Molecular Imaging

The New Frontier for Endoscopic Diagnosis and Personalization in Inflammatory Bowel Disease

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KEYWORDS

• Molecular imaging • Molecular endoscopy • IBD • Fluorescence
• Prediction of response • Fluorescent antibody • Target cell • Mucosa

KEY POINTS

- Molecular endoscopy is ideally based on in vivo visualization of disease-specific perturbations at the molecular level.
- The major challenge for clinical molecular endoscopy procedures is regulatory approval, as fluorescent probes are regarded as new investigational drugs by the authorities and therefore require extensive preclinical efficacy and safety data, as well as fulfillment of "good manufacturing practices"-compliant production requirements.
- Molecular endoscopy has made the transfer into clinical studies in inflammatory bowel disease (IBD) and evidence for the feasibility of this approach is continuously growing. These studies have already improved our understanding of mucosal drug distribution, enhanced our understanding of the mechanism of action of anti-inflammatory therapies, and enabled prediction of individual therapeutic response to targeted therapies. Further larger patient studies are warranted to conform validity of these studies.

INTRODUCTION

Inflammatory bowel diseases (IBDs) encompass immune-mediated disorders of the gastrointestinal tract whose main phenotypic entities comprise Crohn's disease and ulcerative colitis.[1,2] These chronic disorders are characterized by a remitting and exacerbating disease course, resulting in lifelong morbidity. Apart from debilitating clinical symptoms, such as diarrhea, rectal bleeding, abdominal pain, urgency, nocturnal

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bowel movements, and fatigue, both IBD subtypes are associated with progressive bowel damage and increased incidence of colitis-associated neoplasia.[3–6] Optimized care of patients with IBD is therefore dependent on adequate monitoring of disease activity, definition of therapeutic targets, and effective anti-inflammatory therapy to optimally avoid long-term bowel damage and modify the disease course.[6,7]

The advent of targeted therapies that are based on increasing understanding of disease immunopathogenesis and aim to selectively inhibit crucial mediators of the inflammatory process has led to major improvements in therapeutic outcomes.[8] These therapies currently encompass the substance classes of anti-tumor necrosis factor (TNF) antibodies, anti-integrin antibodies, anti-interleukin (IL)-12/23 or anti-IL-23p19 antibodies, Janus kinase inhibitors, as well as sphingosine 1-phosphate receptor modulators.[1,9] However, response to therapy is highly heterogenous among patients, reflecting the extraordinarily complex pathogenic nature of IBD.[10] Depending on the chosen endpoint, overall clinical remission rates are at best 50% to the commenced targeted therapy and 30% to 50% of initial responders are prone to lose response in the course of treatment.[11,12] There is currently a lack of available biomarkers that would prognosticate therapeutic effectiveness of these advanced therapies. These biomarkers, which could potentially reflect ongoing biologic process, could then be applied in clinical practice to assist us in assigning therapies to patients with the highest probability of response.[13] Nevertheless, these therapies enabled continuous evolvement of achievable treatment goals that are not merely limited to symptom control alone, but rather to restoration of quality of life, biochemical remission, and ultimately also endoscopic remission. Furthermore, achievement of novel endpoints that could potentially also include histologic remission, transmural healing, and endoscopic barrier healing may further modify the disease course of the patients.[8,9,14] The rationale behind this evolution of treatment goals is based on available evidence that indicates that these are indeed associated with better long-term patient outcome, as defined by reduced risk of relapse, steroid-free remission, decreased hospitalization rates, resection free intervals, less occurrence of disease-associated complications, and lowered risk of neoplastic lesions.[15,16]

