, V. Mundhöhlen- und Pharynxkarzinome: Inzidenz, Mortalität und Überleben in Deutschland. *Bundesgesundheitsblatt—Gesundheitsforschung—Gesundheitsschutz* **2021**, *64*, 941–950. [\[CrossRef\]](#)
2. Ferlay, J.; Soerjomataram, I.; Dikshit, R.; Eser, S.; Mathers, C.; Rebelo, M.; Parkin, D.M.; Forman, D.; Bray, F. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. *Int. J. Cancer* **2015**, *136*, E359–E386. [\[CrossRef\]](#)
3. Forastiere, A.A.; Ismaila, N.; Lewin, J.S.; Nathan, C.A.; Adelstein, D.J.; Eisbruch, A.; Fass, G.; Fisher, S.G.; Laurie, S.A.; Le, Q.-T.; et al. Use of Larynx-Preservation Strategies in the Treatment of Laryngeal Cancer: American Society of Clinical Oncology Clinical Practice Guideline Update. *J. Clin. Oncol.* **2018**, *36*, 1143–1169. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Gao, R.W.; Teraphongphom, N.T.; Van Den Berg, N.S.; Martin, B.A.; Oberhelman, N.J.; Divi, V.; Kaplan, M.J.; Hong, S.S.; Lu, G.; Ertsey, R.; et al. Determination of Tumor Margins with Surgical Specimen Mapping Using Near-Infrared Fluorescence. *Cancer Res.* **2018**, *78*, 5144–5154. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Dietz, A.; Budach, W. *S3-Leitlinie Diagnostik, Therapie, Prävention und Nachsorge des Oro- und Hypopharynxkarzinoms*, Langversion 1.0 n.d.; Office des Leitlinienprogrammes Onkologie: Berlin, Germany, 2024.
6. Batsakis, J.G. Surgical Excision Margins: A Pathologist's Perspective. *Adv. Anat. Pathol.* **1999**, *6*, 140–148. [\[CrossRef\]](#)
7. Looser, K.G.; Shah, J.P.; Strong, E.W. The significance of “positive” margins in surgically resected epidermoid carcinomas. *Head Neck Surg.* **1978**, *1*, 107–111. [\[CrossRef\]](#)
8. Loree, T.R.; Strong, E.W. Significance of positive margins in oral cavity squamous carcinoma. *Am. J. Surg.* **1990**, *160*, 410–414. [\[CrossRef\]](#)
9. Villemure-Poliquin, N.; Roy, È.-M.; Nguyen, S.; Beauchemin, M.; Audet, N. Tumor Bed Margins Versus Specimen Margins in Oral Cavity Cancer: Too Close to Call? *J. Otolaryngol. Head Neck Surg.* **2024**, *53*, 19160216241278653. [\[CrossRef\]](#)
10. Sivrice, M.E.; Akin, V.; Erkilinç, G.; Yasan, H.; Tüz, M.; Okur, E.; Kumbul, Y.Ç.; Çirış, İ.M. Frozen Section Evaluation for Surgical Margins in Laryngeal Squamous Cell Carcinoma: Is it a Reliable Method for Partial and Total Laryngectomies? *Head Neck Pathol.* **2022**, *17*, 172–177. [\[CrossRef\]](#)
11. DiNardo, L.J.; Lin, J.; Karageorge, L.S.; Powers, C.N. Accuracy, Utility, and Cost of Frozen Section Margins in Head and Neck Cancer Surgery. *Laryngoscope* **2000**, *110*, 1773–1776. [\[CrossRef\]](#)
12. Olson, S.M.; Hussaini, M.; Lewis, J.S. Frozen section analysis of margins for head and neck tumor resections: Reduction of sampling errors with a third histologic level. *Mod. Pathol.* **2011**, *24*, 665–670. [\[CrossRef\]](#)
13. Maxwell, J.H.; Thompson, L.D.R.; Brandwein-Gensler, M.S.; Weiss, B.G.; Canis, M.; Purgina, B.; Prabhu, A.V.; Lai, C.; Shuai, Y.; Carroll, W.R.; et al. Early Oral Tongue Squamous Cell Carcinoma: Sampling of Margins from Tumor Bed and Worse Local Control. *JAMA Otolaryngol. Neck Surg.* **2015**, *141*, 1104. [\[CrossRef\]](#)
14. Du, E.; Ow, T.J.; Lo, Y.; Gersten, A.; Schiff, B.A.; Tassler, A.B.; Smith, R.V. Refining the utility and role of Frozen section in head and neck squamous cell carcinoma resection. *Laryngoscope* **2016**, *126*, 1768–1775. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Gandour-Edwards, R.F.; Donald, P.J.; Wiese, D.A. Accuracy of intraoperative frozen section diagnosis in head and neck surgery: Experience at a university medical center. *Head Neck* **1993**, *15*, 33–38. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Woolgar, J.A.; Triantafyllou, A. A histopathological appraisal of surgical margins in oral and oropharyngeal cancer resection specimens. *Oral Oncol.* **2005**, *41*, 1034–1043. [\[CrossRef\]](#)
17. Jäkel, M.C.; Ambrosch, P.; Martin, A.; Steiner, W. Impact of re-resection for inadequate margins on the prognosis of upper aerodigestive tract cancer treated by laser microsurgery. *Laryngoscope* **2007**, *117*, 350–356. [\[CrossRef\]](#)
18. Kwok, P.; Gleich, O.; Hübner, G.; Strutz, J. Prognostic importance of “clear versus revised margins” in oral and pharyngeal cancer. *Head Neck* **2010**, *32*, 1479–1484. [\[CrossRef\]](#)
19. Yuen, P.W.; Lam, K.Y.; Chan, A.C.L.; Wei, W.I.; Lam, L.K. Clinicopathological Analysis of Local Spread of Carcinoma of the Tongue 11The study was supported by a research grant from the University of Hong Kong, grant number 337/048/0014 and 335/048/0081. *Am. J. Surg.* **1998**, *175*, 242–244. [\[CrossRef\]](#)

20. Uppaluri, R.; Chernock, R.; Mansour, M.; Jackson, R.; Rich, J.; Pipkorn, P.; Paniello, R.C.; Puram, S.; Zevallos, J.P.; Annino, D.J.; et al. Enhanced pathologic tumor response with two cycles of neoadjuvant pembrolizumab in surgically resectable, locally advanced HPV-negative head and neck squamous cell carcinoma (HNSCC). *J. Clin. Oncol.* **2021**, *39*, 6008. [\[CrossRef\]](#)
21. Shibata, H.; Saito, S.; Uppaluri, R. Immunotherapy for Head and Neck Cancer: A Paradigm Shift from Induction Chemotherapy to Neoadjuvant Immunotherapy. *Front. Oncol.* **2021**, *11*, 727433. [\[CrossRef\]](#)
22. Lybaert, W.; Vermorken, J.B. Editorial: Insights in head and neck cancer: 2021. *Front. Oncol.* **2023**, *13*, 1180965. [\[CrossRef\]](#)
23. Dietz, A.; Vermorken, J.B. Is Reducing the Extent of Local Treatments Justified After Successful Neoadjuvant Therapy? In *Critical Issues in Head and Neck Oncology*; Vermorken, J.B., Langendijk, J.A., Leemans, C.R., Machiels, J.-P., Nicolai, P., O'Sullivan, B., Eds.; Springer Nature Switzerland: Cham, Switzerland, 2025; pp. 241–259. ISBN 978-3-031-84538-3.
24. Van Keulen, S.; Nishio, N.; Fakurnejad, S.; Birkeland, A.; Martin, B.A.; Lu, G.; Zhou, Q.; Chirita, S.U.; Forouzanfar, T.; Colevas, A.D.; et al. The Clinical Application of Fluorescence-Guided Surgery in Head and Neck Cancer. *J. Nucl. Med.* **2019**, *60*, 758–763. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Pal, R.; Hom, M.E.; Van Den Berg, N.S.; Lwin, T.M.; Lee, Y.-J.; Prilutskiy, A.; Faquin, W.; Yang, E.; Saladi, S.V.; Varvares, M.A.; et al. First Clinical Results of Fluorescence Lifetime-enhanced Tumor Imaging Using Receptor-targeted Fluorescent Probes. *Clin. Cancer Res.* **2022**, *28*, 2373–2384. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Stokes, G.G. XXX. On the change of refrangibility of light. *Philos. Trans. R. Soc. Lond.* **1852**, *142*, 463–562. [\[CrossRef\]](#)
27. Copeman, S.M.; Coke, F.; Gouldsbrough, C. “Activated” (irradiated) fluorescein in the treatment of cancer. *Br. Med. J.* **1929**, *2*, 233–242. [\[CrossRef\]](#)
28. Moore, G. Fluorescein as an Agent in the Differentiation of Normal and Malignant Tissues. *Science* **1947**, *106*, 130–131. [\[CrossRef\]](#)
29. Goldhahn, W.E. Tetracycline fluorescence for the delimitation of brain tumors. *Arzneimittelforschung* **1967**, *17*, 139–141.
