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Beyond Dysplasia Grade: The Role of Biomarkers in Stratifying Risk

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SYNOPSIS

Gastroenterology society guidelines recommend endoscopic surveillance as a means to detect early stage cancer in Barrett's esophagus. However, the incidence of esophageal adenocarcinoma in Western countries continues to rise, suggesting that this strategy may be inadequate. Current surveillance methods rely on the endoscopist's ability to identify suspicious areas of Barrett's esophagus to biopsy, random biopsies, and on the histopathologic diagnosis of dysplasia. This review highlights the challenges of using dysplasia to stratify cancer risk, and addresses the development and use of molecular biomarkers and *in vivo* molecular imaging to detect early neoplasia in Barrett's esophagus.

Keywords

Barrett's esophagus; biomarker; dysplasia; in vivo imaging; confocal laser endomicroscopy; fluorescence endoscopy

INTRODUCTION

Barrett's esophagus is an extremely common condition occurring in approximately 5.6% of adult Americans.¹ The risk of esophageal adenocarcinoma for the general population of patients with non-dysplastic Barrett's esophagus is low, between 0.12% and 0.33% per year.^{2, 3} For patients with high-grade dysplasia, the risk of esophageal adenocarcinoma is approximately 6% per year, while those patients with low-grade dysplasia have a cancer

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incidence rate that lies between that of non-dysplastic Barrett's esophagus and high grade dysplasia.⁴⁵ Gastrointestinal society guidelines recommend endoscopic surveillance using high-definition white light endoscopy with 4-quadrant random biopsies obtained every 1–2 cm, as a means to detect dysplasia and early cancers in Barrett's esophagus.^{3, 6, 7} However, this strategy has limited effectiveness, as rates of esophageal adenocarcinoma have continued to rise.^{8, 9} Among the challenges associated with our current cancer preventive strategy is the endoscopist's ability to identify suspicious areas in the entire field of Barrett's mucosa for targeted biopsies and the reliance on the histopathological diagnosis of dysplasia. To overcome these challenges, new endoscopic imaging techniques are being explored that highlight neoplastic tissue. Some of the new techniques use fluorescent-tagged molecular biomarkers that bind to abnormal tissue and then are visualized during the imaging process. The combination of new imaging techniques and fluorescein-tagged molecular probes has been termed *in vivo* molecular imaging, an emerging technology that has garnered intense interest both for clinicians and scientists.

The following sections will highlight the limitations of using dysplasia to stratify cancer risk for patients with Barrett's esophagus, concepts regarding the development and use of biomarkers *ex vivo* and *in vivo*, and proof-of principle studies demonstrating the potential of *in vivo* imaging using molecular biomarkers to detect early neoplasia in Barrett's esophagus. This review focuses on current concepts supported by recently published key studies.

LIMITATIONS WITH USING DYSPLASIA TO STRATIFY CANCER RISK IN BARRETT'S ESOPHAGUS

Histological analysis of biopsies to identify dysplasia is the current gold standard for assessing cancer risk in patients with Barrett's esophagus. However, there are a number of limitations when relying on the grading of dysplasia to risk stratify patients with Barrett's esophagus. Four-quadrant random biopsies may not detect areas of dysplasia, leading to sampling error and missed cases of dysplasia. Assuming adequate biopsies are taken, technical or processing artifact of the tissue can hinder accurate determine of dysplasia.¹⁰ Histological interpretation of biopsies is more challenging when active inflammation and ulceration are present, which is not uncommon in patients with Barrett's esophagus. Inflammation induces regenerative changes in the Barrett's epithelium and these regenerative changes can mimic dysplasia. This can lead to diagnostic uncertainty and may result in a biopsy specimen labeled "indefinite for dysplasia", leading to the need for repeat endoscopy with biopsies.¹⁰ This issue of inflammation mimicking dysplasia is the rationale underlying the recommendation in the American College of Gastroenterology (ACG) guidelines which advises against taking biopsies in areas of erosive esophagitis until after intensive anti-secretory therapy has healed any mucosal injury.³

