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Molecular endoscopic imaging: the future is bright

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Abstract: The prediction and final survival rate of gastrointestinal cancers are dependent on the stage of disease. The ideal would be to detect those gastrointestinal lesions at early stage or even premalignant forms which are difficult to detect by conventional endoscopy with white light optical imaging as they show minimum or no changes in morphological characteristics and are thus left untreated. The introduction of molecular imaging has greatly changed the pattern for detecting gastrointestinal lesions from purely macroscopic structural imaging to the molecular level. It allows microscopic examination of the gastrointestinal mucosa with endoscopy after the topical or systemic application of molecular probes. In recent years, major advancements in endoscopic instruments and specific molecular probes have been achieved. This review focuses on the current status of endoscopic imaging and highlights the application of molecular imaging in gastrointestinal and hepatic disease in the context of diagnosis and therapy based on recently published literature in this field. We also discuss the challenges of molecular endoscopic imaging, its future directions and potential that could have a tremendous impact on endoscopic research and clinical practice in future.

Keywords: antibody labeling, confocal endomicroscopy, endoscopy, *ex vivo* study, *in vivo* imaging, molecular imaging

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Introduction

Endoscopy is widely used to directly visualize the large epithelial surfaces in hollow organs, such as the esophagus, stomach, and duodenum.[1] The visible structural abnormalities and color change of the mucosal surface are the basis of disease diagnosis. The success of the evaluation substantially relies on the ability of the endoscopist to visualize those abnormal patterns created in the image by the reflected light. Although standard endoscopy allows for the collection of biopsies and their histological analysis for interpretation in clinical decision making, this method is lacking quantitative evaluation parameters and is established on gross morphological deviations. Many small or depressed neoplastic lesions are undetected when using this method. Therefore, it is important to develop a method for the early detection of malignant and inflammatory lesions to improve disease prognosis and the patient's quality of life. The biochemical features of the tissue can be revealed if substantial data are properly analyzed. The intersection of electronic imaging and molecular biology has great potential in allowing clinicians to observe tissues beyond the gross anatomical structures and understand the biological phenomena.[2] Moreover, this technique accomplishes the quantitative assessments of tissue functions based on their specific molecular expression profiles.

Several medical imaging modalities, such as positron emission tomography (PET), computed tomography (CT), ultrasound (US), single-photon-emission computed tomography (SPECT), and magnetic resonance imaging (MRI), are being used clinically in combination with exogenously administered contrast agents to visualize the tissue morphology *in vivo*. Although these techniques have positive impact on patient care, they also have clear limitations with regard to the evaluation of important tumor features or inflammations.[3–5] Moreover, they are very expensive and largely dependent on morphological changes

in the tissue, and some of them rely on radioactive compounds.

Molecular imaging can be broadly defined as the detection, spatial localization, and quantification of specific molecular targets for characterizing biological processes at the cellular and molecular levels[6,7] and has drawn increasing interest with regard to clinical diagnosis because it considers the authentic biochemical events driving the disease condition. Thus, physicians require specialized instrumentation and imaging agents to visualize specific cellular markers.[8]

The molecular imaging system can be elegantly assigned two roles in clinical applications.[9] The first role is diagnostic imaging, which is used to localize the targeted molecules of a specific disease. Its success mainly depends on the identification of a suitable molecular marker, which represents the disease to be investigated. The second role is molecular targeting therapy for treating those diseases. The same molecular probes can be loaded with an agent that delivers therapy to the targeted cells.

This review focuses on recent imaging technology developments in the field of endoscopy, application of molecular endoscopic imaging (MEI) in disease diagnosis, and therapy based on recently published literature in this field, and its future role in internal medicine.

Components of MEI

Gastrointestinal endoscopy has made great progress over the last decade.[10] In addition, the rapid technological advancement of optics and mechanics continues to facilitate ongoing progress and innovation in the field of endoscopy. However, due to its dependency on morphological change, the use of conventional endoscopy in diagnostics is essentially limited. This can be overcome by MEI, which is established based on three basic points for application to clinical settings: (a) exploration of molecular markers specifically expressed in cancer or disease conditions; (b) development of molecular probes or imaging agents; and (c) suitable device for the acquisition of quality images containing higher-level information in simple form.

Molecular targets

Cancers or inflammations may occur in all gastrointestinal tract (GIT) segments. In Europe and the United States,[11–13] the colorectal carcinoma and dysplasia in Barrett's esophagus (BE) are more frequent, whereas in Japan,[14] cancer mortality is more frequently associated with gastric sites. Generally, disease-specific biological target structures located on the cell's surface or in the cytoplasm are overexpressed in the tissue. The epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2/neu), vascular endothelial growth factor (VEGF), and carcinoembryonic antigen (CEA) are highly expressed in digestive tract cancers,[14–18] whereas the somatostatin receptors (SSTRs) are overexpressed in neuroendocrine tumors.[19] Markers can also consist of proteolytic enzymes such as cathepsin[20] and matrix metalloproteinases (MMPs).[21]

Molecular probes

To visualize the molecular targets in tissues, exogenous targeting agents called molecular probes are administered into the body to interact with a disease-specific marker. The ideal imaging agent should contain several properties, such as high affinity toward the molecular target, low unspecific background signals, safe toxicity profile, deep tissue penetration, rapid clearance from non-target tissue, non-immunogenicity, stability, low cost, and high scale synthesis.[22] There exist several categories of molecular imaging agents, including high-molecular-weight antibodies, engineered protein fragments, peptides, small molecules, aptamers, and various nanoparticles. While each type of agent has a different size range, in this review, the pharmacokinetic and binding properties, and their advantages and disadvantages, will not be discussed in detail because a detailed description of the widely used exogenous targeting agents has been provided in our previous review.[8]

The probes can be labeled with a variety of fluorophores. Most of the current imaging devices are restricted within the range of 480–520 nm. Fluorescein is the only visible fluorophore approved by the US Food and Drug Administration (FDA) for human use.[23] Therefore, fluorescein isothiocyanate (FITC), which has a maximum excitation and emission of 488 and 515 nm, respectively, is widely used to label the molecular probes. Alternatively, the Alexa 488 or DyLight 488, for example, could also be used, because they are generally more stable and brighter than common dyes. These fluorophores are stable and

provide high image resolution with reduced depth. To achieve deep tissue penetration and less interference from autofluorescence, near-infrared (NIR) fluorophores such as the indocyanine green (ICG), Cy5, Cy5.5, and IRDye800, which emit in the range of 665–900 nm, can be used in clinical studies to generate similar fluorescence.[24–26] However, the toxicity of each material needs to be evaluated properly before it is approved for clinical use.

According to the method of administration, the use of imaging agents can raise safety concerns. There are two possible approaches toward applying molecular probes during *in vivo* imaging. Topical and intravenous application has advantages and limitations for both of these approaches.[11,12,18] MI with topical application can be performed within a few minutes. Local concentration may be higher than that of intravenous administration and induce less immunogenicity and fewer side effects. In addition, local application cannot be used for a large mucosal area. Conversely, systemic application requires lead time for an imaging agent to be distributed throughout the body. Moreover, it can generate antigenic effects and be excreted out of the body, due to its possible toxicity. However, intravenous application produces deep tissue penetration and can be efficient for large cancer tissues.

During MEI, devices should be able to characterize tiny molecular changes in the gastrointestinal (GI) tract by detecting the specific molecular probes binding to the target structures with sufficient sensitivity. Devices for the detection of cellular details during GI endoscopy can be categorized into endoscopic instruments for wide-field detection and tiny devices for on-site characterization.