30. Homasson, J.P.; Bonniot, J.P.; Angebault, M.; Renault, P.; Carnot, F.; Santelli, G. Fluorescence as a guide to bronchial biopsy. *Thorax* **1985**, *40*, 38–40. [\[CrossRef\]](#)
31. Harris, D.M.; Werkhaven, J. Endogenous porphyrin fluorescence in tumors. *Lasers Surg. Med.* **1987**, *7*, 467–472. [\[CrossRef\]](#)
32. Sheridan, R.L.; Schomaker, K.T.; Lucchina, L.C.; Hurley, J.; Yin, L.M.; Tompkins, R.G.; Jerath, M.; Torri, A.; Greaves, K.W.; Bua, D.P.; et al. Burn Depth Estimation by Use of Indocyanine Green Fluorescence: Initial Human Trial. *J. Burn Care Rehabil.* **1995**, *16*, 602–604. [\[CrossRef\]](#)
33. Kitai, T.; Inomoto, T.; Miwa, M.; Shikayama, T. Fluorescence navigation with indocyanine green for detecting sentinel lymph nodes in breast cancer. *Breast Cancer Tokyo Jpn.* **2005**, *12*, 211–215. [\[CrossRef\]](#)
34. Nguyen, Q.T.; Tsien, R.Y. Fluorescence-guided surgery with live molecular navigation—A new cutting edge. *Nat. Rev. Cancer* **2013**, *13*, 653–662. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Van Dam, G.M.; Themelis, G.; Crane, L.M.A.; Harlaar, N.J.; Pleijhuis, R.G.; Kelder, W.; Sarantopoulos, A.; de Jong, J.S.; Arts, H.J.G.; van der Zee, A.G.J.; et al. Intraoperative tumor-specific fluorescence imaging in ovarian cancer by folate receptor- $\alpha$  targeting: First in-human results. *Nat. Med.* **2011**, *17*, 1315–1319. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Gioux, S.; Choi, H.S.; Frangioni, J.V. Image-guided surgery using invisible near-infrared light: Fundamentals of clinical translation. *Mol. Imaging* **2010**, *9*, 237–255. [\[CrossRef\]](#)
37. Barth, C.W.; Gibbs, S. Fluorescence image-guided surgery: A perspective on contrast agent development. In *Proceedings of the Molecular-Guided Surgery: Molecules, Devices, and Applications VI*; Gibbs, S.L., Pogue, B.W., Gioux, S., Eds.; SPIE: San Francisco, CA, USA, 2020; p. 18.
38. Dindere, M.E.; Tanca, A.; Rusu, M.; Liehn, E.A.; Bucur, O. Intraoperative Tumor Detection Using Pafolacianine. *Int. J. Mol. Sci.* **2022**, *23*, 12842. [\[CrossRef\]](#)
39. Zhao, H.; Peng, P.; Luo, Z.; Liu, H.; Sun, J.; Wang, X.; Jia, Q.; Yang, Z. Comparison of hexaminolevulinate (HAL)—Guided versus white light transurethral resection for NMIBC: A systematic review and meta-analysis of randomized controlled trials. *Photodiagnosis Photodyn. Ther.* **2023**, *41*, 103220. [\[CrossRef\]](#)
40. Galema, H.A.; Van Ginhoven, T.M.; Franssen, G.J.H.; Hofland, J.; Bouman, C.G.O.T.; Verhoef, C.; Vahrmeijer, A.L.; Hutteman, M.; Hilling, D.E.; Keereweer, S. Fluorescence-guided surgery using methylene blue to improve identification of metastatic small intestinal neuroendocrine tumours. *Br. J. Surg.* **2023**, *110*, 541–544. [\[CrossRef\]](#)
41. OncoNano Medicine. A Study to Evaluate ONM-100, an Intraoperative Fluorescence Imaging Agent for the Detection of Cancer. Available online: <https://www.clinicaltrials.gov/study/NCT03735680> (accessed on 1 July 2025).
42. Iagaru, A. Panitumumab-IRDye800 and 89Zr-Panitumumab in Identifying Metastatic Lymph Nodes in Patients with Squamous Cell Head and Neck Cancer. Available online: <https://clinicaltrials.gov/study/NCT03733210?tab=history&a=4> (accessed on 1 July 2025).
43. University Medical Center Groningen. Image Guided Surgery for Margin Assessment of Head and Neck Cancer Using Cetuximab-IRDye800CW cONjugate (ICON). Available online: <https://clinicaltrials.gov/study/NCT03134846> (accessed on 1 July 2025).

44. University of Texas Southwestern Medical Center. A Phase 2a, Single-dose, Open-label Study to Evaluate Diagnostic Performance and Safety of Pegsitacianine, an Intraoperative Fluorescence Imaging Agent for the Detection of Cancer, in Patients with Unknown Primary Head and Neck Cancer (Illuminate Study). Available online: <https://clinicaltrials.gov/study/NCT05576974> (accessed on 1 July 2025).
45. FluoGuide A/S An Open-Label, Non-Randomized, Single Center, Single Dose, Exploratory Phase II Trial of FG001 (an Imaging Agent) for Localization of Oral and Oropharyngeal Squamous Cell Carcinoma. Available online: <https://www.clinicaltrialsregister.eu/ctr-search/trial/2022-001361-12/DK> (accessed on 1 July 2025).
46. Demétrio De Souza França, P.; Kossatz, S.; Brand, C.; Karassawa Zandoni, D.; Roberts, S.; Guru, N.; Adilbay, D.; Mauguen, A.; Valero Mayor, C.; Weber, W.A.; et al. A phase I study of a PARP1-targeted topical fluorophore for the detection of oral cancer. *Eur. J. Nucl. Med. Mol. Imaging* **2021**, *48*, 3618–3630. [\[CrossRef\]](#)
47. Wang, J.; Li, S.; Wang, K.; Zhu, L.; Yang, L.; Zhu, Y.; Zhang, Z.; Hu, L.; Yuan, Y.; Fan, Q.; et al. A c-MET-Targeted Topical Fluorescent Probe cMBP-ICG Improves Oral Squamous Cell Carcinoma Detection in Humans. *Ann. Surg. Oncol.* **2023**, *30*, 641–651. [\[CrossRef\]](#)
48. Jingbo, W.; Yuan, Y.; Tao, X. ASO Author Reflections: Topically Applied Targeted Near-Infrared Fluorescence Imaging Probe Helps Detect Head and Neck Squamous Cell Carcinoma. *Ann. Surg. Oncol.* **2023**, *30*, 652. [\[CrossRef\]](#)
49. Mizushima, T.; Ohnishi, S.; Shimizu, Y.; Hatanaka, Y.; Hatanaka, K.C.; Hosono, H.; Kubota, Y.; Natsuzaka, M.; Kamiya, M.; Ono, S.; et al. Fluorescent imaging of superficial head and neck squamous cell carcinoma using a  $\gamma$ -glutamyltranspeptidase-activated targeting agent: A pilot study. *BMC Cancer* **2016**, *16*, 411. [\[CrossRef\]](#) [\[PubMed\]](#)
50. Chance, B. Near-Infrared Images Using Continuous, Phase-Modulated, and Pulsed Light with Quantitation of Blood and Blood Oxygenation <sup>a</sup>. *Ann. N. Y. Acad. Sci.* **1998**, *838*, 29–45. [\[CrossRef\]](#) [\[PubMed\]](#)
51. Zhu, B.; Sevic-Muraca, E.M. A review of performance of near-infrared fluorescence imaging devices used in clinical studies. *Br. J. Radiol.* **2015**, *88*, 20140547. [\[CrossRef\]](#)
52. Vahrmeijer, A.L.; Hutteman, M.; van der Vorst, J.R.; van de Velde, C.J.H.; Frangioni, J.V. Image-guided cancer surgery using near-infrared fluorescence. *Nat. Rev. Clin. Oncol.* **2013**, *10*, 507–518. [\[CrossRef\]](#)
53. Richards-Kortum, R.; Sevic-Muraca, E. Quantitative optical spectroscopy for tissue diagnosis. *Annu. Rev. Phys. Chem.* **1996**, *47*, 555–606. [\[CrossRef\]](#)
54. Dobie, C.; Skropeta, D. Insights into the role of sialylation in cancer progression and metastasis. *Br. J. Cancer* **2021**, *124*, 76–90. [\[CrossRef\]](#)
55. Stowell, S.R.; Ju, T.; Cummings, R.D. Protein Glycosylation in Cancer. *Annu. Rev. Pathol. Mech. Dis.* **2015**, *10*, 473–510. [\[CrossRef\]](#)
56. Zhang, Y.; Yang, M.; Portney, N.G.; Cui, D.; Budak, G.; Ozbay, E.; Ozkan, M.; Ozkan, C.S. Zeta potential: A surface electrical characteristic to probe the interaction of nanoparticles with normal and cancer human breast epithelial cells. *Biomed. Microdevices* **2008**, *10*, 321–328. [\[CrossRef\]](#)
57. Le, W.; Chen, B.; Cui, Z.; Liu, Z.; Shi, D. Detection of cancer cells based on glycolytic-regulated surface electrical charges. *Biophys. Rep.* **2019**, *5*, 10–18. [\[CrossRef\]](#)
58. Nishino, M.; Matsuzaki, I.; Musangile, F.Y.; Takahashi, Y.; Iwahashi, Y.; Warigaya, K.; Kinoshita, Y.; Kojima, F.; Murata, S. Measurement and visualization of cell membrane surface charge in fixed cultured cells related with cell morphology. *PLoS ONE* **2020**, *15*, e0236373. [\[CrossRef\]](#)
59. Sun, S.; Wang, Y.; Pang, S.; Wu, X. Combination of the glycated hemoglobin levels and prognostic nutritional index as a prognostic marker in patients with acute coronary syndrome and type 2 diabetes mellitus. *Lipids Health Dis.* **2024**, *23*, 12. [\[CrossRef\]](#) [\[PubMed\]](#)
60. Chehelgerdi, M.; Chehelgerdi, M.; Allela, O.Q.B.; Pecho, R.D.C.; Jayasankar, N.; Rao, D.P.; Thamaraiyani, T.; Vasanthan, M.; Viktor, P.; Lakshmaiya, N.; et al. Progressing nanotechnology to improve targeted cancer treatment: Overcoming hurdles in its clinical implementation. *Mol. Cancer* **2023**, *22*, 169. [\[CrossRef\]](#) [\[PubMed\]](#)
61. Iwasa, J.; Marshall, W.F.; Karp, G. *Karp's Cell and Molecular Biology: Concepts and Experiments*, 9th ed.; Wiley: Hoboken, NJ, USA, 2020; ISBN 978-1-119-59824-4.