Even without the presence of inflammation, histological interpretation of dysplasia is challenging. There are no scientifically validated morphologic features to distinguish low-grade dysplasia (LGD) from high-grade dysplasia (HGD), leading to variations in interpretation between pathologists.^{10–12} In addition to the difficulties in distinguishing low-grade from high-grade dysplasia, there is also a substantial disagreement among pathologists

when distinguishing HGD from intramucosal cancer.^{10, 13} To compound the problem, there is a high degree of inter-observer and intra-observer variability in the diagnosis and grading of dysplasia in Barrett's esophagus, which has been seen in multiple studies.^{13, 14} In studies in which expert gastrointestinal pathologists reviewed histopathology slides from cases of non-dysplastic and dysplastic (both LGD and HGD) Barrett's esophagus, the inter-observer agreement for non-dysplastic BE was fair to moderate, with a kappa statistic (K) of 0.2-0.58, higher for HGD/carcinoma (K=0.43-0.65), and lower for LGD (K=0.11-0.4)¹²⁻¹⁴ These findings among expert gastrointestinal pathologists with a *research interest in Barrett's esophagus* emphasizes the limitations of using dysplasia to stratify the risk of cancer in patients with Barrett's esophagus. As dysplasia is an imperfect measure of cancer risk, new techniques are needed to improve identification of patients who may progress from non-dysplastic Barrett's esophagus to cancer.

BIOMARKER DEVELOPMENT: CANCER HALLMARKS

Biomarkers have been used in multiple diseases to assess risk of cancer, predict response to treatment, and estimate prognosis.¹⁵ Many of these biomarkers are derived from cellular and tissue features specific to cancer. In 2000, Hanahan and Weinberg proposed the concept that genetic alterations of different molecular pathways alter normal cellular physiologic function, allowing cells to acquire essential cancer "hallmarks" that enable them to transform into malignant cells (Figure 1). These essential cancer hallmarks are core physiological attributes allowing cells to: 1) proliferate without exogenous stimulation, 2) resist growth-inhibitory signals, 3) avoid apoptosis, 4) resist cell senescence, 5) develop new vascular supplies (angiogenesis) and 6) invade and metastasize.¹⁶ In 2011, the same authors proposed two additional requisite physiological hallmarks: 1) cancer cells must reprogram their energy metabolism to support their malignant proliferation and 2) cancer cells must evade destruction by immune cells including T and B lymphocytes, macrophages, and natural killer cells (Figure 1).¹⁷ Finally, the acquisition of these cancer attributes is accelerated by two enabling features: 1) genome instability and mutation, which facilitate the genetic alterations essential for tumorigenesis, and 2) tumor-promoting inflammation, which supports the capabilities endowed by the core hallmarks. Many of these cancer hallmarks have been developed into biomarkers for different diseases. This is also a factor in BE, as some of these cancer hallmarks are present in metaplastic Barrett's cells even before they exhibit the histological features of dysplasia.¹⁸ In the future, molecular biomarkers may become better predictors of neoplastic progression than dysplasia.

BIOMARKER DEVELOPMENT FOR BARRETT'S ESOPHAGUS IN THE PRECISION MEDICINE ERA

The United States Precision Medicine Initiative, announced by President Barack Obama during the 2015 State of the Union Address, aspires to improve health by collecting clinical and biomarker data from patients with the same disease and then integrating these findings to reclassify the disease into subtypes (Figure 2).^{15, 19} The goal of the precision medicine approach is the development of better biomarkers for disease, however the magnitude of data collected and the speed of analysis has been substantially accelerated by this initiative. The

idea is to determine which pieces of data from the large quantity of collected data (i.e. histology, RNA, protein, etc.) are the best predictors of disease risk, treatment response, and/or prognosis, and quickly move these data forward into the biomarker development pipeline.¹⁵

Classification of tumor subtypes is already underway, with discovery of mutations and targeted therapies that impact treatment of certain tumor subtypes. For example, colorectal cancers with mutant Ras respond to different chemotherapy than colorectal cancers negative for the Ras mutation.²⁰ Categorization of breast cancers has led to targeted chemotherapy, as tumors with the HER2 mutation respond to specific chemotherapy targeted to the mutation, while other breast cancers do not.²¹