Autofluorescence imaging (AFI) detects the lesion based on different fluorescence emissions among the various tissue types. Commercial endoscopes are combined with high-resolution white light endoscopy (WLE) with reflectance and fluorescence filters and excite the tissue using a short-wavelength light source. AFI can be used for cancer screening tests without the administration of fluorescence probes and can be improved by the combination of high-definition white light imaging and narrow-band imaging (NBI) to develop tri-modal imaging video endoscopes.[27] However, it is recognized that WLE can miss various early lesions.[28–30]

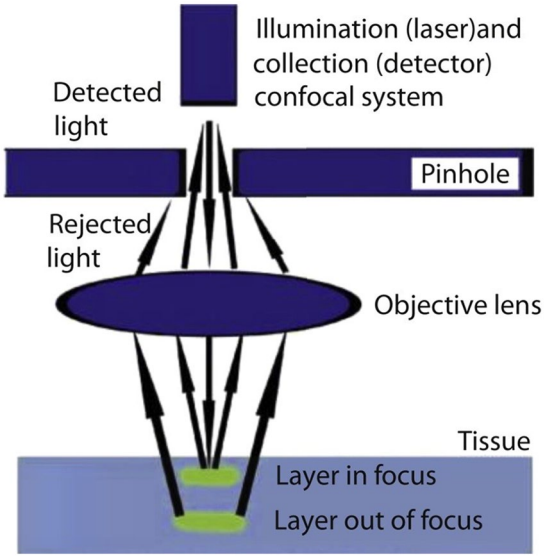

Figure 1. Schematic diagram of confocal laser endomicroscopy principles.[33]

To surpass those limitations, chromoendoscopic technique can be used.[31,32] This is an image-enhanced method where a chemical substance is applied to gastrointestinal mucosa and based on the response to that chemical component, normal or modified mucosal pattern can be detected. The technique can be used to identify BE, chronic ulcerative colitis, and cancers of the stomach, or colon. However, the staining process is time-consuming and requires additional cost. Moreover, the use of different staining materials and then demonstration of data are also very challenging. The coupling of confocal imaging method into conventional endoscopy allows microscopic examination of GIT in real time during endoscopy.[33]

The principles of confocal laser endomicroscopy (CLE) are based on conventional confocal microscopy systems. An area of suspicious tissue is exposed to a low-power blue laser light, and then the reflected fluorescent light from the tissue is subsequently detected by the same lens (Figure 1). Illumination and reflection occur in the same focal plane. Therefore, the system produces high-resolution images of a distinct point by rejecting out of focus light. The biological tissue image is constructed through the horizontal and vertical scanning of the area.

To obtain the confocal images, intravenous or topically applied exogenous fluorescence agents are required. To date, there are two FDA-approved clinically available CLE platforms.[34,35]

One is an endoscope-based system (eCLE) developed by Pentax Corporation (Tokyo, Japan) where miniaturized confocal microscope is integrated in the tip of the endoscope and a fiber optic cable delivers blue laser light (488 nm) to the microscope. Images are collected at a scan rate of 0.7–1.2 frames per second with an imaging depth of 0–250 μm. The device has a field of view of 475 × 475 μm and lateral resolution of 0.7 μm. The second system is a flexible confocal mini probe consisting of a fiber optic bundle with an integrated distal lens connected to a laser scanning unit. This system provides laser excitation at 488 and 660 nm, and lateral and axial resolution ranges from 1.4 to 3.5 μm and from 10 to 15 μm, respectively. In addition, the system provides faster image acquisition with 12 frames per second and a field of view of 240–600 μm. Different specifications with regard to the probe diameter and imaging plane depth are available. A large number of clinical studies have demonstrated the effectiveness of this technology and have been performed in real time for the *in vivo* diagnosis of gastrointestinal diseases.[34,36–38]

Current status of MEI

Barrett's esophagus

Barrett's esophagus is a known precursor to esophageal adenocarcinoma (EAC), which is a malignancy that has been **considerably** on the rise in the Western world over the last few years and has a 5-year survival rate of 10–15%.[39] BE is characterized by the replacement of the squamous epithelium of the esophageal mucosa with a columnar intestinal epithelium containing goblet cells.[40,41] However, the surveillance of BE patients is critical for early detection and the localization of dysplasia. Although clinical guidelines have recommended periodic endoscopic surveillance for the detection of dysplasia and early cancer in patients with BE, conventional screening using WLE has significant limitations, due to the flat appearance of premalignant dysplasia, which remains invisible because these conditions are not distinguishable from the surrounding mucosa. Therefore, a targeted molecular imaging strategy is required for early detection. Several studies (Table 1) have been performed to use MEI for the detection of high-grade dysplasia (HGD) and early-stage adenocarcinoma with regard to BE.

In an *ex vivo* study, Bird-Lieberman and colleagues[42] described a molecular imaging approach, wherein fluorescence endoscopy and a fluorescently labeled lectin wheat germ agglutinin (WGA) were used to investigate the changes in the glycan expression on the epithelial cell surface, which is associated with the progression of BE toward adenocarcinoma.

Wang's group demonstrated the potential of topically administering a fluorescently labeled

Table 1. Summary of major molecular endoscopic imaging studies of gastrointestinal tract.

Study	Technique	Molecular probe	Model used	References
Esophagus				
Barrett's dysplasia	Fluorescence endoscopy	Wheat germ agglutinin (lectin)	*Ex vivo* (human biopsies	Bird-Lieberman and colleagues[42]
Barrett's neoplasia	Confocal laser endomicroscopy	ASYNYDA (peptide)	*In vivo*	Sturm and colleagues[43]
Barrett's neoplasia	Wide-field fluorescence endoscopy	ASYNYDA (peptide)	*In vivo*	Joshi and colleagues[44]
Barrett's esophagus	Wide-field near-infrared fluorescence molecular endoscopy High-definition wide-field endoscopy	Anti-VEGF-A antibody	*In vivo*	Nagengast and colleagues[45]
Barrett's esophageal adenocarcinoma	Confocal laser endomicroscopy	ASYNYDA (peptide)	*In vivo*	Dassie and colleagues[46]

(Continued)

Table 1. (Continued)