62. Webb, B.A.; Chimenti, M.; Jacobson, M.P.; Barber, D.L. Dysregulated pH: A perfect storm for cancer progression. *Nat. Rev. Cancer* **2011**, *11*, 671–677. [\[CrossRef\]](#)
63. Hao, G.; Xu, Z.P.; Li, L. Manipulating extracellular tumour pH: An effective target for cancer therapy. *RSC Adv.* **2018**, *8*, 22182–22192. [\[CrossRef\]](#)
64. Ward, C.; Meehan, J.; Gray, M.E.; Murray, A.F.; Argyle, D.J.; Kunkler, I.H.; Langdon, S.P. The impact of tumour pH on cancer progression: Strategies for clinical intervention. *Explor. Target. Anti-Tumor Ther.* **2020**, *1*, 71–100. [\[CrossRef\]](#)
65. Wu, P.; Zhu, Y.; Liu, S.; Xiong, H. Modular Design of High-Brightness pH-Activatable Near-Infrared BODIPY Probes for Noninvasive Fluorescence Detection of Deep-Seated Early Breast Cancer Bone Metastasis: Remarkable Axial Substituent Effect on Performance. *ACS Cent. Sci.* **2021**, *7*, 2039–2048. [\[CrossRef\]](#)

66. Yokomizo, S.; Henary, M.; Buabeng, E.R.; Fukuda, T.; Monaco, H.; Baek, Y.; Manganiello, S.; Wang, H.; Kubota, J.; Ulumben, A.D.; et al. Topical pH Sensing NIR Fluorophores for Intraoperative Imaging and Surgery of Disseminated Ovarian Cancer. *Adv. Sci.* **2022**, *9*, 2201416. [\[CrossRef\]](#)
67. Kim, S.Y.; Podder, A.; Lee, H.; Cho, Y.-J.; Han, E.H.; Khatun, S.; Sessler, J.L.; Hong, K.S.; Bhuniya, S. Self-assembled amphiphilic fluorescent probe: Detecting pH-fluctuations within cancer cells and tumour tissues. *Chem. Sci.* **2020**, *11*, 9875–9883. [\[CrossRef\]](#)
68. Tian, M.; Wu, R.; Xiang, C.; Niu, G.; Guan, W. Recent Advances in Fluorescent Probes for Cancer Biomarker Detection. *Molecules* **2024**, *29*, 1168. [\[CrossRef\]](#)
69. Zhang, R.R.; Schroeder, A.B.; Grudzinski, J.J.; Rosenthal, E.L.; Warram, J.M.; Pinchuk, A.N.; Eliceiri, K.W.; Kuo, J.S.; Weichert, J.P. Beyond the margins: Real-time detection of cancer using targeted fluorophores. *Nat. Rev. Clin. Oncol.* **2017**, *14*, 347–364. [\[CrossRef\]](#)
70. Baart, V.M.; Van Duijn, C.; Van Egmond, S.L.; Dijkmeester, W.A.; Jansen, J.C.; Vahrmeijer, A.L.; Sier, C.F.M.; Cohen, D. EGFR and  $\alpha v \beta 6$  as Promising Targets for Molecular Imaging of Cutaneous and Mucosal Squamous Cell Carcinoma of the Head and Neck Region. *Cancers* **2020**, *12*, 1474. [\[CrossRef\]](#)
71. Barberio, M.; Benedicenti, S.; Pizzicannella, M.; Felli, E.; Collins, T.; Jansen-Winkel, B.; Marescaux, J.; Viola, M.G.; Diana, M. Intraoperative Guidance Using Hyperspectral Imaging: A Review for Surgeons. *Diagnostics* **2021**, *11*, 2066. [\[CrossRef\]](#)
72. Wu, Z.; Doondea, J.B.; Gholami, A.M.; Janning, M.C.; Lemeer, S.; Kramer, K.; Eccles, S.A.; Gollin, S.M.; Grenman, R.; Walch, A.; et al. Quantitative Chemical Proteomics Reveals New Potential Drug Targets in Head and Neck Cancer. *Mol. Cell. Proteomics* **2011**, *10*, M111.011635. [\[CrossRef\]](#)
73. Abdelazeem, K.N.M.; Nguyen, D.; Corbo, S.; Darragh, L.B.; Matsumoto, M.W.; Van Court, B.; Neupert, B.; Yu, J.; Olimpo, N.A.; Osborne, D.G.; et al. Manipulating the EphB4-ephrinB2 axis to reduce metastasis in HNSCC. *Oncogene* **2025**, *44*, 130–146. [\[CrossRef\]](#)
74. Al-Jamaei, A.A.H.; Subramanyam, R.V.; Helder, M.N.; Forouzanfar, T.; Van Der Meij, E.H.; Al-Jamei, S.; De Visscher, J.G.A.M. A narrative review of the role of Eph receptors in head and neck squamous cell carcinoma. *Oral Dis.* **2024**, *30*, 833–845. [\[CrossRef\]](#) [\[PubMed\]](#)
75. Utispan, K.; Koontongkaew, S. Mucin 1 regulates the hypoxia response in head and neck cancer cells. *J. Pharmacol. Sci.* **2021**, *147*, 331–339. [\[CrossRef\]](#) [\[PubMed\]](#)
76. Odenthal, J.; Rijpkema, M.; Bos, D.; Wagena, E.; Croes, H.; Grenman, R.; Boerman, O.; Takes, R.; Friedl, P. Targeting CD44v6 for fluorescence-guided surgery in head and neck squamous cell carcinoma. *Sci. Rep.* **2018**, *8*, 10467. [\[CrossRef\]](#)
77. Mack, B.; Gires, O. CD44s and CD44v6 Expression in Head and Neck Epithelia. *PLoS ONE* **2008**, *3*, e3360. [\[CrossRef\]](#) [\[PubMed\]](#)
78. Ciulean, I.S.; Fischer, J.; Quaiser, A.; Bach, C.; Abken, H.; Tretbar, U.S.; Fricke, S.; Koehl, U.; Schmiedel, D.; Grunwald, T. CD44v6 specific CAR-NK cells for targeted immunotherapy of head and neck squamous cell carcinoma. *Front. Immunol.* **2023**, *14*, 1290488. [\[CrossRef\]](#)
79. Andersen, A.O.; Christensen, A.; Straede, K.; Lawaetz, M.; Hahn, C.H.; Rubek, N.; Wessel, I.; Lelkaitis, G.; Kiss, K.; Paaske, N.; et al. Optical molecular imaging in oral- and oropharyngeal squamous cell carcinoma using a novel uPAR-targeting near-infrared imaging agent FG001 (ICG-glu-glu-AE105): An explorative phase II clinical trial. *Theranostics* **2025**, *15*, 52–67. [\[CrossRef\]](#)
80. Christensen, A.; Grønhoj, C.; Jensen, J.; Lelkaitis, G.; Kiss, K.; Juhl, K.; Charabi, B.; Mortensen, J.; Kjær, A.; Von Buchwald, C. Expression patterns of uPAR, TF and EGFR and their potential as targets for molecular imaging in oropharyngeal squamous cell carcinoma. *Oncol. Rep.* **2022**, *48*, 147. [\[CrossRef\]](#)
81. Dirheimer, L.; Pons, T.; François, A.; Lamy, L.; Cortese, S.; Marchal, F.; Bezdetnaya, L. Targeting of 3D oral cancer spheroids by  $\alpha v \beta 6$  integrin using near-infrared peptide-conjugated IRDye 680. *Cancer Cell Int.* **2024**, *24*, 228. [\[CrossRef\]](#)
82. Lauwerends, L.J.; Zweedijk, B.E.; Galema, H.A.; Neijenhuis, L.K.A.; Dekker-Ensink, N.G.; Baatenburg De Jong, R.J.; Verhoef, C.; Bhairosingh, S.S.; Kuppen, P.J.K.; Vahrmeijer, A.L.; et al. Tumour Marker Expression in Head and Neck Malignancies to Identify Potential Targets for Intraoperative Molecular Near-Infrared Imaging. *Mol. Diagn. Ther.* **2024**, *28*, 811–820. [\[CrossRef\]](#) [\[PubMed\]](#)
83. Yuan, Y.; Fan, T.; Wang, J.; Yuan, Y.; Tao, X. Near-infrared imaging of head and neck squamous cell carcinoma using indocyanine green that targets the  $\alpha v \beta 6$  peptide. *J. Biomed. Opt.* **2024**, *29*, 046002. [\[CrossRef\]](#) [\[PubMed\]](#)
84. Nystrom, M.L.; McCulloch, D.; Weinreb, P.H.; Violette, S.M.; Speight, P.M.; Marshall, J.F.; Hart, I.R.; Thomas, G.J. Cyclooxygenase-2 Inhibition Suppresses  $\alpha v \beta 6$  Integrin-Dependent Oral Squamous Carcinoma Invasion. *Cancer Res.* **2006**, *66*, 10833–10842. [\[CrossRef\]](#)