Adequate imaging of the mucosa has therefore gained increasing importance for the evaluation of disease activity and the rational management of patients with IBD. High-definition video endoscopy is currently accepted as the gold standard for the detection and characterization of mucosal inflammation in patients with IBD, complemented by dye-based and virtual chromoendoscopy in the long-term follow-up during surveillance. Further advances in endoscopic imaging techniques have additionally enhanced our ability to visualize mucosal inflammation and structural alterations during endoscopic procedures.[17] Despite these advancements, applied imaging modalities are limited to detecting morphologic features alone, subsequently confirmed by histologic analysis of obtained mucosal biopsies.[18] They are not able to allow detailed analysis of molecular processes that drive mucosal inflammation in IBD, thereby limiting insights into disease pathology and characterization of disease-specific perturbations. The visualization of molecular targets has been the subject of an increasing number of preclinical and clinical studies in IBD to address a variety of clinically relevant problems. Here, molecular endoscopy has been at the forefront of recent developments. It is therefore based on the application of labeled probes directed toward a defined molecular mucosal target to allow visualization with dedicated light sources at a cellular level.[17,18]

In this narrative review, the authors give an overview of current findings of molecular endoscopy approaches in IBD. They also illustrate how so far performed studies have led to a better understanding of mucosal drug distribution in the intestinal tissue,

enhanced understanding of the mode of action of targeted therapies, and enabled prediction of individual therapeutic response. They also describe how further translation of these molecular endoscopy approaches into clinical use can be achieved. They could not include all studies that are pertinent to the discussed subject but mentioned data that are relevant to the broad scope of this review.

DISCUSSION

Basic Principles of Molecular Endoscopy

Molecular endoscopy is based on in vivo visualization and characterization of mucosal features on the basis of disease-specific molecular alterations. This approach prerequisites the identification of specific cellular proteins that are critically involved in the immunopathogenesis of the disease. These insights have enabled the translation of scientific findings into molecular imaging studies. This is best exemplified by studies in the cancer field, where Cathepsin B, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), Claudin-1, and tyrosine–protein kinase Met (c-Met) have been used for the enhanced detection of colonic adenoma, and EGFR and vascular endothelial growth factor for colorectal cancer. Furthermore, HER2, certain glycans, and cyclophilin A were used for better detection of Barrett's neoplasia in the esophagus.[17] The chosen molecular targets should enable a sufficient target-to-background ratio, as it is over-expressed in the investigated tissue area in comparison to its surrounding.[18]

Another critical factor for molecular endoscopy studies is the selection of an adequate molecular probe that should demonstrate high specificity against the chosen target structure to increase target visualization by enhancing the contrast. The ideal molecular probe would further possess high target affinity, rapid binding kinetics, long-lasting binding capacities, deep tissue penetration, low immunogenicity, in vivo stability, and a convincing safety profile.[18,19] The most widely used probes in so far conducted preclinical and clinical studies are lectins, peptides, activatable enzymes, antibodies, antibody fragments, nanobodies, affibodies, and peptides. These probes are then labeled by fluorescent dyes as optical reporters, such as high-affinity fluorophores that provide a distinct fluorescence emission spectrum, which can be detected by dedicated fluorescence endoscopes. Manufacturing fluorescent molecular probes is an arduous challenge, as it prerequisites a multidisciplinary team that is able to generate the probes in a good manufacturing practice (GMP)-conform setting for subsequent in vivo use in clinical studies. There are different routes of administration for the selected probes, with respective benefits and pitfalls. Topical administration is performed by spraying the fluorescent probe onto the luminal surface of the tissue during endoscopy and the unbound probe is rinsed off with water after an appropriate incubation time. This method allows application of a higher local concentration of the probe and ensures lower systemic concentrations, markedly reducing the risk of systemic toxicity. However, topical administration is restricted to the detection of focal disease only in areas with larger mucosal surface (eg, colon) and necessitates a clean surface. Systemic administration of probes ensures standardized systemic distribution and enables binding to subsurface target structures. However, this approach needs a lead time prior to the examination, resulting in an additional patient visit, and there is a heightened probability of unwanted systemic side effects and allergic reactions.[18,19] Applied devices for molecular endoscopy in the gastrointestinal tract can be subsumed into instruments for macroscopic wide-field detection and point devices for on-site characterization. Clinical studies have so far been performed with modified fiber-based endoscopy systems, where a fiber is inserted through the