Study	Technique	Molecular probe	Model used	References
Barrett's neoplasia	White light endoscopy Narrow-band imaging Autofluorescence imaging	Wheat germ agglutinin (lectin)	*Ex vivo*	Neves and colleagues[47]
Barrett's dysplasia	Wide-field endoscopy (multispectral light scattering)	No external contrast agents used	Both *in vivo* and *ex vivo*	Qiu and colleagues[48]
Stomach				
Gastric intestinal metaplasia	Confocal laser endomicroscopy	No external contrast agents used	*In vivo*	Guo and colleagues[49]
Gastric cancer	Confocal laser endomicroscopy	Anti-MG7 antibody	*In vivo* (mouse xenograft)	Li and colleagues[50]
Gastric neoplasia and cancer	Fluorescence molecular tomography Fluorescence reflectance imaging	Cathepsin Matrix metalloproteinase (MMP)	Both *in vivo* and *ex vivo*	Ding and colleagues[51]
Gastric cancer	Confocal laser endomicroscopy	GEBP11 (peptide)	Both *in vivo* and *ex vivo*	Liu and colleagues[52]
Colon				
Colon polyp	Wide-field fluorescence endoscopy	GE-137 (peptide)	*In vivo*	Burggraaf and colleagues[53]
Colorectal adenoma	Wide-field fluorescence endoscopy	Bevacizumab (antibody)	Both *in vivo* and *ex vivo*	Hartmans and colleagues[54]
Sessile serrated adenomas	Wide-field fluorescence endoscopy	KCCFPAQ (peptide)	*In vivo*	Joshi and colleagues[55]
Colitis	Wide-field fluorescence endoscopy	gGlu-HMRG (enzyme)	*In vivo*	Mitsunaga and colleagues[56]
Colorectal neoplasia	Confocal laser endomicroscopy	Anti-EGFR antibody	*In vivo*	Liu and colleagues[57]
Crohn's disease	Confocal laser endomicroscopy	Anti-TNF antibody	*In vivo*	Atreya and colleagues[58]
Colorectal adenomatous polyps	White light endoscopy	*Ulex europaeus* agglutinin-1 functionalized mesoporous silica nanoparticles	Both *in vivo* and *ex vivo*	Chen and colleagues[59]
Ulcerative colitis	Confocal laser endomicroscopy	VRPMPLQ (peptide)	*Ex vivo*	De Palma and colleagues[60]
Colonic adenoma	Confocal laser endomicroscopy	VRPMPLQ (peptide)	*In vivo*	Hsiung and colleagues[12]
Colitis	Confocal laser endomicroscopy	Acriflavinium	*In vivo*	Neumann and colleagues[61]

EGFR, epidermal growth factor receptor; gGlu-HMRG, γ-glutamyl hydroxymethyl rhodamine green; MMP, matrix metalloproteinase; TNF, tumor necrosis factor.

synthetic peptide.[43,44,62] The FITC-labeled peptide ASYNYDA, which was identified by the phage display, was administered in 25 patients with BE for *in vivo* imaging using a confocal laser endomicroscope.[43] The CLE highlighted areas of HGD after and before EAC at depths of 50 μm exhibited specific peptide binding to neoplastic crypts, while the squamous epithelium did not exhibit peptide binding. In another study, the researchers used the same peptide to achieve the wide-field detection of dysplasia.[44] The results identified HGD and EAC with a sensitivity of 76% and specificity of 94%.

Nagengast and colleagues demonstrated the feasibility of concurrently using wide-field near-infrared fluorescence molecular endoscopy (NIR-FME) and WLE to detect dysplastic and early EAC lesions in patients with BE by applying a fluorescently labeled antibody (VEGF) both topically and systemically (Figure 2). The study was conducted in 20 patients. The NIR-FME topical administration of the tracer achieved an improvement of 33% over the WLE for dysplasia detection.

Many tumors present a multiplicity of cell surface and proteomic markers, and these molecular signatures are divergent across patients. Therefore, the simultaneous multi-fluorophore imaging of multiple molecular targets is needed to increase the sensitivity of cancer diagnosis and improve personalized treatment. The nanoparticles can be particularly useful for this purpose as they offer unique physicochemical properties.[63] Particularly, they have large surface area-to-volume ratio suitable for proper functionalization and strong fluorescent labeling for targeting minute amounts of various molecular markers. Therefore, they can enhance signals and potentially contribute to the detection of early-stage disease conditions. Several preclinical and clinical studies using nanoparticles have been carried out intended to diagnose gastrointestinal lesions.

In such an *in vivo* study, Dassie and colleagues[46] injected NPs intravenously in 17 rats in which gastroesophageal reflux was induced by surgical esophagogastric–jejunal anastomosis to develop an experimental model of Barrett's EAC. NPs were prepared from polysaccharides, grafted with ASYNYDA peptide which has the affinity for esophageal cancer cells, and then loaded with DCM (4-(dicyanomethylene)-2-methyl-6-(4-dimethylaminostyryl)-4H-pyran) as imaging agents. The confocal laser endomicroscope was used to collect fluorescence images. The fluorescence signal was detected in rats affected by esophageal cancer, whereas control non-operated rats ($n = 13$) with normal esophagus showed no binding of nanoparticles.

Recently, an *ex vivo* study[47] was conducted to demonstrate the feasibility of a topically applied NIR dye-labeled lectin for the endoscopic detection of early neoplasia in BE. Hence, 29 endoscopic mucosal resection (EMR) specimens from 17 patients were assessed. WGA conjugated with NIR fluorophore (IR800CW)[64] was introduced topically before performing visualization through NIR imaging. These studies confirmed the ability of nanoparticles to serve as a potential tool for cancer diagnosis.

Qiu and colleagues[48] developed a multispectral light scattering endoscopic imaging system to investigate entire esophageal lining and detect suspected subcellular dysplastic changes. The system was a combination of light scattering spectroscopy (LSS) with a collimated broadband light beam from a fiber optic probe and requires no external contrast agents. In the patient-based diagnosis study, 55 out of 57 cases were identified correctly demonstrating a sensitivity of 96% and a specificity of 97% of the technique. In addition, the accuracy (90%) was evaluated through biopsy-based diagnosis study where LSS data were compared with pathology reports of 411 biopsies from 24 patients.

Gastric cancer

Gastric cancer is the second major cause of death from cancer in the world and the most commonly occurring cancer in Asia specifically in Japan, Korea, and China.[65] There were over 1 million new cases in 2018 worldwide.[66] Despite therapeutic advances, the gastric cancer survival rate is relatively worse compared with other solid malignancies. Since the symptoms are not visible at the onset of the disease, the prognosis remains very poor and patients are often diagnosed in the later stages. Several preclinical and *ex vivo* human studies have illustrated the potential of MEI in the early detection of gastric cancer and treatment monitoring.

In a prospective study, Guo and colleagues[49] used CLE for the diagnosis and classification of gastric intestinal metaplasia (GIM). A total of 267 sites from 53 patients were evaluated and GIM was