Incredible technological advances have now accelerated biomarker discovery and development. DNA, RNA, proteins, cell metabolites, microbial products, and host cell products are being measured by “omics” techniques, such as genomics and metabolomics. The profiles derived from these “omics” techniques can then be used to identify viable biomarkers.¹⁵ Specifically, over the last 10 years, the Cancer Genome Atlas (TCGA) collaborative initiative has performed “omic” profiling of advanced stage tumors using multiple platforms, such as DNA sequencing for mutations and RNA sequencing for micro-RNA expression, to gain insight into the molecular alterations associated with cancer.²² This effort has uncovered several hundred genes that are potential drivers of cancer formation. In addition, performance of multiple “omics” arrays has allowed re-categorization of several tumor types. For example, low grade gliomas are traditionally classified based on histology, but this classification suffers from large intra- and inter-observer variability and does not adequately predict clinical outcomes.²³ By combining data from multiple “omics” profiles from low grade gliomas, a new classification was developed, comprised of 3 molecular subtypes strongly associated with overall survival, outperforming histological classification for prediction of survival.²³ Similar to the study of gliomas, the histological classification in Barrett’s esophagus suffers from the same issues, as histological classification does not always accurately predict clinical outcomes. Using the precision medicine approach to categorize molecular subtypes of Barrett’s esophagus holds promise for the future of biomarker development (Figure 2).

Using a precision medicine-type approach, Stachler *et al.* performed whole-exome sequencing on DNA extracted from esophageal adenocarcinoma and Barrett’s esophagus from the same patient.²⁴ Using complex bioinformatics analyses, they found that the majority of esophageal adenocarcinomas harbored a p53 mutation and that the same p53 mutation could be detected in the non-dysplastic Barrett’s metaplasia of patients that progressed to cancer. Interestingly, this study found two general pathways for neoplastic transformation in Barrett’s esophagus (Figure 3). They found a minority of tumors progressed along the traditional pathway of carcinogenesis, involving the step-wise accumulation of alterations in the p53 and p16 tumor suppressor genes, followed by oncogene activation, and then development of genomic instability. In contrast, the majority of tumors in the study developed through a “genome-doubled pathway”. In this pathway, the cell first acquired a p53 mutation that gave that cell a growth advantage and allowed it to expand throughout the mucosa. These p53-mutant cells then underwent whole genome

doubling, an alteration which was primarily detected in areas of dysplasia. Whole genome doubling was then followed by genomic instability and oncogene amplification, resulting in malignancy. The investigators proposed that the genome-doubled pathway may be a more rapid pathway to cancer development in Barrett's esophagus, and may possibly explain the failure of endoscopic surveillance strategies to stem the rising incidence of esophageal adenocarcinoma.²⁴ Using the precision medicine approach, perhaps Barrett's esophagus could be stratified into molecular subtypes, based on p53 immunostaining with or without whole genome doubling. This could potentially improve our ability to risk stratify neoplastic progression and guide treatment decisions for patients with Barrett's esophagus (Figure 2).

USE OF BIOMARKER PANELS TO ASSESS CANCER RISK IN BARRETT'S ESOPHAGUS

Recently, biomarker studies have focused on panels of biomarkers to attempt to determine the risk of neoplasia in Barrett's esophagus patients.^{25–27} For each of these studies, the selection of the biomarkers was based on the biology underlying cancer development, as determined by basic and translational research studies. A few of the more promising predictive biomarkers for cancer progression in Barrett's esophagus have been validated in large studies; most with retrospective validation, while some studies include both a retrospective validation followed by prospective evaluation in a cohort of patients. Biomarker development and validation is challenging and lengthy process. The studies illustrated below demonstrate the potential for biomarker-based prediction of cancer risk in patients with Barrett's esophagus. However, at this time the guidelines from the American College of Gastroenterology and American Gastroenterological Association recommend against routine use of biomarkers in management of Barrett's esophagus.^{3, 28}

Methylation Arrays

One study examined gene methylation arrays in Barrett's esophagus and esophageal adenocarcinoma.²⁵ Genes with differential methylation were identified and applied to cohort of samples including 60 non-dysplastic Barrett's esophagus, 9 esophageal adenocarcinomas, and 28 dysplastic Barrett's esophagus. From this retrospective validation, the investigators identified a 4-gene panel that was able to discriminate between non-dysplastic Barrett's esophagus and Barrett's-associated neoplasia with a high area-under-the-curve (AUC) of 0.988. They then prospectively used the 4-gene panel in 61 patients with non-dysplastic Barrett's esophagus, 20 patients with HGD/cancer, and 17 patients with LGD to assess risk of neoplastic progression. They found that the risk of developing dysplasia and cancer was higher in the patients with more methylation.