working channel of a conventional endoscope. This fiber conducts the excitation light to the tissue and leads the emitted signal back through a band-pass filter to a near-infrared-fluorescence camera system.[18] Confocal laser endomicroscopy (CLE) on the other hand enables in vivo microscopic imaging of the tissue with subcellular resolution. It is currently available as a flexible fiber-optic bundle device that can pass through the instrument channel of the endoscope. It utilizes laser light with a wavelength of 488 nm that is directed through a pinhole onto a defined point of the tissue. The intensity of the light reflected off a given point, which would be the fluorescent probe in molecular endoscopy, is then measured in order to compute a virtual image from these data, enabling histologic imaging with 1000 fold magnification in real time. Importantly, the reflected light passes through a pinhole while scattered light from outside the plane of interest is not detected, enabling increased spatial resolution.[17]

Molecular Endoscopy Studies in Inflammatory Bowel Disease

The growing therapeutic armamentarium in IBD has enabled us to choose from a variety of different substances that specifically target an inflammatory pathway involved in the immunopathogenesis of IBD, but we are still not able to identify patients with a heightened probability of response prior to the commencement of the treatment.[20] Choosing the most appropriate biologic therapy at the right time has important implications for the patient's probable response. As already described earlier, there is an urgent clinical need to establish predictive markers of response to available targeted therapies in IBD. Such an approach would enable us to prevent a delay of initiating an effective treatment and ensure a substantial benefit for the individual patient. Treatment with an effective therapy also reduces the risk of being exposed to potential systemic side effects of an ineffective therapy.[21] An ideal predictive biomarker would be derived from insights into the underlying molecular pathologic processes that drive the initiation and perpetuation of intestinal inflammation in IBD. The identified biomarker would optimally need to integrate the mode of action of the therapeutic substance and the temporarily distinct functions of specific signaling pathways in intestinal inflammation.[22] Furthermore, there is currently insufficient knowledge regarding drug distribution in the inflamed mucosa of patients with IBD. Previous studies have indicated that patients with active disease in spite of detectable levels of anti-TNF in the serum may need higher doses of anti-TNF antibodies to neutralize tissue inflammation.[23] Heightened understanding of the variable presence and distribution of targeted therapies in the intestinal tissue would help to predict efficacious dosing in an individual patient. These areas of heightened clinical relevance have recently been addressed by different molecular endoscopy studies in IBD.

Mode of Action of Therapeutic Antibodies and Prediction of Therapeutic Response in Patients with Inflammatory Bowel Disease

Several studies have shown that TNF levels are markedly increased in the serum and intestinal tissue of patients with IBD, centrally regulating the intestinal inflammatory process in multiple ways.[24] Here, studies have shown that the transmembrane precursor protein mTNF expressed on mucosal immune cells is the pivotal factor in driving the inflammatory reaction in IBD, thereby also representing the decisive target for effective anti-TNF therapy. Application of anti-TNF agents to block the interaction between mTNF on CD14+ macrophages and TNF receptor 2 has been shown to induce therapeutically relevant T-cell apoptosis.[25]

Thus, a correlation between enumeration of mucosal TNF expression and the efficiency of the TNF antibody directed against it was plausible and subsequently analyzed for the first time in a molecular endoscopy clinical study. Here, mTNF expression on

intestinal cells in vivo was used for predicting individual patient response to subsequently initiated anti-TNF therapy in patients with 25 biologic-naïve Crohn's disease. Detection of mTNF-bearing mucosal cells was achieved by topical application of a GMP-conform fluorescent anti-TNF antibody and CLE was employed to assess the number of mTNF-positive mucosal cells. It was not only possible to visualize intestinal mTNF-positive cells in vivo in real-time, but there was moreover even a correlation between mucosal mTNF expression in the area of highest endoscopic inflammation and subsequent clinical response to commenced anti-TNF therapy. Crohn's disease patients with high amounts of mTNF+ cells showed significantly higher response rates at week 12 (92%) as compared to patients with low amounts of mTNF+ cells (15%). The clinical response in patients with high mTNF expression was sustained over a follow-up period of 1 year and resulted in higher probability of endoscopic remission and less corticosteroid use. The sensitivity and specificity for prediction were 92% and 85%, respectively. The positive predictive value was 85% and the negative predictive value was 92%. Interestingly, the in vivo molecular endoscopy findings had a higher statistically significant correlation with clinical response than ex vivo immunohistochemical staining of intestinal biopsies taken from the investigated area.[26] Here, molecular endoscopy was used for the first time to stratify patients into possible responders and nonresponders to targeted therapies.