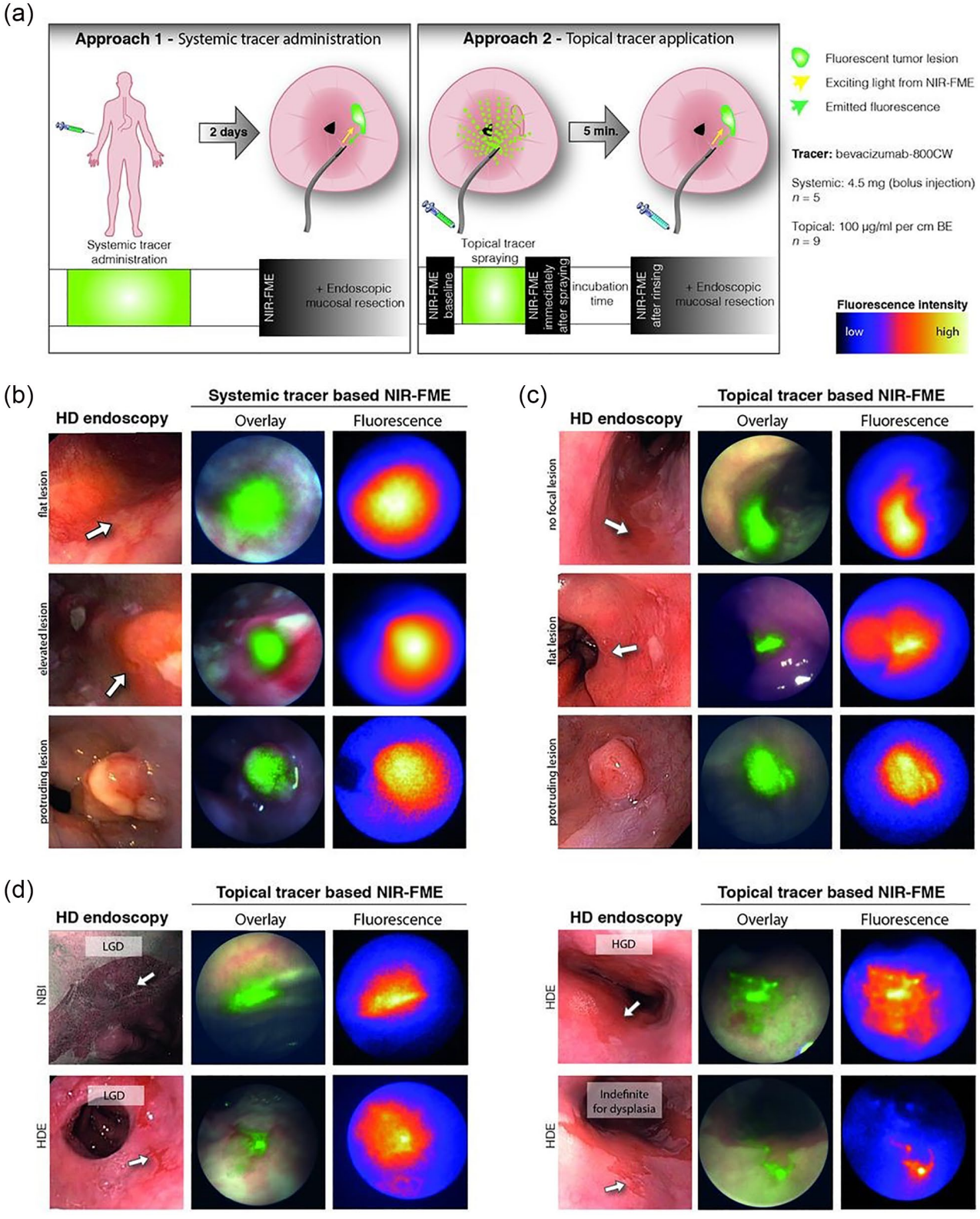

Figure 2. NIR-FME of esophageal adenocarcinoma and dysplasia through targeting VEGF. (a) Schematic overview of systemic and topical approaches; summary of results showing all lesions identified by NIR-FME inspection following (b) systemic and (c) topical tracer application; (d) dysplastic lesions missed by HD-WLE and NBI were visualized by NIR-FME.[45]
BE, Barrett's esophagus; HDE, high-definition endoscopy; HGD, high-grade dysplasia; LGD, low-grade dysplasia; NBI, narrow-band imaging; NIR-FME, near-infrared fluorescence molecular endoscopy; VEGF, vascular endothelial growth factor; WLE, white light endoscopy.

identified based on (a) the shape of the goblet cells, (b) the presence of absorptive cells or brush border, and (c) the architecture of vessels and crypts. The sensitivity and specificity of CLE for GIM were 98.13% and 95.33%, respectively, compared with conventional endoscopy with a sensitivity of 36.88% and specificity of 91.59%.

Li and colleagues[50] injected an Alexa Fluor 488-labeled antibody against MG7 (a tumor-associated antigen overexpressed in human gastric cancer) into a xenograft mouse model. After 48 h, *in vivo* imaging was performed with the FIVE1 confocal endomicroscopy instrument. The xenograft tumors revealed a specific cellular signal. The non-tumor tissue or mice injected with non-specific control antibodies did not exhibit a specific signal.

Ding and colleagues[51] conducted a preclinical study using murine models and demonstrated the feasibility of using activatable molecular probes and near-infrared fluorescence (NIRF) imaging for the detection of gastric neoplasia and cancer, both *in vivo* and *ex vivo*. In this study, two activatable molecular probes, namely, cathepsin and MMP, were injected 24 and 6 h, respectively, before quantitative tomographic NIRF imaging was performed. The study compared $Smad4^{+/-}$ mice with gastric neoplasia to wild-type controls. Molecular imaging *in vivo* revealed an intense activation for both the cathepsin B and MMP probes.

Liu and colleagues conducted a study to evaluate the feasibility of real-time molecular imaging for GEBP11 (a new nine amino acid vascular homing peptide, screened and identified using phage display technology) in gastric cancer using CLE (Figure 3). The investigation was performed on tumor-bearing mice models and surgical specimens of patients with gastric cancer. It was confirmed that GEBP11 could specifically bind to co-cultured human umbilical vein endothelial cells (co-HUVECs). The results revealed that the GEBP11 peptide can be used as a potential candidate for the molecular imaging of gastric cancer.

Colorectal polyps

Colorectal polyps are small growths of tissue containing clump of cells on the lining of the colon or large intestine and can vary in size and number. Recently, the specific categorization of polyps has drawn a substantial amount of interest. The detection of polyps relies on the type of polyps and the experience of the endoscopist. Because of their location in the proximal colon and covered with a mucus cap, detection of right-sided sessile serrated adenomas (SSAs)/polyps is more challenging.[67] Serrated polyps can be classified into hyperplastic or mixed polyps and were previously known to have little potential for malignancy. However, due to the advancements in the molecular understanding of colon cancer, several research studies provided evidence that some serrated polyps may act as the precursor lesions for the development of colorectal cancers (CRCs).[68]

The first in-human molecular imaging study with intravenous application of fluorescent agent was conducted by Burggraaf and colleagues.[53] Cy5-labeled GE-137 peptide was injected into 15 CRC patients. GE-137 specifically binds with c-Met, a human tyrosine kinase heavily expressed in CRC. After 3 h of incubation, wide-field NIR fluorescence imaging specifically identified all tubular adenoma overexpressing c-Met. The visualization included 38 grossly visible colon polyps which were already seen through white light as well as an additional nine small, flat lesions that were not visible with white light alone. No apparent toxicity was noticed in the study, therefore demonstrating that molecular imaging in detecting colorectal polyps is feasible and safe in humans.

In a proof-of-concept dose escalation study,[54] fluorescence molecular endoscopy (FME) was used to detect colorectal adenoma using a fluorescently labeled antibody bevacizumab-800CW, which is active against the VEGF-A, overexpressed in colorectal adenomas. In this study, 17 patients with familial adenomatous polyposis received an intravenous injection with different doses of antibody. After 3 days, NIR-FME detected even very small dysplastic adenomas ($<$3 mm). 25 mg of bevacizumab-800CW was identified as the best-performing tracer dose which was below the conventional therapeutic dose of 5–10 mg/kg and displayed no side effects. Spectroscopy analyses of fresh resected specimens and microscopy of formalin-fixed and paraffin-embedded (FFPE) tissues confirmed the findings.