Mutational Load

Another study examined the mutational load, determined from loss of heterozygosity and microsatellite instability of 10 genomic loci associated with tumor suppressor genes, as a potential biomarker for determining risk of dysplasia and cancer in Barrett's esophagus.²⁶ The mutational load score was compared between 23 patients with non-dysplastic Barrett's esophagus or those with LGD who progressed to HGD/cancer and 46 patients with non-dysplastic Barrett's esophagus or those with LGD who did not progress. The mutational load

score was significantly higher in patients who progressed to HGD/cancer than in patients who did not. Using a mutational load score of >1 in baseline mucosal biopsies, accuracy for predicting progression to dysplasia and cancer was 89.9%.

Histopathologic Biomarker Classification System

In another study, the investigators developed a 15-feature histopathologic biomarker classification system (p53, p16, COX-2, HER2/neu, and others) to risk stratify patients with Barrett's esophagus.²⁷ They then tested this biomarker classification system in a validation set of 38 patients who progressed to HGD or cancer and 145 patients who did not progress. Based on study findings, the 15-feature classification system was able to risk stratify Barrett's esophagus patients into low, intermediate, and high risk for progression over 5 years. The area under the ROC curve for prediction of 5 year progression to HGD or cancer was 0.804. The hazard ratio for progression to HGD or cancer in the high risk vs. low risk group was 9.42.

P53 IMMUNOSTAINING: SO CLOSE AND PERHAPS NOT SO FAR AWAY

Among the potential molecular biomarkers in Barrett's esophagus, immunostaining for p53 protein alterations has advanced the farthest into clinical practice. p53 is tumor suppressor gene that is activated in response to DNA injury. Activation of p53 decreases cell proliferation, a process that prevents cells with damaged DNA from undergoing mitosis and perpetuating the genomic damage. If the DNA injury is severe and irreparable, then p53 induces cell destruction through apoptosis. p53 is frequently inactivated in a number of human tumors, including Barrett's-associated cancers.

In response to DNA injury, normal (wild-type) p53 protein rapidly accumulates and then is rapidly degraded, making it difficult to detect p53 protein expression in normal biopsy samples. In contrast, mutant p53 protein is stable and overexpression can be easily detected in tissue samples by immunostaining techniques. In addition to overexpression, mutant p53 can also lead to loss of expression in tissue samples, which can also be evaluated by immunostaining. In a recent case-control study performed in the Netherlands, p53 protein expression was determined by immunostaining in more than 12,000 biopsies from 49 cases of patients with Barrett's esophagus who progressed to HGD or cancer and from 586 control patients whose Barrett's esophagus did not progress to neoplasia.²⁹ Aberrant p53 expression, defined as overexpression or loss of expression, was identified in 49% of biopsies from progressors compared to 14% in control biopsies who did not progress to HGD or cancer. With aberrant p53 expression, the overall relative risk of neoplastic progression increased by a factor of 6.2.²⁹ Aberrant p53 expression in non-dysplastic Barrett's esophagus was associated with an increased relative risk (RR 4.3) of neoplastic progression, whereas an even higher relative risk (RR 12.2) was seen for low grade dysplasia.²⁹

More recently, p53 immunostaining was prospectively evaluated as a predictor of progression to HGD and esophageal adenocarcinoma.³⁰ Patients with Barrett's esophagus referred for treatment of HGD or early cancer and patients undergoing Barrett's esophagus surveillance had p53 immunostaining of their Barrett's mucosal biopsies. Patients without a diagnosis of HGD or esophageal adenocarcinoma were then followed for a median of 71

months, and 12% progressed to HGD or cancer during this time.³⁰ Aberrant p53 expression was significantly higher in the baseline biopsies of those patients that progressed to HGD or cancer (63.6%) than in those that did not progress (7.5%).³⁰ Multivariate analysis demonstrated that aberrant p53 expression in baseline Barrett's mucosal biopsies was a significant and independent predictor of progression to neoplasia (hazard ratio, HR 17).³⁰