85. Sun, J.Y.; Shen, J.; Thibodeaux, J.; Huang, G.; Wang, Y.; Gao, J.; Low, P.S.; Dimitrov, D.S.; Sumer, B.D. In vivo optical imaging of folate receptor- $\beta$  in head and neck squamous cell carcinoma. *Laryngoscope* **2014**, *124*, E312–E319. [\[CrossRef\]](#)
86. Puré, E.; Blomberg, R. Pro-tumorigenic roles of fibroblast activation protein in cancer: Back to the basics. *Oncogene* **2018**, *37*, 4343–4357. [\[CrossRef\]](#)
87. Wang, C.; Xu, X.; Folaron, M.; Gunn, J.R.; Hodge, S.; Chen, E.Y.; Hoopes, P.J.; Tichauer, K.M.; Samkoe, K.S. Improved Discrimination of Tumors with Low and Heterogeneous EGFR Expression in Fluorescence-Guided Surgery Through Paired-Agent Protocols. *Mol. Imaging Biol.* **2023**, *25*, 110–121. [\[CrossRef\]](#)

88. Li, D.; Li, X.; Li, J.; Wang, Y.; Tan, F.; Li, X. Development of a fibroblast activation protein-targeted PET/NIR dual-modality probe and its application in head and neck cancer. *Front. Bioeng. Biotechnol.* **2023**, *11*, 1291824. [\[CrossRef\]](#)
89. Vaughan, H.J.; Green, J.J.; Tzeng, S.Y. Cancer-Targeting Nanoparticles for Combinatorial Nucleic Acid Delivery. *Adv. Mater.* **2020**, *32*, 1901081. [\[CrossRef\]](#)
90. Nakamura, Y.; Mochida, A.; Choyke, P.L.; Kobayashi, H. Nanodrug Delivery: Is the Enhanced Permeability and Retention Effect Sufficient for Curing Cancer? *Bioconjug. Chem.* **2016**, *27*, 2225–2238. [\[CrossRef\]](#)
91. Rosenthal, E.L.; Moore, L.S.; Tipirneni, K.; de Boer, E.; Stevens, T.M.; Hartman, Y.E.; Carroll, W.R.; Zinn, K.R.; Warram, J.M. Sensitivity and Specificity of Cetuximab-IRDye800CW to Identify Regional Metastatic Disease in Head and Neck Cancer. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* **2017**, *23*, 4744–4752. [\[CrossRef\]](#)
92. Rosenthal, E.L.; Warram, J.M.; De Boer, E.; Chung, T.K.; Korb, M.L.; Brandwein-Gensler, M.; Strong, T.V.; Schmalbach, C.E.; Morlandt, A.B.; Agarwal, G.; et al. Safety and Tumor Specificity of Cetuximab-IRDye800 for Surgical Navigation in Head and Neck Cancer. *Clin. Cancer Res.* **2015**, *21*, 3658–3666. [\[CrossRef\]](#) [\[PubMed\]](#)
93. Warram, J.M.; De Boer, E.; Van Dam, G.M.; Moore, L.S.; Bevans, S.L.; Walsh, E.M.; Young, E.S.; Carroll, W.R.; Stevens, T.M.; Rosenthal, E.L. Fluorescence imaging to localize head and neck squamous cell carcinoma for enhanced pathological assessment. *J. Pathol. Clin. Res.* **2016**, *2*, 104–112. [\[CrossRef\]](#) [\[PubMed\]](#)
94. Mukkamala, R.; Lindeman, S.D.; Kragness, K.A.; Shahriar, I.; Srinivasarao, M.; Low, P.S. Design and characterization of fibroblast activation protein targeted pan-cancer imaging agent for fluorescence-guided surgery of solid tumors. *J. Mater. Chem. B* **2022**, *10*, 2038–2046. [\[CrossRef\]](#)
95. Ellington, A.D.; Szostak, J.W. Selection in vitro of single-stranded DNA molecules that fold into specific ligand-binding structures. *Nature* **1992**, *355*, 850–852. [\[CrossRef\]](#)
96. Ellington, A.D.; Szostak, J.W. In vitro selection of RNA molecules that bind specific ligands. *Nature* **1990**, *346*, 818–822. [\[CrossRef\]](#)
97. Camorani, S.; Crescenzi, E.; Fedele, M.; Cerchia, L. Oligonucleotide aptamers against tyrosine kinase receptors: Prospect for anticancer applications. *Biochim. Biophys. Acta BBA-Rev. Cancer* **2018**, *1869*, 263–277. [\[CrossRef\]](#)
98. Affinito, A.; Quintavalle, C.; Esposito, C.L.; Roscigno, G.; Giordano, C.; Nuzzo, S.; Ricci-Vitiani, L.; Scognamiglio, I.; Minic, Z.; Pallini, R.; et al. Targeting Ephrin Receptor Tyrosine Kinase A2 with a Selective Aptamer for Glioblastoma Stem Cells. *Mol. Ther. Nucleic Acids* **2020**, *20*, 176–185. [\[CrossRef\]](#)
99. Santana-Viera, L.; Dassie, J.P.; Rosàs-Lapeña, M.; Garcia-Monclús, S.; Chicón-Bosch, M.; Pérez-Capó, M.; Pozo, L.D.; Sanchez-Serra, S.; Almacellas-Rabaiget, O.; Maqueda-Marcos, S.; et al. Combination of protein and cell internalization SELEX identifies a potential RNA therapeutic and delivery platform to treat EphA2-expressing tumors. *Mol. Ther.-Nucleic Acids* **2023**, *32*, 758–772. [\[CrossRef\]](#)
100. Cheng, S.; Jacobson, O.; Zhu, G.; Chen, Z.; Liang, S.H.; Tian, R.; Yang, Z.; Niu, G.; Zhu, X.; Chen, X. PET imaging of EGFR expression using an 18F-labeled RNA aptamer. *Eur. J. Nucl. Med. Mol. Imaging* **2019**, *46*, 948–956. [\[CrossRef\]](#)
101. Thomas, B.J.; Awan, S.Z.; Joshi, T.; Daniels, M.A.; Porciani, D.; Burke, D.H. Anti-EGFR aptamer exhibits direct anti-cancer effects in NSCLC cells harboring EGFR L858R mutations. *Npj Precis. Oncol.* **2024**, *8*, 271. [\[CrossRef\]](#)
102. Wang, D.-L.; Song, Y.-L.; Zhu, Z.; Li, X.-L.; Zou, Y.; Yang, H.-T.; Wang, J.-J.; Yao, P.-S.; Pan, R.-J.; Yang, C.J.; et al. Selection of DNA aptamers against epidermal growth factor receptor with high affinity and specificity. *Biochem. Biophys. Res. Commun.* **2014**, *453*, 681–685. [\[CrossRef\]](#) [\[PubMed\]](#)
103. Tian, Y.; Tang, C.; Shi, G.; Wang, G.; Du, Y.; Tian, J.; Zhang, H. Novel fluorescent GLUT1 inhibitor for precision detection and fluorescence image-guided surgery in oral squamous cell carcinoma. *Int. J. Cancer* **2022**, *151*, 450–462. [\[CrossRef\]](#) [\[PubMed\]](#)
104. Zmay, G.; Koshmanova, A.; Narodov, A.; Gorbushin, A.; Voronkovskii, I.; Grek, D.; Luzan, N.; Kolovskaya, O.; Shchugoreva, I.; Artyushenko, P.; et al. Visualization of Brain Tumors with Infrared-Labeled Aptamers for Fluorescence-Guided Surgery. *J. Am. Chem. Soc.* **2024**, *146*, 24989–25004. [\[CrossRef\]](#)
105. Kichkailo, A.S.; Narodov, A.A.; Komarova, M.A.; Zmay, T.N.; Zmay, G.S.; Kolovskaya, O.S.; Erakhtin, E.E.; Glazyrin, Y.E.; Veprintsev, D.V.; Moryachkov, R.V.; et al. Development of DNA aptamers for visualization of glial brain tumors and detection of circulating tumor cells. *Mol. Ther. Nucleic Acids* **2023**, *32*, 267–288. [\[CrossRef\]](#)
106. Wang, J.; Fang, X.; Zhang, C.; Ji, H.; Pang, Q.; Li, X.; Luo, Z.; Wu, Q.; Zhang, L. Development of Aptamer-Based Molecular Tools for Rapid Intraoperative Diagnosis and In Vivo Imaging of Serous Ovarian Cancer. *ACS Appl. Mater. Interfaces* **2021**, *13*, 16118–16126. [\[CrossRef\]](#)
107. Li, C.-H.; Kuo, T.-R.; Su, H.-J.; Lai, W.-Y.; Yang, P.-C.; Chen, J.-S.; Wang, D.-Y.; Wu, Y.-C.; Chen, C.-C. Fluorescence-Guided Probes of Aptamer-Targeted Gold Nanoparticles with Computed Tomography Imaging Accesses for in Vivo Tumor Resection. *Sci. Rep.* **2015**, *5*, 15675. [\[CrossRef\]](#)