Joshi and colleagues[55] developed a small fluorescently labeled peptide that binds specifically to SSAs using the phage display technique for the

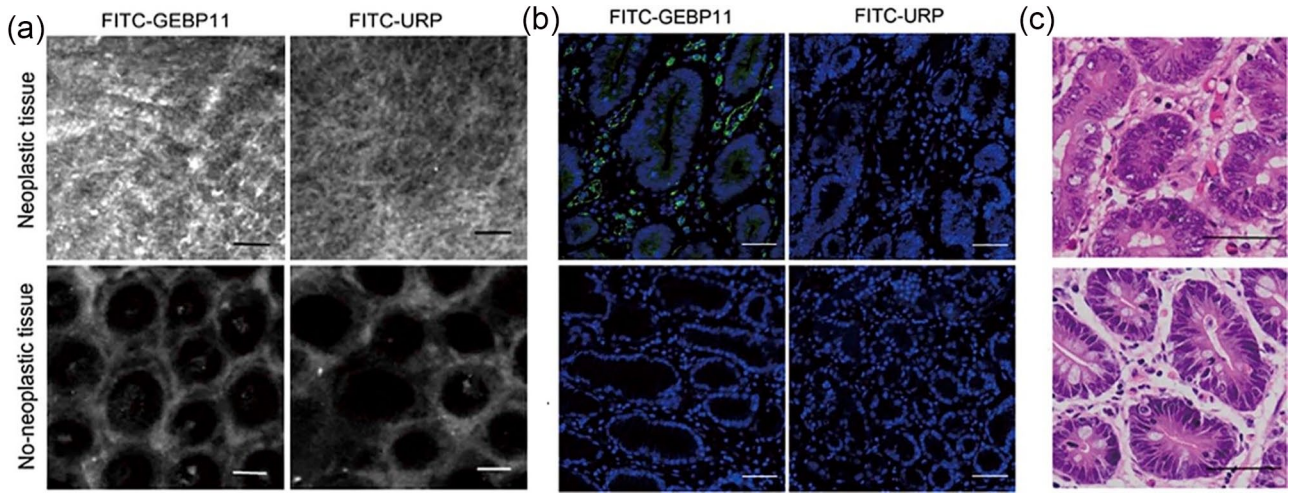

Figure 3. Real-time molecular imaging in gastric cancer. (a) CLE of neoplastic and non-neoplastic tissue specimens after incubating samples with FITC-conjugated GEBP11 (homing peptide) or URP; (b) confocal laser microscopy after nuclear counterstaining; (c) H&E staining of tumor and healthy tissue.[52]
CLE, confocal laser endomicroscopy; FITC, fluorescein isothiocyanate; H&E, hematoxylin and eosin; URP, unrelated peptide.

wide-field imaging of lesions in the proximal colon after topical administration (76.4 μmol/L) with subsequent *ex vivo* quantification. This study successfully distinguished the SSAs from the normal colonic mucosa with a sensitivity of 89% and specificity of 92%. In the *ex vivo* quantification, the peptide bound to the SSAs had a significantly higher mean fluorescence intensity than that bound to the hyperplastic polyps.

Inflammatory bowel disease and CRC

In a preclinical study, to enzymatically monitor colitis-associated colon cancer (CAC), activatable fluorescent probe γ-glutamyl hydroxymethyl rhodamine green (gGlu-HMRG) was topically administered to mice and incubated for 5 min to detect the γ-glutamyl transpeptidase, which is a cell surface enzyme that metabolizes glutathione and is associated with cancer.[56] Wide-field endoscopy was used to collect rhodamine green fluorescence from colonic neoplasia. Hence, 52 mice were tested and the results revealed that gGlu-HMRG can improve the endoscopic detection of CAC. Similarly, protease-activatable smart probes,[13,69] the MMP activatable probe,[70] or fluorescently labeled peptides[71,72] can be used to detect colonic neoplasms and dysplastic polyps.

In a prospective study,[57] first, the molecular imaging of EGFR in humans was conducted *in vivo*. After the topical application of the fluorescent-labeled molecular probe against EGFR for a total of 37 patients with neoplastic lesions in the colon or rectum, the CLE was used to determine the EGFR-specific fluorescence intensity. The study detected a specific fluorescence signal in 18 out of 19 and 12 out of 18 patients with CRC and colorectal adenoma, respectively. Conversely, the normal mucosa did not exhibit fluorescence. Thus, this study demonstrated that the application of CLE in combination with a fluorescent-labeled antibody could be used in molecular imaging to diagnose colorectal neoplasia.

In a similar study, *in vivo* molecular imaging was performed by Atreya and colleagues[58] for patients with Crohn's disease. The topical administration of the fluorescently labeled monoclonal antibody adalimumab against the membrane-bound tumor necrosis factor (mTNF) to 25 patients led to the detection of intestinal $mTNF^+$ immune cells during CLE imaging. Patients with high numbers of $mTNF^+$ immune cells exhibited higher response rates to adalimumab therapy compared with patients who had low numbers of $mTNF^+$ cells.

Chen and colleagues[59] used fluorescently labeled nanoparticles as targeted endoscopic contrast agents for the detection of premalignant colonic lesions. *Ex vivo*/*in vivo* and microscopic studies demonstrated that FITC-labeled mesoporous silica nanoparticles (MSNs) coated with lectin *Ulex europaeus* agglutinin-1 (UEA1) could be useful to detect polyps and early CRCs through

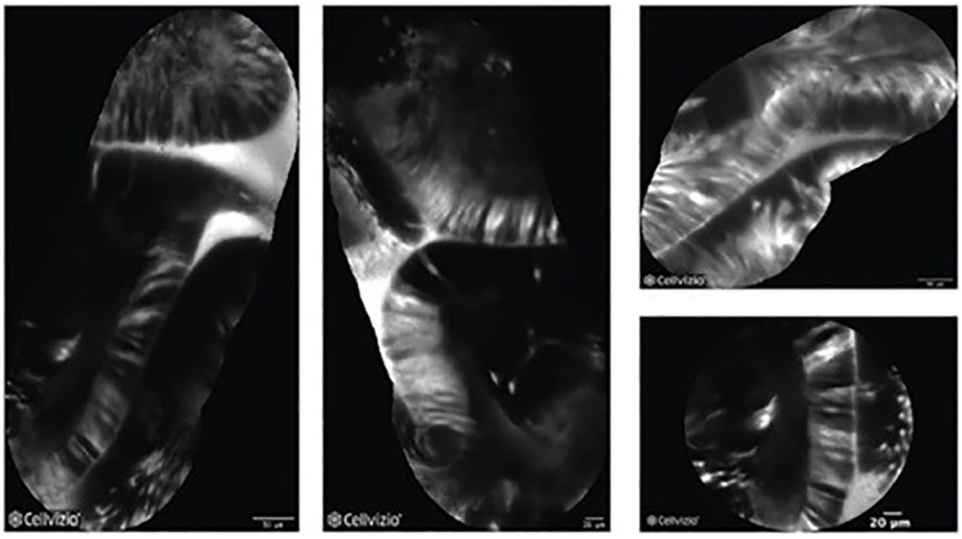

Figure 4. Dysplastic mucosa at CLE with heptapeptide (VRPMPLQ). VRPMPLQ/CLE images showing dysplastic colonocytes, obtained from different patients. The observed active binding of the peptide to the colonocytes determines a strong increase in fluorescence.[60]
CLE, confocal laser endomicroscopy.

targeting α-L-fucose, a glycosylation component often involved with tumorogenesis.

Recently, an *ex vivo* pilot study was conducted to assess the feasibility of combining a fluorescent-labeled molecular probe and CLE to detect dysplasia associated with ulcerative colitis (Figure 4). The heptapeptide VRPMPLQ with a predicted high binding affinity for dysplastic tissue was synthesized using phage display technology and labeled with fluorescein. Eleven lesions from nine patients were investigated by staining the specimens with a fluorescent-labeled peptide and visualized using confocal imaging.