A similar approach was also applied for another targeted therapy, the anti-integrin antibody vedolizumab, where molecular endoscopy study was applied ex vivo in patients with Crohn's disease. Here, fluorescent vedolizumab was applied to obtain intestinal biopsies, which were taken from the area of highest inflammation, to assess the number of alpha4beta7 ($\alpha4\beta7$)-positive cells via CLE before the commencement of vedolizumab treatment. Patients with $\alpha4\beta7$-expressing mucosal cells in pericryptal regions demonstrated response to subsequent vedolizumab therapy, whereas patients with none of these cells did not.[27] In another molecular endoscopy study that applied fluorescent antibodies ex vivo to obtained biopsies and visualized target cell expression via CLE, and subsequently computer-aided quantitative image analysis was performed. Ex vivo, increased binding of labeled infliximab and vedolizumab at baseline predicted response to therapy in ulcerative colitis (Area Under the Receiver Operating Characteristics [AUROC], 83%; accuracy 77%; Positive predictive value [PPV] 89%; NPV 50%), but not in Crohn's disease (AUROC 58%).[28] A recently published ex vivo study used dual-band CLE that allowed to simultaneously identify 2 distinct markers using 2 different wavelengths of excitation. In terms of clinical response and remission, endoscopic improvement and histologic response, fluorescent vedolizumab staining tended to be higher in obtained biopsies of responder patients compared to nonresponders at week 22 in ulcerative colitis. The results were similar in terms of clinical remission and endoscopic improvement with a sensitivity of 78% and a specificity of 85% (P=.05).[29]

Mode of Action of Therapeutic Antibodies and Drug Distribution in Patients with Inflammatory Bowel Disease

Gabriëls and colleagues[30] recently published an elegantly conducted phase 1 feasibility study, where in vivo fluorescence molecular imaging was applied in conjunction with intravenously applied fluorescent vedolizumab to visualize mucosal vedolizumab distribution and identify mucosal target cells in patients with IBD. The in vivo molecular endoscopy procedure demonstrated heightened uptake of fluorescent vedolizumab in the inflamed intestinal tissue. Ex vivo fluorescence microscopy in the intestinal biopsies taken from all in vivo investigated sites showed deep penetration and heterogeneous distribution of fluorescent vedolizumab in the inflamed tissue.

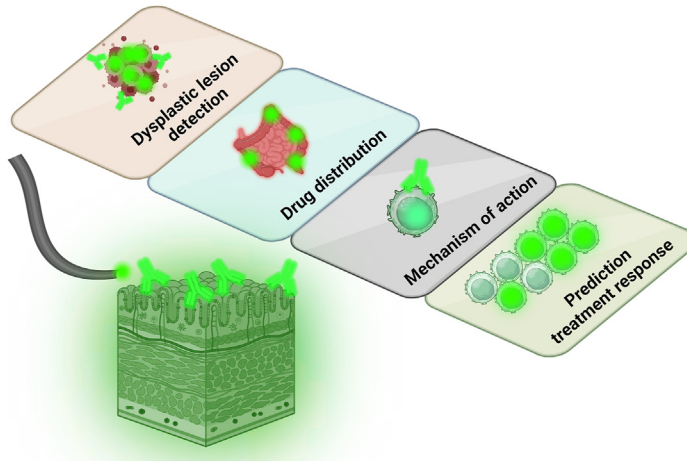

Fig. 1. Preclinical and clinical in vivo molecular endoscopy studies addressing unmet needs in IBD. Studies have so far been conducted in detecting dysplastic lesions in an experimental colitis model, as well as in vivo clinical studies depicting drug distribution in the inflamed mucosa, elucidating the mode of action of anti-inflammatory therapies and predicting individual therapeutic response. (Image created using BioRender.com.)