108. Fasting, C.; Schalley, C.A.; Weber, M.; Seitz, O.; Hecht, S.; Koksche, B.; Darnedde, J.; Graf, C.; Knapp, E.; Haag, R. Multivalency as a Chemical Organization and Action Principle. *Angew. Chem. Int. Ed.* **2012**, *51*, 10472–10498. [\[CrossRef\]](#)
109. Möser, C.; Lorenz, J.; Sajfutdinow, M.; Smith, D. Pinpointed Stimulation of EphA2 Receptors via DNA-Templated Oligovalence. *Int. J. Mol. Sci.* **2018**, *19*, 3482. [\[CrossRef\]](#)

110. Dai, B.; Hu, Y.; Duan, J.; Yang, X.-D. Aptamer-guided DNA tetrahedron as a novel targeted drug delivery system for MUC1-expressing breast cancer cells in vitro. *Oncotarget* **2016**, *7*, 38257–38269. [\[CrossRef\]](#) [\[PubMed\]](#)
111. Liu, X.; Wu, L.; Wang, L.; Jiang, W. A dual-targeting DNA tetrahedron nanocarrier for breast cancer cell imaging and drug delivery. *Talanta* **2018**, *179*, 356–363. [\[CrossRef\]](#)
112. Van Zundert, I.; Spezzani, E.; Brillas, R.R.; Paffen, L.; Yurchenko, A.; De Greef, T.F.A.; Albertazzi, L.; Bertucci, A.; Patiño, T. Unveiling DNA Origami Interaction Dynamics on Living Cell Surfaces by Single Particle Tracking. *bioRxiv* **2024**, in press. [\[CrossRef\]](#)
113. Van Schaik, J.E.; Halmos, G.B.; Witjes, M.J.H.; Plaat, B.E.C. An overview of the current clinical status of optical imaging in head and neck cancer with a focus on Narrow Band imaging and fluorescence optical imaging. *Oral Oncol.* **2021**, *121*, 105504. [\[CrossRef\]](#)
114. Steinkamp, P.J.; Voskuil, F.J.; Van Der Vegt, B.; Doff, J.J.; Schepman, K.-P.; De Visscher, S.A.H.J.; Kelder, W.; Jayalakshmi, Y.; Gao, J.; Sumer, B.D.; et al. A Standardized Framework for Fluorescence-Guided Margin Assessment for Head and Neck Cancer Using a Tumor Acidosis Sensitive Optical Imaging Agent. *Mol. Imaging Biol.* **2021**, *23*, 809–817. [\[CrossRef\]](#)
115. Preziosi, A.; Cirelli, C.; Waterhouse, D.; Privitera, L.; De Coppi, P.; Giuliani, S. State of the art medical devices for fluorescence-guided surgery (FGS): Technical review and future developments. *Surg. Endosc.* **2024**, *38*, 6227–6236. [\[CrossRef\]](#)
116. Kanniyappan, U.; Wang, B.; Yang, C.; Ghassemi, P.; Litorja, M.; Suresh, N.; Wang, Q.; Chen, Y.; Pfefer, T.J. Performance test methods for near-infrared fluorescence imaging. *Med. Phys.* **2020**, *47*, 3389–3401. [\[CrossRef\]](#)
117. Kriukova, E.; LaRochelle, E.; Pfefer, T.J.; Kanniyappan, U.; Gioux, S.; Pogue, B.; Ntziachristos, V.; Gorpas, D. Impact of signal-to-noise ratio and contrast definition on the sensitivity assessment and benchmarking of fluorescence molecular imaging systems. *J. Biomed. Opt.* **2024**, *30*, S13703. [\[CrossRef\]](#)
118. Ochoa, M.I.; Ruiz, A.; LaRochelle, E.; Reed, M.; Berber, E.; Poultides, G.; Pogue, B.W. Assessment of open-field fluorescence guided surgery systems: Implementing a standardized method for characterization and comparison. *J. Biomed. Opt.* **2023**, *28*, 096007. [\[CrossRef\]](#)
119. Van Beurden, F.; Van Willigen, D.M.; Vojnovic, B.; Van Oosterom, M.N.; Brouwer, O.R.; Der Poel, H.G.V.; Kobayashi, H.; Van Leeuwen, F.W.B.; Buckle, T. Multi-Wavelength Fluorescence in Image-Guided Surgery, Clinical Feasibility and Future Perspectives. *Mol. Imaging* **2020**, *19*, 153601212096233. [\[CrossRef\]](#)
120. Yu, M.; Liu, X.; Wang, S.; Qin, Z.; Hu, B.; Li, Z.; Sun, S. Application of NIR Fluorescent Materials in Imaging and Treatment of Tumors of Different Depths. *Nanomaterials* **2025**, *15*, 811. [\[CrossRef\]](#)
121. Wang, J.; Liu, Y.; Morsch, M.; Lu, Y.; Shangguan, P.; Han, L.; Wang, Z.; Chen, X.; Song, C.; Liu, S.; et al. Brain-Targeted Aggregation-Induced-Emission Nanoparticles with Near-Infrared Imaging at 1550 nm Boosts Orthotopic Glioblastoma Theranostics. *Adv. Mater.* **2022**, *34*, 2106082. [\[CrossRef\]](#)
122. Darwan, D.; Lim, K.R.G.; Wijaya, H.; Lim, Z.C.; Wang, T.; Ang, W.H.; Tan, Z. Deep Fluorescence Imaging by Laser-Scanning Excitation and Artificial Neural Network Processing. *Adv. Opt. Mater.* **2020**, *8*, 2000390. [\[CrossRef\]](#)
123. Lu, G.; van den Berg, N.S.; Martin, B.A.; Nishio, N.; Hart, Z.P.; van Keulen, S.; Fakurnejad, S.; Chirita, S.U.; Raymundo, R.C.; Yi, G.; et al. Tumour-specific fluorescence-guided surgery for pancreatic cancer using panitumumab-IRDye800CW: A phase 1 single-centre, open-label, single-arm, dose-escalation study. *Lancet Gastroenterol. Hepatol.* **2020**, *5*, 753–764. [\[CrossRef\]](#) [\[PubMed\]](#)
124. Xu, M.; Chen, Z.; Zheng, J.; Zhao, Q.; Yuan, Z. Artificial intelligence-aided optical imaging for cancer theranostics. *Semin. Cancer Biol.* **2023**, *94*, 62–80. [\[CrossRef\]](#)
125. Combalia, M.; Garcia, S.; Malvey, J.; Puig, S.; Mülberger, A.G.; Browning, J.; Garcet, S.; Krueger, J.G.; Lish, S.R.; Lax, R.; et al. Deep learning automated pathology in ex vivo microscopy. *Biomed. Opt. Express* **2021**, *12*, 3103. [\[CrossRef\]](#)
126. Ouyang, W.; Aristov, A.; Lelek, M.; Hao, X.; Zimmer, C. Deep learning massively accelerates super-resolution localization microscopy. *Nat. Biotechnol.* **2018**, *36*, 460–468. [\[CrossRef\]](#)
127. Roberts, H.W.; Donati-Bourne, J.F.; Wilson, V.L.; Wilton, J.C. The Use of Live Fluorescence Staining Techniques in Surgery: A Review. *J. Investig. Surg.* **2013**, *26*, 283–293. [\[CrossRef\]](#)
128. Keereweer, S.; Van Driel, P.B.A.A.; Snoeks, T.J.A.; Kerrebijn, J.D.F.; Baatenburg De Jong, R.J.; Vahrmeijer, A.L.; Sterenborg, H.J.C.M.; Löwik, C.W.G.M. Optical Image-Guided Cancer Surgery: Challenges and Limitations. *Clin. Cancer Res.* **2013**, *19*, 3745–3754. [\[CrossRef\]](#)
129. Pfahl, A.; Radmacher, G.K.; Köhler, H.; Maktabi, M.; Neumuth, T.; Melzer, A.; Gockel, I.; Chalopin, C.; Jansen-Winkel, B. Combined indocyanine green and quantitative perfusion assessment with hyperspectral imaging during colorectal resections. *Biomed. Opt. Express* **2022**, *13*, 3145. [\[CrossRef\]](#)
130. Chalopin, C.; Maktabi, M.; Köhler, H.; Cervantes-Sanchez, F.; Pfahl, A.; Jansen-Winkel, B.; Mehdorn, M.; Barberio, M.; Gockel, I.; Melzer, A. Intraoperative Imaging for Procedures of the Gastrointestinal Tract. In *Innovative Endoscopic and Surgical Technology in the GI Tract*; Horgan, S., Fuchs, K.-H., Eds.; Springer International Publishing: Cham, Switzerland, 2021; pp. 365–379, ISBN 978-3-030-78216-0.