Hsiung and colleagues[12] performed the first trial of topically administering oligopeptides in the colon. An M13 phage library was screened to identify the specific septapeptide VRPMPLQ conjugated with fluorescein and tested in 26 patients during colonoscopy. Imaging was performed using a fluorescence confocal microendoscope, demonstrating the preferential binding of peptides to dysplastic colonocytes relative to adjacent normal cells with a sensitivity of 81% and specificity of 78%. Therefore, the identification of dysplasia-targeting peptides and merging with CLE can contribute to the early detection of colon cancer and potentially other epithelial malignancies.

The potential application of CLE to the detection of gut microbiota has been reported by Neumann and colleagues[61] Infection with *Clostridium difficile* was tested in the colon of 80 patients, and single rod-shaped bacteria were visualized with the topical administration of acriflavinium, which indicates that the CLE can potentially be applied to the *in vivo* diagnosis of *Clostridium difficile* infection (CDI)-associated colitis. However, molecular probes specific to bacterial species, such as antibodies and peptides, are required to further advance this field.

Small bowel

The **small intestine** within the GIT constitutes 75% of the total length and 90% of the surface area. It is approximately 6.5 m in an adult, which is much longer than conventional video gastroscopes/colonoscopes. Therefore, the small intestine has been difficult to visualize and examine by traditional endoscopic techniques. Thus, diagnosis of small intestine diseases has always been a challenge for clinicians. Over the past few years, several new endoscopic and radiologic modalities were developed or improved for the investigation of small bowel diseases, for example, capsule endoscopy, deep enteroscopy, computerized tomography, magnetic resonance enterography, and ultrasonography.[73–76] While the techniques have made major improvements in detecting abnormalities in the small bowel, we will not discuss these in detail in this manuscript since it is beyond the scope of this review.

The future is bright

Molecular imaging has revolutionized gastrointestinal endoscopy and clinical research by allowing the identification of both structural and molecular changes in tissues. It can potentially enrich the diagnostic data obtained during the endoscopic procedures. Despite several promising translational and early-stage clinical studies, MI has not yet found its place in clinical routine for the detection of dysplasia or in the decision-making process for the development of treatment strategies. Because molecular imaging is still in the development phase and further improvements are needed, mentioned below are some of the challenges need to be addressed.

Imaging agents

One major challenge is to obtain new specific imaging agents, whose development is costly and requires a multidisciplinary team of chemists, biologists, pharmacists, and clinicians.[77] The development of such agents require time-consuming and complicated experiments on labeling methods, binding, formulation, stability, toxicity, image interpretation.[78] Furthermore, issues related to the pharmacokinetics, half-life, and quality control, for example, are hindering efforts toward improving the production and development of suitable probes. The use of microfluidic technology for synthesizing imaging probes can be advantageous in enhancing their yields, quality, and availability.

New marker discovery

The prognosis and survival rate of GIT cancers are largely related to the stage of the disease. Ideally, lesions should be detected at an early stage, before they become malignant. However, the identification remains difficult for tiny lesions and even impossible for functional disorders. Therefore, researchers should put more effort into discovering new markers heavily expressed during diseased conditions.

Multiple capacity and sensitivity

Another major limitation of MEI is its inability to simultaneously monitor multiple physiologies or molecular targets. To overcome this problem, nanoparticles can be employed to visualize multiple targets or signaling pathways by coupling several specific ligands into a single particle, while the sensitivity can be increased by coupling a strong fluorescent molecule with a single particle.

Overreliance on preclinical experiments

Although preclinical studies using small animals are unavoidable in molecular imaging research, the unbiased correlation of imaging results from the preclinical to the clinical environment is not straightforward due to larger difference in size, general physiology, lifespan, and so on. Therefore, the predictive value of animal trials is expected to increase as much as possible so as to ensure the extraction of high-quality information that is directly relevant to human diseases. The anatomy and physiology between a large animal like porcine and human beings are notably similar. This suggests the justification of using large animal models for interpretation of disease progression in human patients.[79] However, regulatory requirements and safety concerns should be strictly monitored.

Efficiency of endoscopists and devices

Gastrointestinal endoscopy is a field that requires physicians with manifold clinical skills, such as the active manipulation of endoscopic devices, visual identification of tissue morphology, and classification of diseases. Improvements in the practice of endoscopy also depend on the detection efficacy of endoscopists with diverse levels of experience. It is believed that artificial intelligence (AI) can potentially improve a physician's ability to perform medical tasks.[78,80–82] However, it must first overcome the skepticism of medical professionals and patients. Although some progress has been made, further development of endoscopic instruments is also required to integrate molecular imaging into clinical diagnostic settings. Therefore, the associated instruments and techniques should be standardized and their safety and reproducibility should be demonstrated.

Once the existing challenges are properly addressed, it is widely expected that molecular imaging will dramatically advance in a number of areas and significantly improve the time lines and veracity of detecting the presence and extent of certain diseases, when used for diagnostic purposes. Moreover, molecular imaging therapy utilizing target-specific agents to treat cancer offers an attractive and possibly well-tolerated new alternative. They offer the chance to replace chemotherapy and radiotherapy which often

cause side effects, as a result from damage to the healthy cells and tissues near the treatment area. Target-specific therapy based on molecular imaging offers a highly selective approach in the future.

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References

1. Moore J and Maitland D. Endoscopy. In: Moore JE Jr and Maitland DJ (eds) *Biomedical technology and devices handbook*. 2nd ed. New York: Taylor & Francis, 2013, pp. 217–45.
2. Elahi SF and Wang TD. Future and advances in endoscopy. *J Biophotonics* 2011; 4: 471–481.
3. Garcia-Figueiras R, Baleato-Gonzalez S, Padhani AR, *et al*. Advanced imaging of colorectal cancer: from anatomy to molecular imaging. *Insight Imag* 2016; 7: 285–309.
4. McQueen AS and Scott J. CT staging of colorectal cancer: what do you find in the chest? *Clin Radiol* 2012; 67: 352–358.
5. Lim G-H, Koh Cheong WK, Wong KS, *et al*. Natural history of small, 'indeterminate' hepatic lesions in patients with colorectal cancer. *Dis Colon Rectum* 2009; 52: 1487–1491.
6. Weissleder R and Mahmood U. Molecular imaging. *Radiology* 2001; 219: 316–333.
7. Tajiri H. Advances in endoscopic imaging and diagnosis: toward molecular imaging. In: Niwa H, Tajiri H, Nakajima M, *et al*. (eds) *New Challenges in Gastrointestinal Endoscopy*. Tokyo, Japan: Springer, 2008, pp. 29–39.
8. Ahmed S, Strand S, Weinmann-Menke J, *et al*. Molecular endoscopic imaging in cancer. *Digest Endosc* 2018; 30: 719–729.
9. Rollo FD. Molecular imaging: an overview and clinical applications. *Radiol Manage* 2003; 25: 28–32; quiz 33–35.
10. Sivak MV. Gastrointestinal endoscopy: past and future. *Gut* 2006; 55: 1061–1064.
11. Keller R, Winde G, Terpe HJ, *et al*. Fluorescence endoscopy using a fluorescein-labeled monoclonal antibody against carcinoembryonic antigen in patients with colorectal carcinoma and adenoma. *Endoscopy* 2002; 34: 801–807.
12. Hsiung P-L, Hardy J, Friedland S, *et al*. Detection of colonic dysplasia in vivo using a targeted heptapeptide and confocal microendoscopy. *Nat Med* 2008; 14: 454–458.
13. Marten K, Bremer C, Khazaie K, *et al*. Detection of dysplastic intestinal adenomas using enzyme-sensing molecular beacons in mice. *Gastroenterology* 2002; 122: 406–414.
14. Ito S, Muguruma N, Kusaka Y, *et al*. Detection of human gastric cancer in resected specimens using a novel infrared fluorescent anti-human carcinoembryonic antigen antibody with an infrared fluorescence endoscope in vitro. *Endoscopy* 2001; 33: 849–853.
15. Hoetker MS, Kiesslich R, Diken M, *et al*. Molecular in vivo imaging of gastric cancer in a human-murine xenograft model: targeting epidermal growth factor receptor. *Gastrointest Endosc* 2012; 76: 612–620.
16. Foersch S, Kiesslich R, Waldner MJ, *et al*. Molecular imaging of VEGF in gastrointestinal cancer in vivo using confocal laser endomicroscopy. *Gut* 2010; 59: 1046–1055.
17. Kang HW, Torres D, Wald L, *et al*. Targeted imaging of human endothelial-specific marker in a model of adoptive cell transfer. *Lab Invest* 2006; 86: 599–609.
18. Goetz M, Ziebart A, Foersch S, *et al*. In vivo molecular imaging of colorectal cancer with confocal endomicroscopy by targeting epidermal growth factor receptor. *Gastroenterology* 2010; 138: 435–446.
19. Qian ZR, Li T, Ter-Minassian M, *et al*. Association between somatostatin receptor expression and clinical outcomes in neuroendocrine tumors. *Pancreas* 2016; 45: 1386–1393.
20. Tung CH, Mahmood U, Bredow S, *et al*. In vivo imaging of proteolytic enzyme activity using a novel molecular reporter. *Cancer Res* 2000; 60: 4953–4958.
21. Bremer C, Bredow S, Mahmood U, *et al*. Optical imaging of matrix metalloproteinase-2 activity in tumors: feasibility study in a mouse model. *Radiology* 2001; 221: 523–529.