Recent studies such as these highlight the promise for biomarkers, in particular aberrant p53 expression, to predict cancer progression in Barrett's esophagus either alone or in combination with histology. In fact, the British Society for Gastroenterology has given a grade B recommendation (non-randomized, supportive clinical studies) to the use of p53 immunostaining as an adjunct to routine histologic assessment for dysplasia, which may enhance the "performance" of a diagnosis of dysplasia as a risk stratification biomarker for Barrett's esophagus.⁷

CHALLENGES OF ENDOSCOPIC BE SURVEILLANCE AND NEW ADVANCES IN IMAGING

Our current cancer preventive strategy relies on endoscopic surveillance to detect dysplasia. Although there are no data from randomized controlled trials, a number of observational studies demonstrate that surveillance programs allow detection of Barrett's-associated cancers at an earlier stage and are associated with an increase in survival for patients undergoing surveillance compared to patients not undergoing regular endoscopic surveillance (Reviewed in^{3, 6}). However, there are numerous challenges with current endoscopic surveillance protocols. Currently, standard endoscopic surveillance uses high-definition white light endoscopy to visually inspect the mucosa coupled with systematic four-quadrant biopsies obtained at 1–2 cm intervals along the length of the non-dysplastic Barrett's metaplasia, with areas of mucosal irregularity sampled separately.^{3, 28} Thus, it is not surprising that current surveillance programs are expensive, labor-intensive, and time consuming both for patients and physicians.^{3, 6} Studies have found that physician adherence to this surveillance protocol ranges from 30–51%, which is problematic.^{31, 32} Moreover, even if strict adherence to the biopsy protocol is followed, there is still the issue of biopsy sampling error.³³

To overcome these challenges, newer imaging modalities are being developed that can distinguish dysplasia from the surrounding areas of non-dysplastic Barrett's mucosa *in vivo* so that "targeted" rather than random biopsies can be obtained. Such a strategy should reduce cost, reduce time, and increase adherence to surveillance protocols. Endoscopic imaging techniques can be classified into two basic categories, those that image a wide area of Barrett's mucosa, such as narrow band imaging, and those that image a precise area in great detail, such as confocal laser endomicroscopy (CLE).^{34–36} Several of the techniques have been used successfully in Barrett's esophagus patients for screening, surveillance, and identifying dysplasia and early cancers, and have been endorsed by the American Society for Gastrointestinal Endoscopy for detection of dysplasia in Barrett's esophagus.³⁷ The ideal endoscopic imaging technique would have the ability to survey large areas of Barrett's mucosa, but also able to identify small areas of dysplasia or early cancers. Many of the

current endoscopic technologies can accomplish one of these goals, but not both requirements.

IN VIVO ENDOSCOPIC MOLECULAR IMAGING USING BIOMARKERS

Some of the most exciting developments in surveillance of Barrett's esophagus stem from the development of biomarker-based endoscopic imaging. Many of these biomarkers are coupled with current endoscopic imaging techniques. *In vivo* molecular imaging techniques are being developed which use specific biomarker-based contrast enhancement in combination with *in vivo* imaging. The specificity of these contrast agents is guided by the underlying molecular biology of the tissue of interest, such as imaging agents targeted to neoplastic Barrett's esophagus.³⁸ Peptides, antibodies, activated enzymes, and lectins are all being developed for use as molecular biomarkers to specifically target regions of neoplasia during *in vivo* molecular imaging.^{39–42} These biomarkers are often tagged with bright, fluorescent dyes which provide a high level of contrast to enhance visualization. Detection of these fluorescent-tagged molecular biomarkers requires an imaging modality that can detect fluorescence. Current modalities include endoscopes with a single blue light excitation channel (i.e. autofluorescence-capable endoscopes), endoscopes with a spiral scanning fiber with 3 excitation wavelengths (red, green, and blue), and CLE.^{43, 44, 40}