131. McMahon, J.; O'Brien, C.J.; Pathak, I.; Hamill, R.; McNeil, E.; Hammersley, N.; Gardiner, S.; Junor, E. Influence of condition of surgical margins on local recurrence and disease-specific survival in oral and oropharyngeal cancer. *Br. J. Oral Maxillofac. Surg.* **2003**, *41*, 224–231. [\[CrossRef\]](#)
132. Nagaya, T.; Nakamura, Y.A.; Choyke, P.L.; Kobayashi, H. Fluorescence-Guided Surgery. *Front. Oncol.* **2017**, *7*, 314. [\[CrossRef\]](#)
133. Zhu, Y.; Xiao, T.; He, Y.; Hong, X.; Zhou, T.; Da, M.; Ge, S.; Xie, D.; Wang, Z. Application of near-infrared fluorescence imaging in lingual lymph node screening and drainage pattern observation for tongue cancer. *Front. Cell Dev. Biol.* **2022**, *10*, 986575. [\[CrossRef\]](#) [\[PubMed\]](#)
134. Stone, L.D.; Kasten, B.B.; Rao, S.; Gonzalez, M.L.; Stevens, T.M.; Lin, D.; Carroll, W.; Greene, B.; Moore, L.S.; Fuson, A.; et al. Interim Phase II Results Using Panitumumab-IRDye800CW during Transoral Robotic Surgery in Patients with Oropharyngeal Cancer. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* **2024**, *30*, 4016–4028. [\[CrossRef\]](#) [\[PubMed\]](#)
135. Cianchi, F.; Indennitate, G.; Paoli, B.; Ortolani, M.; Lami, G.; Manetti, N.; Tarantino, O.; Messeri, S.; Foppa, C.; Badii, B.; et al. The Clinical Value of Fluorescent Lymphography with Indocyanine Green During Robotic Surgery for Gastric Cancer: A Matched Cohort Study. *J. Gastrointest. Surg. Off. J. Soc. Surg. Aliment. Tract* **2020**, *24*, 2197–2203. [\[CrossRef\]](#)
136. Zhong, Q.; Chen, Q.-Y.; Huang, X.-B.; Lin, G.-T.; Liu, Z.-Y.; Chen, J.-Y.; Wang, H.-G.; Weng, K.; Li, P.; Xie, J.-W.; et al. Clinical implications of Indocyanine Green Fluorescence Imaging-Guided laparoscopic lymphadenectomy for patients with gastric cancer: A cohort study from two randomized, controlled trials using individual patient data. *Int. J. Surg. Lond. Engl.* **2021**, *94*, 106120. [\[CrossRef\]](#)
137. Park, J.-H.; Berlth, F.; Wang, C.; Wang, S.; Choi, J.-H.; Park, S.-H.; Suh, Y.-S.; Kong, S.-H.; Park, D.J.; Lee, H.-J.; et al. Mapping of the perigastric lymphatic network using indocyanine green fluorescence imaging and tissue marking dye in clinically advanced gastric cancer. *Eur. J. Surg. Oncol. J. Eur. Soc. Surg. Oncol. Br. Assoc. Surg. Oncol.* **2022**, *48*, 411–417. [\[CrossRef\]](#)
138. Zweedijk, B.E.; Dalmeijer, S.W.R.; Van Manen, L.; Galema, H.A.; Lauwerends, L.J.; Abbasi, H.; Kremer, B.; Verhoef, C.; Robinson, D.J.; Koppes, S.A.; et al. Molecular-Targeted Fluorescence Lymph Node Imaging Could Play a Clinical Role in the Surgical Setting: A Systematic Review. *Cancers* **2025**, *17*, 1352. [\[CrossRef\]](#)
139. Peloso, A.; Franchi, E.; Canepa, M.C.; Barbieri, L.; Briani, L.; Ferrario, J.; Bianco, C.; Quaretti, P.; Brugnattelli, S.; Dionigi, P.; et al. Combined use of intraoperative ultrasound and indocyanine green fluorescence imaging to detect liver metastases from colorectal cancer. *HPB* **2013**, *15*, 928–934. [\[CrossRef\]](#)
140. Van Der Vorst, J.R.; Schaafsma, B.E.; Hutteman, M.; Verbeek, F.P.R.; Liefers, G.; Hartgrink, H.H.; Smit, V.T.H.B.M.; Löwik, C.W.G.M.; Van De Velde, C.J.H.; Frangioni, J.V.; et al. Near-infrared fluorescence-guided resection of colorectal liver metastases. *Cancer* **2013**, *119*, 3411–3418. [\[CrossRef\]](#)
141. Knospe, L.; Gockel, I.; Jansen-Winkel, B.; Thieme, R.; Niebisch, S.; Moulla, Y.; Stelzner, S.; Lyros, O.; Diana, M.; Marescaux, J.; et al. New Intraoperative Imaging Tools and Image-Guided Surgery in Gastric Cancer Surgery. *Diagnostics* **2022**, *12*, 507. [\[CrossRef\]](#)
142. Jafari, M.D.; Wexner, S.D.; Martz, J.E.; McLemore, E.C.; Margolin, D.A.; Sherwinter, D.A.; Lee, S.W.; Senagore, A.J.; Phelan, M.J.; Stamos, M.J. Perfusion assessment in laparoscopic left-sided/anterior resection (PILLAR II): A multi-institutional study. *J. Am. Coll. Surg.* **2015**, *220*, 82–92.e1. [\[CrossRef\]](#)
143. Liu, D.; Liang, L.; Liu, L.; Zhu, Z. Does intraoperative indocyanine green fluorescence angiography decrease the incidence of anastomotic leakage in colorectal surgery? A systematic review and meta-analysis. *Int. J. Color. Dis.* **2021**, *36*, 57–66. [\[CrossRef\]](#) [\[PubMed\]](#)
144. Arezzo, A.; Bonino, M.A.; Ris, F.; Boni, L.; Cassinotti, E.; Foo, D.C.C.; Shum, N.F.; Brolese, A.; Ciarleglio, F.; Keller, D.S.; et al. Intraoperative use of fluorescence with indocyanine green reduces anastomotic leak rates in rectal cancer surgery: An individual participant data analysis. *Surg. Endosc.* **2020**, *34*, 4281–4290. [\[CrossRef\]](#) [\[PubMed\]](#)
145. Zeh, R.; Sheikh, S.; Xia, L.; Pierce, J.; Newton, A.; Predina, J.; Cho, S.; Nasrallah, M.; Singhal, S.; Dorsey, J.; et al. The second window ICG technique demonstrates a broad plateau period for near infrared fluorescence tumor contrast in glioblastoma. *PLoS ONE* **2017**, *12*, e0182034. [\[CrossRef\]](#)
146. Voskuil, F.J.; Steinkamp, P.J.; Zhao, T.; Van Der Vegt, B.; Koller, M.; Doff, J.J.; Jayalakshmi, Y.; Hartung, J.P.; Gao, J.; Sumer, B.D.; et al. Exploiting metabolic acidosis in solid cancers using a tumor-agnostic pH-activatable nanoprobe for fluorescence-guided surgery. *Nat. Commun.* **2020**, *11*, 3257. [\[CrossRef\]](#)
147. Randall, L.M.; Wenham, R.M.; Low, P.S.; Dowdy, S.C.; Tanyi, J.L. A phase II, multicenter, open-label trial of OTL38 injection for the intra-operative imaging of folate receptor-alpha positive ovarian cancer. *Gynecol. Oncol.* **2019**, *155*, 63–68. [\[CrossRef\]](#)
148. Giuliani, S.; Paraboschi, I.; McNair, A.; Smith, M.; Rankin, K.S.; Elson, D.S.; Paleri, V.; Leff, D.; Stasiuk, G.; Anderson, J. Monoclonal Antibodies for Targeted Fluorescence-Guided Surgery: A Review of Applicability across Multiple Solid Tumors. *Cancers* **2024**, *16*, 1045. [\[CrossRef\]](#)
149. Jiao, J.; Zhang, J.; Yang, F.; Song, W.; Han, D.; Wen, W.; Qin, W. Quicker, deeper and stronger imaging: A review of tumor-targeted, near-infrared fluorescent dyes for fluorescence guided surgery in the preclinical and clinical stages. *Eur. J. Pharm. Biopharm.* **2020**, *152*, 123–143. [\[CrossRef\]](#)

150. Enghard, A.S.; Palaras, A.; Volgger, V.; Stepp, H.; Mack, B.; Libl, D.; Gires, O.; Betz, C.S. Confocal laser endomicroscopy in head and neck malignancies using FITC-labelled EpCAM- and EGF-R-antibodies in cell lines and tumor biopsies. *J. Biophotonics* **2017**, *10*, 1365–1376. [CrossRef]