22. Li M and Wang TD. Targeted endoscopic imaging. *Gastrointest Endosc Clin N Am* 2009; 19: 283–298.
23. Wallace MB, Meining A, Canto MI, *et al*. The safety of intravenous fluorescein for confocal laser endomicroscopy in the gastrointestinal tract. *Aliment Pharmacol Ther* 2010; 31: 548–552.
24. Vakoc BJ, Lanning RM, Tyrrell JA, *et al*. Three-dimensional microscopy of the tumor microenvironment in vivo using optical frequency domain imaging. *Nat Med* 2009; 15: 1219–1223.
25. Mordon S, Devoisselle JM, Soulie-Begu S, *et al*. Indocyanine green: physicochemical factors affecting its fluorescence in vivo. *Microvasc Res* 1998; 55: 146–152.
26. Ogawa M, Kosaka N, Choyke PL, *et al*. In vivo molecular imaging of cancer with a quenching near-infrared fluorescent probe using conjugates of monoclonal antibodies and indocyanine green. *Cancer Res* 2009; 69: 1268–1272.
27. Curvers WL, van Vilsteren FG, Baak LC, *et al*. Endoscopic trimodal imaging versus standard video endoscopy for detection of early Barrett's neoplasia: a multicenter, randomized, crossover study in general practice. *Gastrointest Endosc* 2011; 73: 195–203.
28. Yalamarthi S, Witherspoon P, McCole D, *et al*. Missed diagnoses in patients with upper gastrointestinal cancers. *Endoscopy* 2004; 36: 874–879.
29. Rex DK, Cutler CS, Lemmel GT, *et al*. Colonoscopic miss rates of adenomas determined by back-to-back colonoscopies. *Gastroenterology* 1997; 112: 24–28.
30. Van Rijn JC, Reitsma JB, Stoker J, *et al*. Polyp miss rate determined by tandem colonoscopy: a systematic review. *Am J Gastroenterol* 2006; 101: 343–350.
31. Wong Kee Song LM, Adler DG, Chand B, *et al*. Chromoendoscopy. *Gastrointest Endosc* 2007; 66: 639–649.
32. Buchner AM. The role of chromoendoscopy in evaluating colorectal dysplasia. *Gastroenterol Hepatol (N Y)* 2017; 13: 336–347.
33. Chauhan SS, Abu Dayyeh BK, Bhat YM, *et al*. Confocal laser endomicroscopy. *Gastrointest Endosc* 2014; 80: 928–938.
34. Goetz M, Malek NP and Kiesslich R. Microscopic imaging in endoscopy: endomicroscopy and endocytoscopy. *Nat Rev Gastroenterol Hepatol* 2013; 11: 11–18.
35. Wallace MB and Fockens P. Probe-based confocal laser endomicroscopy. *Gastroenterology* 2009; 136: 1509–1513.
36. Neumann H, Kiesslich R, Wallace MB, *et al*. Confocal laser endomicroscopy: technical advances and clinical applications. *Gastroenterology* 2010; 139: 388–392, 392.e1–e2.
37. Neumann H, Vieth M, Raithel M, *et al*. Confocal laser endomicroscopy for the in vivo detection of intraepithelial neoplasia in Peutz-Jeghers polyps. *Endoscopy* 2010; 42: E139–E140.
38. De Palma GD, Wallace MB and Giovannini M. Confocal laser endomicroscopy. *Gastroenterol Res Pract* 2012; 2012: 216209.
39. Wang KL, Wu T-T, Choi IS, *et al*. Expression of epidermal growth factor receptor in esophageal and esophagogastric junction adenocarcinomas association with poor outcome. *Cancer* 2007; 109: 658–667.
40. Spechler SJ. Barrett's esophagus and esophageal adenocarcinoma: pathogenesis, diagnosis, and therapy. *Med Clin North Am* 2002; 86: 1423–1445, vii.
41. Shaheen N and Ransohoff DF. Gastroesophageal reflux, Barrett esophagus, and esophageal cancer: scientific review. *JAMA* 2002; 287: 1972–1981.
42. Bird-Lieberman EL, Neves AA, Lao-Sirieix P, *et al*. Molecular imaging using fluorescent lectins permits rapid endoscopic identification of dysplasia in Barrett's esophagus. *Nat Med* 2012; 18: 315–321.
43. Sturm MB, Joshi BP, Lu S, *et al*. Targeted imaging of esophageal neoplasia with a fluorescently labeled peptide: first in-human results. *Sci Transl Med* 2013; 5: 184ra61.
44. Joshi BP, Duan X, Kwon RS, *et al*. Multimodal endoscope can quantify wide-field fluorescence detection of Barrett's neoplasia. *Endoscopy* 2016; 48: A1–A13.
45. Nagengast WB, Hartmans E, Garcia-Allende PB, *et al*. Near-infrared fluorescence molecular endoscopy detects dysplastic oesophageal lesions using topical and systemic tracer of vascular endothelial growth factor A. *Gut* 2019; 68: 7–10.
46. Dassie E, Arcidiacono D, Wasiak I, *et al*. Detection of fluorescent organic nanoparticles by confocal laser endomicroscopy in a rat model of Barrett's esophageal adenocarcinoma. *Int J Nanomed* 2015; 10: 6811–6823.
47. Neves AA, Di Pietro M, O'Donovan M, *et al*. Detection of early neoplasia in Barrett's

esophagus using lectin-based near-infrared imaging: an ex vivo study on human tissue. *Endoscopy* 2018; 50: 618–625.