The performed molecular endoscopy study provides first in vivo insights into drug distribution in patients with IBD. This might be of potentially high clinical and therapeutic relevance, as patients with endoscopic response had higher tissue levels of vedolizumab than patients who did not respond.[31] The used molecular endoscopy approach might therefore be potentially used to further elucidate mechanism of failure to respond to initiated anti-inflammatory antibody treatment. Furthermore, the performed study also shed much needed light on the mechanism of action of vedolizumab in IBD, which may consist of further modes than merely binding to gut homing T cells and inhibiting their trafficking to the intestinal mucosa. In performed multiplex immunohistochemistry staining analyses, binding between fluorescent vedolizumab and plasma cells, as well as intracellular fluorescent vedolizumab localization in eosinophils and macrophages could be demonstrated.[30] Altogether, this was the first study to visualize the macroscopic and microscopic distribution of an intravenously administered fluorescent anti-inflammatory-targeted therapy in the gut of patients with IBD.

SUMMARY AND OUTLOOK

Molecular endoscopy that visualizes mucosal properties in vivo due to molecular alterations rather than their morphologic structure has transitioned from preclinical studies to clinical trials. The performed studies have addressed research areas that indicate an unmet clinical need in IBD. These include elucidation of the mechanism of action of available anti-inflammatory therapies, intestinal distribution of targeted therapies, and also prediction of individual therapeutic response (**Fig. 1**). The so far conducted studies are encouraging and biologically sound to prove causation and not just correlation of identified predictive biomarkers, but they have so far only been done in small cohorts and need to be validated in larger, ideally multicentric studies. Furthermore, an additional area has also been addressed by at least a preclinical molecular endoscopy study, where detection of neoplastic mucosal lesions through improved imaging

contrasts was studied. This is especially relevant in surveillance colonoscopies in patients with persistent colonic inflammation, where sufficient differentiation between surrounding mucosal inflammation and neoplastic lesions is often not possible. In the performed study, a topically applied enzymatically activatable probe γ-Glutamyl hydroxymethyl rhodamine green (gGlu-HMRG), which fluoresces in the presence of γ-glutamyl transpeptidase, was applied in an experimental model for colitis-associated cancer. The charm of the method consisted in the application of an activatable probe, which only emits signals in the presence of an enzyme that is specifically associated with neoplastic lesions. Fluorescence colonoscopy allowed the detection of gGlu-HMRG fluorescent lesions in tissue with high-grade dysplasia or cancer even in diminutive lesions. Remarkably, these lesions were visible against a background of persisting microscopic but not endoscopically active inflammation. Further studies are warranted in this regard.[32] The translation of preclinical findings into clinical trials in molecular endoscopy is challenging, as this requires sufficient generation of preclinical data (eg, toxicity, stability, binding affinity, specificity) and a multidisciplinary team to ensure GMP-conform manufacturing of the fluorescent probe. As with all biomarkers in the IBD field, there is the need for independent validation cohorts to ensure generalizability and reproducibility of data. Here, well-characterized, sufficiently powered, longitudinal prospective cohort studies are needed. After sufficient validation, a biomarker-stratified interventional clinical trial should evaluate whether the discovered biomarker is indeed capable of improving clinical outcomes by facilitating personalized medicine.[13] Nevertheless, the available exciting data of the first molecular endoscopy studies clearly emphasize the potential of this method, which might have an impact on improved future diagnostic and therapeutic algorithms.[17–19]

CLINICS CARE POINTS

- Identification of specific cellular proteins that are critically involved in the immunopathogenesis of IBD is prerequisite for molecular endoscopy studies.
- A GMP-conform fluorescent probe with high specificity and a compatible endoscopic device for visualization of the mucosal target is important for in vivo molecular endoscopy studies.
- Mucosal drug distribution, demonstration of the mechanism of action of targeted therapies, prediction of individual therapeutic response, and visualization of dysplastic lesions are ongoing areas of research in molecular endoscopy studies.
- Well-characterized, sufficiently powered, longitudinal prospective cohort studies are needed to validate the findings of so far conducted molecular endoscopy studies.

DISCLOSURE

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