151. Thariat, J.; Etienne-Grimaldi, M.-C.; Grall, D.; Bensadoun, R.-J.; Cayre, A.; Penault-Llorca, F.; Veracini, L.; Francoual, M.; Formento, J.-L.; Dassonville, O.; et al. Epidermal Growth Factor Receptor Protein Detection in Head and Neck Cancer Patients: A Many-Faceted Picture. *Clin. Cancer Res.* **2012**, *18*, 1313–1322. [CrossRef]
152. Spiegelberg, D.; Kuku, G.; Selvaraju, R.; Nestor, M. Characterization of CD44 variant expression in head and neck squamous cell carcinomas. *Tumor Biol.* **2014**, *35*, 2053–2062. [CrossRef] [PubMed]
153. Orian-Rousseau, V. CD44, a therapeutic target for metastasising tumours. *Eur. J. Cancer* **2010**, *46*, 1271–1277. [CrossRef]
154. Chen, J.; Zhou, J.; Lu, J.; Xiong, H.; Shi, X.; Gong, L. Significance of CD44 expression in head and neck cancer: A systemic review and meta-analysis. *BMC Cancer* **2014**, *14*, 15. [CrossRef]
155. Staibano, S.; Merolla, F.; Testa, D.; Iovine, R.; Mascolo, M.; Guarino, V.; Castellone, M.D.; Di Benedetto, M.; Galli, V.; Motta, S.; et al. OPN/CD44v6 overexpression in laryngeal dysplasia and correlation with clinical outcome. *Br. J. Cancer* **2007**, *97*, 1545–1551. [CrossRef]
156. Brown, R.L.; Reinke, L.M.; Damerow, M.S.; Perez, D.; Chodosh, L.A.; Yang, J.; Cheng, C. CD44 splice isoform switching in human and mouse epithelium is essential for epithelial-mesenchymal transition and breast cancer progression. *J. Clin. Investig.* **2011**, *121*, 1064–1074. [CrossRef]
157. Fakurnejad, S.; Van Keulen, S.; Nishio, N.; Engelen, M.; Van Den Berg, N.S.; Lu, G.; Birkeland, A.; Baik, F.; Colevas, A.D.; Rosenthal, E.L.; et al. Fluorescence molecular imaging for identification of high-grade dysplasia in patients with head and neck cancer. *Oral Oncol.* **2019**, *97*, 50–55. [CrossRef]
158. De Wit, J.G.; Vonk, J.; Voskuil, F.J.; De Visscher, S.A.H.J.; Schepman, K.-P.; Hooghiemstra, W.T.R.; Linssen, M.D.; Elias, S.G.; Halmos, G.B.; Plaat, B.E.C.; et al. EGFR-targeted fluorescence molecular imaging for intraoperative margin assessment in oral cancer patients: A phase II trial. *Nat. Commun.* **2023**, *14*, 4952. [CrossRef]
159. Rakuten Medical, Inc. ASP-1929 Photoimmunotherapy (PIT) Study in Patients with Recurrent Head/Neck Cancer. Available online: <https://clinicaltrials.gov/study/NCT05182866> (accessed on 1 July 2025).
160. Erasmus University Rotterdam. Fluorescence-guided Surgery Using cRGD-ZW800-1 in Oral Cancer. Available online: <https://clinicaltrials.eu/trial/study-on-improving-oral-cancer-surgery-using-crgd-zw800-1-fluorescent-imaging-for-patients-with-oral-cancer/> (accessed on 1 July 2025).
161. Vanderbilt-Ingram Cancer Center Evaluating the Use of Dual Imaging Techniques for Detection of Disease in Patients with Head and Neck Cancer. Available online: <https://clinicaltrials.gov/study/NCT05945875> (accessed on 1 July 2025).
162. Meeks, N.; James, S.; Krishnan, G.; Wodeyar, A.; Tanaka, H.; Kasten, B.B.; Lee, Y.-J.; Hom, M.E.; Rosenthal, E.L.; Warram, J.M. Background Tissue with Native Target Expression Can Determine Presence of Nodal Metastasis in Head and Neck Squamous Cell Carcinoma Patients Infused with Targeted Fluorescent Tracers. *Mol. Imaging Biol.* **2025**, *27*, 333–340. [CrossRef]
163. University Medical Center Groningen. Real-time Margin Assessment in Head and Neck Cancer (LIGHTNING). Available online: <https://clinicaltrials.gov/study/NCT05499065> (accessed on 1 July 2025).
164. Witjes, M.J.H. Clinical challenges in image-guided surgery in cancer. In *Proceedings of the Molecular-Guided Surgery: Molecules, Devices, and Applications XI*; Gibbs, S.L., Tichauer, K.M., Eds.; SPIE: San Francisco, CA, USA, 2025; p. 39.
165. Erasmus Medical Center. Fluorescence-guided Surgery in Laryngeal- and Hypopharyngeal Cancer: A Feasibility Trial (STELLAR). Available online: <https://clinicaltrials.gov/study/NCT05752149> (accessed on 1 July 2025).
166. Pal, R.; Lwin, T.M.; Krishnamoorthy, M.; Collins, H.R.; Chan, C.D.; Prilutskiy, A.; Nasrallah, M.P.; Dijkhuis, T.H.; Shukla, S.; Kendall, A.L.; et al. Fluorescence lifetime of injected indocyanine green as a universal marker of solid tumours in patients. *Nat. Biomed. Eng.* **2023**, *7*, 1649–1666. [CrossRef]
167. Dirheimer, L.; Cortese, S.; Dolivet, G.; Merlin, J.L.; Marchal, F.; Mastronicola, R.; Bezdetnaya, L. Fluorescence Imaging-Assessed Surgical Margin Detection in Head and Neck Oncology by Passive and Active Targeting. *Mol. Diagn. Ther.* **2025**, *29*, 465–481. [CrossRef]
168. Voskuil, F.J.; De Jongh, S.J.; Hooghiemstra, W.T.R.; Linssen, M.D.; Steinkamp, P.J.; De Visscher, S.A.H.J.; Schepman, K.-P.; Elias, S.G.; Meersma, G.-J.; Jonker, P.K.C.; et al. Fluorescence-guided imaging for resection margin evaluation in head and neck cancer patients using cetuximab-800CW: A quantitative dose-escalation study. *Theranostics* **2020**, *10*, 3994–4005. [CrossRef]
169. Wu, C.; Gleysteen, J.; Teraphongphom, N.T.; Li, Y.; Rosenthal, E. In-vivo optical imaging in head and neck oncology: Basic principles, clinical applications and future directions. *Int. J. Oral Sci.* **2018**, *10*, 10. [CrossRef] [PubMed]
170. Alekseev, M.; Rybakov, E.; Shelygin, Y.; Chernyshov, S.; Zarodnyuk, I. A study investigating the perfusion of colorectal anastomoses using fluorescence angiography: Results of the FLAG randomized trial. *Color. Dis. Off. J. Assoc. Coloproctol. G. B. Irel.* **2020**, *22*, 1147–1153. [CrossRef] [PubMed]
171. Vu, A.; Farah, C. Narrow band imaging: Clinical applications in oral and oropharyngeal cancer. *Oral Dis.* **2016**, *22*, 383–390. [CrossRef]

172. Davies, K.; Connolly, J.M.; Dockery, P.; Wheatley, A.M.; Olivo, M.; Keogh, I. Point of care optical diagnostic technologies for the detection of oral and oropharyngeal squamous cell carcinoma. *Surg. J. R. Coll. Surg. Edinb. Irel.* **2015**, *13*, 321–329. [\[CrossRef\]](#)
173. Green, B.; Tsiroyannis, C.; Brennan, P.A. Optical diagnostic systems for assessing head and neck lesions. *Oral Dis.* **2016**, *22*, 180–184. [\[CrossRef\]](#)
174. Green, B.; Cobb, A.R.M.; Brennan, P.A.; Hopper, C. Optical diagnostic techniques for use in lesions of the head and neck: Review of the latest developments. *Br. J. Oral Maxillofac. Surg.* **2014**, *52*, 675–680. [\[CrossRef\]](#)
175. Arens, C.; Dreyer, T.; Malzahn, K.; Glanz, H. Direct and indirect autofluorescence laryngoscopy in the diagnosis of laryngeal cancer and its precursor lesions. *Otolaryngol. Pol. Pol. Otolaryngol.* **2004**, *58*, 197–203.