48. Qiu L, Chuttani R, Pleskow DK, *et al*. Multispectral light scattering endoscopic imaging of esophageal precancer. *Light Sci Appl* 2018; 7: 17174.

49. Guo Y-T, Li Y-Q, Yu T, *et al*. Diagnosis of gastric intestinal metaplasia with confocal laser endomicroscopy in vivo: a prospective study. *Endoscopy* 2008; 40: 547–553.

50. Li Z, Zuo XL, Li CQ, *et al*. In vivo molecular imaging of gastric cancer by targeting MG7 antigen with confocal laser endomicroscopy. *Endoscopy* 2013; 45: 79–85.

51. Ding S, Blue RE, Chen Y, *et al*. Molecular imaging of gastric neoplasia with near infrared fluorescent (NIRF) activatable probes. *Mol Imaging* 2012; 11: 507–515.

52. Liu L, Yin J, Liu C, *et al*. In vivo molecular imaging of gastric cancer in human-murine xenograft models with confocal laser endomicroscopy using a tumor vascular homing peptide. *Cancer Lett* 356: 891–898.

53. Burggraaf J, Kamerling IMC, Gordon PB, *et al*. Detection of colorectal polyps in humans using an intravenously administered fluorescent peptide targeted against c-Met. *Nat Med* 2015; 21: 955–961.

54. Hartmans E, Tjalma JJJ, Linssen MD, *et al*. Potential red-flag identification of colorectal adenomas with wide-field fluorescence molecular endoscopy. *Theranostics* 2018; 8: 1458–1467.

55. Joshi BP, Dai Z, Gao Z, *et al*. Detection of sessile serrated adenomas in the proximal colon using wide-field fluorescence endoscopy. *Gastroenterology* 2017; 152: 1002.e9–1013.e9.

56. Mitsunaga M, Kosaka N, Choyke PL, *et al*. Fluorescence endoscopic detection of murine colitis-associated colon cancer by topically applied enzymatically rapid-activatable probe. *Gut* 2013; 62: 1179–1186.

57. Liu J, Zuo X, Li C, *et al*. In vivo molecular imaging of epidermal growth factor receptor in patients with colorectal neoplasia using confocal laser endomicroscopy. *Cancer Lett* 2013; 330: 200–207.

58. Atreya R, Neumann H, Neufert C, *et al*. In vivo imaging using fluorescent antibodies to tumor necrosis factor predicts therapeutic response in Crohn's disease. *Nat Med* 2014; 20: 313–318.

59. Chen N-T, Souris JS, Cheng S-H, *et al*. Lectin-functionalized mesoporous silica nanoparticles for endoscopic detection of premalignant colonic lesions. *Nanomedicine* 2017; 13: 1941–1952.

60. De Palma GD, Colavita I, Zambrano G, *et al*. Detection of colonic dysplasia in patients with ulcerative colitis using a targeted fluorescent peptide and confocal laser endomicroscopy: a pilot study. *PLoS ONE* 2017; 12: e0180509.

61. Neumann H, Gunther C, Vieth M, *et al*. Confocal laser endomicroscopy for in vivo diagnosis of Clostridium difficile associated colitis—a pilot study. *PLoS ONE* 2013; 8: e58753.

62. Sturm MB, Piraka C, Elmunzer BJ, *et al*. In vivo molecular imaging of Barrett's esophagus with confocal laser endomicroscopy. *Gastroenterology* 2013; 145: 56–58.

63. Prasad M, Lambe UP, Brar B, *et al*. Nanotherapeutics: an insight into healthcare and multi-dimensional applications in medical sector of the modern world. *Biomed Pharmacother* 2018; 97: 1521–1537.

64. ClinicalTrials.gov Bethesda (Maryland): National Library of Medicine (US). 200 Feb 29. Identifier CT02129933, VEGF-targeted [Internet] fluorescence near-infrared (NIR) endoscopy in (pre) malignant esophageal lesions (VICE) 24 April 2014, https://clinicaltrials.gov/ct2/show/NCT02129933

65. Leung WK, Wu MS, Kakugawa Y, *et al*. Screening for gastric cancer in Asia: current evidence and practice. *Lancet Oncol* 2008; 9: 279–287.

66. Bray F, Ferlay J, Soerjomataram I, *et al*. Global Cancer Statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 68: 394–424, http://gco.iarc.fr/

67. Huang CS, Farraye FA, Yang S, *et al*. The clinical significance of serrated polyps. *Am J Gastroenterol* 2011; 106: 229–240.

68. Michalopoulos G and Tzathas C. Serrated polyps of right colon: guilty or innocent? *Ann Gastroenterol* 2013; 26: 212–219.

69. Alencar H, Funovics MA, Figueiredo J, *et al*. Colonic adenocarcinomas: near-infrared microcatheter imaging of smart probes for early detection–study in mice. *Radiology* 2007; 244: 232–238.

70. Yoon SM, Myung SJ, Kim IW, *et al*. Application of near-infrared fluorescence imaging using

a polymeric nanoparticle-based probe for the diagnosis and therapeutic monitoring of colon cancer. *Dig Dis Sci* 2011; 56: 3005–3013.

71. Joshi BP, Liu Z, Elahi SF, *et al*. Near-infrared-labeled peptide multimer functions as phage mimic for high affinity, specific targeting of colonic adenomas in vivo (with videos). *Gastrointest Endosc* 2012; 76: 1197.e1–e5–206. e1–e5.
72. Liu Z, Miller SJ, Joshi BP, *et al*. In vivo targeting of colonic dysplasia on fluorescence endoscopy with near-infrared octapeptide. *Gut* 2013; 62: 395–403.
73. Gauci J, Sammut L, Sciberras M, *et al*. Small bowel imaging in Crohn's disease patients. *Ann Gastroenterol* 2018; 31: 395–405.
74. Aktas H and Mensink PB. Small bowel diagnostics: current place of small bowel Endoscopy. *Best Pract Res Clin Gastroenterol* 2012; 26: 209–220.
75. Eliakim R. Video capsule endoscopy of the small bowel. *Curr Opin Gastroenterol* 2008; 24: 159–163.
76. Eliakim R. Video capsule endoscopy of the small bowel. *Curr Opin Gastroenterol* 2013; 29: 133–139.
77. De Vries EG, Oude Munnink TH, van Vugt MA, *et al*. Toward molecular imaging-driven drug development in oncology. *Cancer Discov* 2011; 1: 25–28.
78. Van der Sommen F, Curvers WL and Nagengast WB. Novel developments in endoscopic mucosal imaging. *Gastroenterology* 2018; 154: 1876–1886.
79. Swindle MM, Makin A, Herron AJ, *et al*. Swine as models in biomedical research and toxicology testing. *Vet Pathol* 2012; 49: 344–356.
80. Mori Y, Kudo S-E and Mohmed HEN, *et al*. Artificial intelligence and upper gastrointestinal endoscopy: current status and future perspective. *Dig Endosc* 2018; 31: 378–388.
81. Mori Y, Kudo S, Berzin TM, *et al*. Computer-aided diagnosis for colonoscopy. *Endoscopy* 2017; 49: 813–819.
82. Alagappan M, Brown JRG, Mori Y, *et al*. Artificial intelligence in gastrointestinal endoscopy: the future is almost here. *World J Gastrointest Endosc* 2018; 10: 239–249.