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

Received: 30 April 2019; revised manuscript accepted: 10 July 2019.

Introduction
Endoscopy is widely used to directly visualize the large epithelial surfaces in hollow organs, such as the esophagus, stomach, and duodenum. 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. 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. 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\cite{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.\cite{8}

The molecular imaging system can be elegantly assigned two roles in clinical applications.\cite{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.\cite{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,\cite{11,12,13} the colorectal carcinoma and dysplasia in Barrett’s esophagus (BE) are more frequent, whereas in Japan,\cite{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,\cite{14-18} whereas the somatostatin receptors (SSTRs) are overexpressed in neuroendocrine tumors.\cite{19} Markers can also consist of proteolytic enzymes such as cathepsin\cite{20} and matrix metalloproteinases (MMPs).\cite{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.\cite{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.\cite{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.\cite{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. 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. 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 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. However, it is recognized that WLE can miss various early lesions.

To surpass those limitations, chromoendoscopic technique can be used. 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.

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.
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 colleagues42 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

| Study | Technique | Molecular probe | Model used | References |
|-------|-----------|----------------|------------|------------|
| Esophagus | | | | |
| Barrett’s dysplasia | Fluorescence endoscopy | Wheat germ agglutinin (lectin) | Ex vivo (human biopsies) | Bird-Lieberman and colleagues42 |
| Barrett’s neoplasia | Confocal laser endomicroscopy | ASNYDA (peptide) | In vivo | Sturm and colleagues43 |
| Barrett’s neoplasia | Wide-field fluorescence endoscopy | ASNYDA (peptide) | In vivo | Joshi and colleagues44 |
| Barrett’s esophagus | Wide-field near-infrared fluorescence molecular endoscopy | Anti-VEGF-A antibody | In vivo | Nagengast and colleagues45 |
| Barrett’s esophageal adenocarcinoma | Confocal laser endomicroscopy | ASNYDA (peptide) | In vivo | Dassie and colleagues46 |

(Continued)
| Study                        | Technique                                      | Molecular probe                     | Model used                        | References                        |
|-----------------------------|-----------------------------------------------|-------------------------------------|-----------------------------------|-----------------------------------|
| Barrett’s neoplasia         | White light endoscopy                         | Wheat germ agglutinin (lectin)      | Ex vivo                           | Neves and colleagues⁴⁷           |
|                             | Narrow-band imaging                           |                                     |                                   |                                   |
|                             | Autofluorescence imaging                      |                                     |                                   |                                   |
| Barrett’s dysplasia         | Wide-field endoscopy (multispectral light scattering) | No external contrast agents used   | Both in vivo and ex vivo          | Qiu and colleagues⁴⁸             |
| Stomach                     |                                               |                                     |                                   |                                   |
| Gastric intestinal metaplasia | Confocal laser endomicroscopy                  | No external contrast agents used    | In vivo                           | Guo and colleagues⁴⁹             |
| Gastric cancer              | Confocal laser endomicroscopy                  | Anti-MG7 antibody                   | In vivo (mouse xenograft)         | Li and colleagues⁵⁰              |
| Gastric neoplasia and cancer| Fluorescence molecular tomography             | Cathepsin Matrix metalloproteinase (MMP) | Both in vivo and ex vivo          | Ding and colleagues⁵¹             |
|                             | Fluorescence reflectance imaging              |                                     |                                   |                                   |
| Gastric cancer              | Confocal laser endomicroscopy                  | GEBP11 (peptide)                    | Both in vivo and ex vivo          | Liu and colleagues⁵²             |
| Colon                       |                                               |                                     |                                   |                                   |
| Colon polyp                 | Wide-field fluorescence endoscopy              | GE-137 (peptide)                    | In vivo                           | Burggraaf and colleagues⁵³       |
| Colorectal adenoma          | Wide-field fluorescence endoscopy              | Bevacizumab (antibody)              | Both in vivo and ex vivo          | Hartmans and colleagues⁵⁴       |
| Sessile serrated adenomas   | Wide-field fluorescence endoscopy              | KCCFPAQ (peptide)                   | In vivo                           | Joshi and colleagues⁵⁵           |
| Colitis                     | Wide-field fluorescence endoscopy              | gGlu-HMRG (enzyme)                  | In vivo                           | Mitsunaga and colleagues⁵⁶       |
| Colorectal neoplasia        | Confocal laser endomicroscopy                  | Anti-EGFR antibody                  | In vivo                           | Liu and colleagues⁵⁷             |
| Crohn’s disease             | Confocal laser endomicroscopy                  | Anti-TNF antibody                   | In vivo                           | Atreya and colleagues⁵⁸         |
| Colorectal adenomatous polyps | White light endoscopy                         | Ulex europaeus agglutinin-1 functionalized mesoporous silica nanoparticles | Both in vivo and ex vivo          | Chen and colleagues⁵⁹           |
| Ulcerative colitis          | Confocal laser endomicroscopy                  | VRPMPLQ (peptide)                   | Ex vivo                           | De Palma and colleagues⁶⁰       |
| Colonic adenoma             | Confocal laser endomicroscopy                  | VRPMPLQ (peptide)                   | In vivo                           | Hsiung and colleagues¹²          |
| Colitis                     | Confocal laser endomicroscopy                  | Acriflavinium                       | In vivo                           | Neumann and colleagues¹¹         |

EGFR, epidermal growth factor receptor; gGlu-HMRG, γ-glutamyl hydroxymethyl rhodamine green; MMP, matrix metalloproteinase; TNF, tumor necrosis factor.
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. 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. 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. 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 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 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) 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 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. There were over 1 million new cases in 2018 worldwide. 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 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. 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 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 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. 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).

The first in-human molecular imaging study with intravenous application of fluorescent agent was conducted by Burggraaf and colleagues. 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, 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 developed a small fluorescently labeled peptide that binds specifically to SSAs using the phage display technique for the
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 (γGlu-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. Wide-field endoscopy was used to collect rhodamine green fluorescence from colonic neoplasia. Hence, 52 mice were tested and the results revealed that γGlu-HMRG can improve the endoscopic detection of CAC. Similarly, protease-activatable smart probes, the MMP activatable probe, or fluorescently labeled peptides can be used to detect colonic neoplasms and dysplastic polyps.

In a prospective study, 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 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 used fluorescently labeled nanoparticles as targeted endoscopic contrast agents for the detection of premalignant colonic lesions. Ex 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...

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**Figure 3.** Real-time molecular imaging in gastric cancer. (a) CLE of neoplastic and non-neoplastic tissue specimens after incubating samples with FITC-conjugated GEPB11 (homing peptide) or URP; (b) confocal laser microscopy after nuclear counterstaining; (c) H&E staining of tumor and healthy tissue. CLE, confocal laser endomicroscopy; FITC, fluorescein isothiocyanate; H&E, hematoxylin and eosin; URP, unrelated peptide.
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 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. 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. 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. The development of such agents require time-consuming and complicated experiments on labeling methods, binding, formulation, stability, toxicity, image interpretation. 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. 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. 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.

**Funding**
The authors received no financial support for the research, authorship, and/or publication of this article.

**Conflict of interest statement**
The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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