176. Vila, P.M.; Park, C.W.; Pierce, M.C.; Goldstein, G.H.; Levy, L.; Gurudutt, V.V.; Polydorides, A.D.; Godbold, J.H.; Teng, M.S.; Genden, E.M.; et al. Discrimination of benign and neoplastic mucosa with a high-resolution microendoscope (HRME) in head and neck cancer. *Ann. Surg. Oncol.* **2012**, *19*, 3534–3539. [\[CrossRef\]](#)
177. Kallaway, C.; Almond, L.M.; Barr, H.; Wood, J.; Hutchings, J.; Kendall, C.; Stone, N. Advances in the clinical application of Raman spectroscopy for cancer diagnostics. *Photodiagnosis Photodyn. Ther.* **2013**, *10*, 207–219. [\[CrossRef\]](#) [\[PubMed\]](#)
178. Santos, L.F.; Wolthuis, R.; Koljenović, S.; Almeida, R.M.; Puppels, G.J. Fiber-Optic Probes for in Vivo Raman Spectroscopy in the High-Wavenumber Region. *Anal. Chem.* **2005**, *77*, 6747–6752. [\[CrossRef\]](#) [\[PubMed\]](#)
179. Zhang, L.; Wu, Y.; Zheng, B.; Su, L.; Chen, Y.; Ma, S.; Hu, Q.; Zou, X.; Yao, L.; Yang, Y.; et al. Rapid histology of laryngeal squamous cell carcinoma with deep-learning based stimulated Raman scattering microscopy. *Theranostics* **2019**, *9*, 2541–2554. [\[CrossRef\]](#) [\[PubMed\]](#)
180. Weber, A.; Enderle-Ammour, K.; Kurowski, K.; Metzger, M.C.; Poxleitner, P.; Werner, M.; Rothweiler, R.; Beck, J.; Straehle, J.; Schmelzeisen, R.; et al. AI-Based Detection of Oral Squamous Cell Carcinoma with Raman Histology. *Cancers* **2024**, *16*, 689. [\[CrossRef\]](#)
181. Barroso, E.M.; Smits, R.W.H.; Bakker Schut, T.C.; Ten Hove, I.; Hardillo, J.A.; Wolvius, E.B.; Baatenburg De Jong, R.J.; Koljenović, S.; Puppels, G.J. Discrimination between Oral Cancer and Healthy Tissue Based on Water Content Determined by Raman Spectroscopy. *Anal. Chem.* **2015**, *87*, 2419–2426. [\[CrossRef\]](#)
182. Liao, Z.; Lizio, M.G.; Corden, C.; Khout, H.; Rakha, E.; Notingher, I. Feasibility of integrated high-wavenumber Raman imaging and fingerprint Raman spectroscopy for fast margin assessment in breast cancer surgery. *J. Raman Spectrosc.* **2020**, *51*, 1986–1995. [\[CrossRef\]](#)
183. Koljenović, S.; Bakker Schut, T.C.; Wolthuis, R.; De Jong, B.; Santos, L.; Caspers, P.J.; Kros, J.M.; Puppels, G.J. Tissue characterization using high wave number Raman spectroscopy. *J. Biomed. Opt.* **2005**, *10*, 031116. [\[CrossRef\]](#)
184. Grimbergen, M.C.M.; Van Swol, C.F.P.; Van Moorselaar, R.J.A.; Uff, J.; Mahadevan-Jansen, A.; Stone, N. Raman spectroscopy of bladder tissue in the presence of 5-aminolevulinic acid. *J. Photochem. Photobiol. B* **2009**, *95*, 170–176. [\[CrossRef\]](#)
185. Carr, J.A.; Franke, D.; Caram, J.R.; Perkinson, C.F.; Saif, M.; Askoxylakis, V.; Datta, M.; Fukumura, D.; Jain, R.K.; Bawendi, M.G.; et al. Shortwave infrared fluorescence imaging with the clinically approved near-infrared dye indocyanine green. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 4465–4470. [\[CrossRef\]](#)
186. Lauwerends, L.J.; Abbasi, H.; Bakker Schut, T.C.; Van Driel, P.B.A.A.; Hardillo, J.A.U.; Santos, I.P.; Barroso, E.M.; Koljenović, S.; Vahrmeijer, A.L.; Baatenburg De Jong, R.J.; et al. The complementary value of intraoperative fluorescence imaging and Raman spectroscopy for cancer surgery: Combining the incompatibles. *Eur. J. Nucl. Med. Mol. Imaging* **2022**, *49*, 2364–2376. [\[CrossRef\]](#)
187. Röhrich, M. Fibroblast Activation Protein Inhibitor PET Imaging in Head and Neck Cancer. *PET Clin.* **2023**, *18*, 315–323. [\[CrossRef\]](#)
188. Linz, C.; Brands, R.C.; Kertels, O.; Dierks, A.; Brumberg, J.; Gerhard-Hartmann, E.; Hartmann, S.; Schirbel, A.; Serfling, S.; Zhi, Y.; et al. Targeting fibroblast activation protein in newly diagnosed squamous cell carcinoma of the oral cavity—Initial experience and comparison to [<sup>18</sup>F]FDG PET/CT and MRI. *Eur. J. Nucl. Med. Mol. Imaging* **2021**, *48*, 3951–3960. [\[CrossRef\]](#)
189. Syed, M.; Flechsig, P.; Liermann, J.; Windisch, P.; Staudinger, F.; Akbaba, S.; Koerber, S.A.; Freudlsperger, C.; Plinkert, P.K.; Debus, J.; et al. Fibroblast activation protein inhibitor (FAPI) PET for diagnostics and advanced targeted radiotherapy in head and neck cancers. *Eur. J. Nucl. Med. Mol. Imaging* **2020**, *47*, 2836–2845. [\[CrossRef\]](#) [\[PubMed\]](#)
190. Han, D.; Wu, J.; Han, Y.; Wei, M.; Han, S.; Lin, R.; Sun, Z.; Yang, F.; Jiao, D.; Xie, P.; et al. A novel anti-PSMA human scFv has the potential to be used as a diagnostic tool in prostate cancer. *Oncotarget* **2016**, *7*, 59471–59481. [\[CrossRef\]](#) [\[PubMed\]](#)
191. Mazzocco, C.; Fracasso, G.; Germain-Genevois, C.; Dugot-Senant, N.; Figini, M.; Colombatti, M.; Grenier, N.; Couillaud, F. In vivo imaging of prostate cancer using an anti-PSMA scFv fragment as a probe. *Sci. Rep.* **2016**, *6*, 23314. [\[CrossRef\]](#) [\[PubMed\]](#)
192. Van Driel, P.B.A.A.; Van Der Vorst, J.R.; Verbeek, F.P.R.; Oliveira, S.; Snoeks, T.J.A.; Keereweert, S.; Chan, B.; Boonstra, M.C.; Frangioni, J.V.; Van Bergen En Henegouwen, P.M.P.; et al. Intraoperative fluorescence delineation of head and neck cancer with a fluorescent Anti-epidermal growth factor receptor nanobody. *Int. J. Cancer* **2014**, *134*, 2663–2673. [\[CrossRef\]](#)
193. Mahalingam, S.M.; Dudkin, V.Y.; Goldberg, S.; Klein, D.; Yi, F.; Singhal, S.; O'Neil, K.T.; Low, P.S. Evaluation of a Centyrin-Based Near-Infrared Probe for Fluorescence-Guided Surgery of Epidermal Growth Factor Receptor Positive Tumors. *Bioconjug. Chem.* **2017**, *28*, 2865–2873. [\[CrossRef\]](#)

194. Elliott, J.T.; Marra, K.; Evans, L.T.; Davis, S.C.; Samkoe, K.S.; Feldwisch, J.; Paulsen, K.D.; Roberts, D.W.; Pogue, B.W. Simultaneous In Vivo Fluorescent Markers for Perfusion, Protoporphyrin Metabolism, and EGFR Expression for Optically Guided Identification of Orthotopic Glioma. *Clin. Cancer Res.* **2017**, *23*, 2203–2212. [[CrossRef](#)]
195. Gao, Z.; Li, G.; Li, X.; Zhou, J.; Duan, X.; Chen, J.; Joshi, B.P.; Kuick, R.; Khoury, B.; Thomas, D.G.; et al. In vivo near-infrared imaging of ErbB2 expressing breast tumors with dual-axes confocal endomicroscopy using a targeted peptide. *Sci. Rep.* **2017**, *7*, 14404. [[CrossRef](#)]

**Disclaimer/Publisher's Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.