Lectins

One molecular imaging study examined fluorescent-tagged lectins as a biomarker for Barrett's esophagus. Lectins are carbohydrate-binding proteins that have specificity for particular glycans located on the cell surface. Glycan expression has been shown to be altered in cancers of the pancreas, colon, and breast.⁴⁵ Using a lectin protein expression array, Bird-Lieberman *et al.* identified the lectin *Triticum vulgare* agglutinin (WGA), from wheat germ, as binding with high affinity to tissue samples of squamous esophagus.⁴² When tissue samples of Barrett's metaplasia and dysplasia were analyzed using 2 independent lectin arrays, the binding of WGA decreased as the degree of dysplasia increased. WGA was then fluorescently-tagged, incubated with biopsies obtained from patients with non-dysplastic and dysplastic (low-grade and high-grade) Barrett's esophagus at the bedside *ex vivo*, and then imaged with a fluorescent camera. Compared to non-dysplastic Barrett's biopsies, fluorescence intensities were lower in those biopsies containing dysplasia. These findings were further studied using esophagectomy specimens from patients with Barrett's esophagus containing HGD or cancer. The esophageal mucosa was treated with fluorescently-labeled WGA and imaged with a fluorescence endoscope. This demonstrated reductions in fluorescence intensity in areas of Barrett's-associated dysplasia when compared with non-dysplastic Barrett's esophagus or squamous mucosa.⁴²

Peptides

A second study identified a short peptide (ASYNYDA) that binds preferentially to neoplastic Barrett's esophagus.³⁹ This peptide was then fluorescently-tagged and used for *in vitro*, *ex vivo* and *in vivo* imaging. *In vitro*, confocal fluorescence microscopy demonstrated binding of this peptide to the plasma membrane of esophageal adenocarcinoma cell lines FLO1, OE33, and OE19, but not to the non-dysplastic Barrett's cell line Q-hTERT.³⁹ *Ex*

vivo imaging using a fluorescence-stereomicroscope of Barrett's esophagus with HGD confirmed specificity in binding of the peptide to Barrett's neoplasia, with a significantly higher fluorescence intensity for HGD and esophageal adenocarcinoma than for squamous mucosa and non-dysplastic Barrett's mucosa. The investigators then prospectively examined 25 patients with a history of HGD or esophageal adenocarcinoma. The esophageal mucosa was sprayed with the fluorescent peptide and CLE was performed. *In vivo* imaging of the squamous mucosa showed no binding of the peptide and non-dysplastic Barrett's mucosa demonstrated some fluorescent signal by peptide binding (Figure 4 B&C). In contrast, HGD (Figure 4D) and esophageal adenocarcinoma showed intense and specific peptide binding to the neoplastic crypts; histology confirmed the presence of neoplasia (Figure 4E). The investigators found a sensitivity of 75% and a specificity of 97% for the fluorescent-tagged peptide to detect HGD and esophageal adenocarcinoma, with an area under the receiver operating characteristic (ROC) curve of 0.91.³⁹

This fluorescent-tagged peptide specific for Barrett's-associated neoplasia was then tested in a prospective study of 50 patients with Barrett's esophagus using a multimodal fluorescence-reflectance endoscope. The patients had been referred for endoscopic therapy for Barrett's mucosa with HGD or early cancer.⁴¹ Imaging was then performed using a wide-field fluorescence-reflectance endoscope modified to match the fluorescence spectrum of fluorescein. Areas of the mucosa were evaluated by combining the fluorescence and reflectance images.⁴¹ After optimizing the target to background ratio of mean fluorescence intensity, the investigators found a sensitivity of 76% and a specificity of 94% for the peptide to detect HGD and esophageal adenocarcinoma, with an area under the ROC curve of 0.884.⁴¹

Antibodies

Another potential molecular imaging method for identification of dysplasia in Barrett's esophagus is the use of targeted antibodies. One study in a rat model of Barrett's esophagus and esophageal adenocarcinoma used a fluorescent-labeled anti-HER2 antibody to identify areas of dysplasia and cancer.⁴⁶ In *ex vivo* esophageal specimens treated with an anti-HER2 antibody, the fluorescence intensity of esophageal adenocarcinoma was significantly higher than in normal squamous mucosa or non-dysplastic Barrett's mucosa. CLE imaging of rat esophagi was then performed after injection of the fluorescent-labeled anti-HER2 antibody. *In vivo* fluorescence intensity was higher in esophageal adenocarcinoma than in normal mucosa or non-dysplastic Barrett's mucosa demonstrating the future potential for antibody-based molecular imaging in Barrett's esophagus.

SUMMARY

Endoscopic surveillance is currently recommended to detect dysplasia and cancer at an early stage in Barrett's mucosa. Despite its limitations, dysplasia is still the gold standard biomarker to stratify cancer risk in patients with Barrett's esophagus. Among the potential molecular biomarkers, immunostaining for p53 has advanced the farthest into clinical practice with the British Society of Gastroenterology suggesting its use as an adjunct to the routine histologic assessment for dysplasia. Newer biomarker panels incorporating multiple

molecular targets are in development. *In vivo* molecular imaging, which uses fluorescent-labeled biomarkers shows promise for use in detecting early neoplasia arising from Barrett's mucosa.

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KEY POINTS

- Gastroenterology society guidelines recommend endoscopic surveillance of Barrett's esophagus using high-definition white light endoscopy with 4-quadrant random biopsies to detect dysplasia and early cancers.
- The presence of dysplasia is the current gold standard biomarker for cancer risk in Barrett's esophagus.
- Precision medicine and new biomarker development techniques have the potential to improve cancer risk assessment and response to treatment for patients with Barrett's esophagus by identifying specific subtypes based on biomarker expression.
- Immunostaining for p53 is recommended by the British Society of Gastroenterology as an adjunct to histological assessment of dysplasia in patients with Barrett's esophagus.
- Early proof-of-principal studies demonstrate the promise of fluorescent-tagged molecular biomarkers to enhance the sensitivity and specificity of neoplasia detection during *in vivo* imaging for patients with Barrett's esophagus.

Figure 1.

Cancer Hallmarks. The core physiologic attributes of cancer cells are shown in the green boxes. In general, oncogene activation is the way in which cells can proliferate without exogenous stimulation and inactivation of tumor suppressor genes (TSG) is a common way in which cells resist growth-inhibitory signals. The two additional requisite physiological attributes of cancer cells are shown in the orange circles. The rounded boxes in blue are the enabling features that accelerate the rate at which cells acquire the core and the additional 2 physiologic attributes of cancer cells.

Figure 2.

Theoretical Re-Classification of Barrett's Esophagus Based on Biomarkers in the Era of Precision Medicine. Patients with Barrett's esophagus would be classified based on molecular subtypes such as aberrant p53 immunostaining (IHC). Large amounts of data collected from clinical medicine, "omics", and epidemiologic associations will be analyzed and synthesized, to develop more precise molecular subtypes. These molecular subtypes will not only predict progression to cancer, but may also guide treatment decisions for patients with Barrett's esophagus.

Figure 3.

Two Proposed Pathways for Neoplastic Transformation in Barrett's Metaplasia. Metaplastic Barrett's cells first acquire a mutation leading to inactivation of p53. In the traditional pathway, there is step-wise accumulation of alterations in tumor suppressor genes such as p16. This is followed by the activation of oncogenes and genomic instability, finally resulting in cancer formation. In the genome-doubled pathway, the p53-mutant Barrett's cells undergo whole genome doubling, followed by genomic instability and oncogene amplification, resulting in cancer formation. It has been proposed that the genome-doubled pathway more rapidly progresses to cancer than the traditional step-wise accumulation of alterations in tumor suppressor genes and oncogenes.

Figure 4.

In vivo Molecular Imaging using CLE and a Fluorescein-Tagged Molecular Peptide in Barrett's Esophagus. Representative white-light (A) and peptide-based fluorescence images of normal squamous epithelium (B), non-dysplastic Barrett's metaplasia (C), and dysplasia (D) obtained using CLE. (E) Histology (H&E) confirms dysplasia in (D). Black arrows indicate the white-light images of the mucosa depicted in panels B–E. Images courtesy of Thomas D. Wang, M.D., Ph.D.