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Molecular Imaging for Guiding Oncologic Prognosis and Therapy in Esophageal Adenocarcinoma

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Abstract

In the last 30 years, the incidence of esophageal adenocarcinoma has skyrocketed. Sadly, advances in treatment have not followed the same trend, and the prognosis for patients with esophageal adenocarcinoma remains poor with a 5-year survival rate of only 15%. Like most cancers, early detection is the key to improving prognosis, but this outcome has proven difficult in the esophagus for several reasons: 1) patients present with advanced disease because “alarm symptoms” such as dysphagia occur at a late stage, and 2) high-grade dysplasia (HGD) and early adenocarcinoma (ACA) are not visible on routine surveillance endoscopy. Currently, the recommended surveillance strategy involves collection of random biopsies, an imperfect technique that is limited by sampling error and is infrequently used because of the considerable time and cost it requires. Even in patients with biopsy-proven dysplasia, adequate guidance for clinical management decisions is still lacking. Dysplasia alone is not an entirely reliable biomarker for the risk of progression to adenocarcinoma because the natural history of this condition is extremely variable. Clearly, there is a need for additional biomarkers that can better characterize this disease, and thus improve our ability to treat patients on an individual basis. As we better understand the molecular changes that lead to the development of this cancer, new molecular biomarkers are needed to allow for more personalized diagnoses, surveillance and treatment. Targeted agents against EGFR, HER2/neu and VEGF are currently being evaluated for their role in combination chemotherapy for metastatic esophageal adenocarcinoma. As these studies progress, a reliable approach for determining receptor status in individual patients is essential. Molecular imaging uses fluorescent probes that target specific cell surface receptors, and has the potential to evaluate an individual patient’s gene expression profile. By topically applying fluorescent probes to dysplastic epithelium during endoscopy, a variety of receptors can be visualized, and the response to treatment can be monitored in real time. This technique can mitigate the limitations of current surveillance protocols, allow for improved cancer detection, and be used for truly personalized treatment in the future.

Introduction

Esophageal adenocarcinoma has the fastest rising incidence of any cancer in the US and has increased >350% in the past 30 years as shown in Figure 1 (1). Despite recent advances in our understanding of the molecular mechanisms of esophageal adenocarcinoma, the

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prognosis remains poor with a 5-year survival rate of only 15% (2). This dismal outcome is likely the result of the rapid vascular and lymphatic spread of this disease as well as the poor clinical response to chemotherapy and radiation. Additionally, patients typically seek medical attention at a very late stage where alarm symptoms such as dysphagia occur only after the tumor has already formed. However, if this cancer is detected early, patients have a relatively good outcome following surgical resection and neoadjuvant chemotherapy.

The progression of normal esophageal mucosa to cancer is reflected histologically by the transformation of squamous epithelium into intestinal metaplasia followed by dysplasia and then adenocarcinoma. The metaplastic phase is known as Barrett's esophagus, which is defined by the American College of Gastroenterology (ACG) as a change in the esophageal epithelium of any length that can be recognized on endoscopy and is confirmed on pathology to include Goblet cells (3,4). Barrett's is thought to be a complication of longstanding GERD via the following mechanism: GERD causes acute mucosal injury which promotes a wound healing response and cellular proliferation that results in the replacement of the squamous lining of the distal esophagus by columnar epithelium containing specialized intestinal metaplasia.

Diagnosis of Barrett's Esophagus

Barrett's affects roughly 1.5% of the adult population (5) and is usually diagnosed in patients complaining of symptoms such as heartburn, regurgitation and dysphagia. When these patients subsequently undergo endoscopy, those with Barrett's often have an irregular squamocolumnar junction (Z-line) proximal to the gastroesophageal (GE) junction. This visual finding is concerning for Barrett's esophagus, thus biopsies are usually taken to confirm intestinal metaplasia on pathology.

Endoscopic Surveillance of Barrett's Esophagus

Once Barrett's esophagus is diagnosed, endoscopic surveillance is recommended with random 4-quadrant biopsies for detection of dysplasia. Current recommendations suggest endoscopic surveillance every 2 to 3 years for patients with metaplasia (6). The cost-effectiveness of this surveillance interval is often questioned because the rate of conversion from Barrett's to adenocarcinoma is only 0.5% per year (7). However, arguments for more frequent surveillance include: 1) the majority of adenocarcinomas occur from within Barrett's esophagus and 2) the risk of esophageal adenocarcinoma is about 30–40 times higher in patients with Barrett's compared to those without (8,9).

Method of Identification of Dysplastic Tissue for Biopsy

In addition to the frequency of surveillance, the most effective method has been debated as well. The Seattle protocol has been validated and thus is recommended. This strategy involves collecting a comprehensive set of biopsies in 4 quadrants at 1–2 cm intervals using jumbo forceps. This technique is labor intensive, and the extensive tissue processing and pathological evaluation can be expensive. Accordingly, this protocol is infrequently used by community gastroenterologists. In addition, HGD and early adenocarcinoma are flat in architecture thus not visible on routine endoscopy. Therefore, a more sensitive imaging technique is needed to identify early disease over a length of Barrett's esophagus that can be up to 15 cm, even in the presence of inflammation. Several novel endoscopic methods for detection of dysplasia are being developed, including cytology (10); chromoendoscopy (11); fluorescence spectroscopy (12); reflectance spectroscopy (12); light-scattering spectroscopy (12); trimodal spectroscopy (13); Raman spectroscopy (14); optical coherence tomography (15); narrow-band imaging (NBI) (16), and confocal endomicroscopy (14). No method has

yet effectively demonstrated the ability to identify focal cellular changes over a large surface area, largely because these methods are in general non-specific.

Histologic Analysis of Biopsied Tissue

Surveillance methods are also limited because the diagnosis of dysplasia is largely subjective, and considerable intra- and inter-observer variation frequently occurs. The histological diagnosis of dysplasia in Barrett's esophagus challenges even the most experienced pathologists. As shown in Fig. 2, when intestinal metaplasia transforms into dysplastic epithelium, it is often difficult to appreciate the presence of invasion through the basement membrane into the lamina propria. These features are especially difficult to appreciate on biopsies that are small in size with random orientation. Furthermore, the appearance of dysplasia can have significant heterogeneity, and the diagnostic architectural features, including nuclear enlargement, hyperchromasia, cell stratification, and increased mitoses, are often seen in regenerative epithelium, a process that is common in the setting of acid reflux. The difficulty in distinguishing regenerative from dysplastic changes has resulted in the designation of specimens that are indefinite for dysplasia, further confounding the clarity of diagnosis. In addition, the ability of dysplasia to predict the natural history of this disease has shown inconsistent results. For HGD followed for 3–7.3 years, the cancer incidence ranged from 14–56% (17–20). Also, the absence of HGD on 2 subsequent biopsies does not seem to exclude future progression to cancer (21).

Need for Improved Methods of Risk Stratification

These limitations are worrisome given that clinical management decisions are currently based on the histopathologic diagnosis. The current surveillance algorithm recommends that low-grade dysplasia (LGD) be followed by endoscopic surveillance twice the first year and annually thereafter (6). The risk of development of esophageal adenocarcinoma in patients with LGD is only 0.6–1.6%/year (6). HGD should be treated with endoscopic therapy or surgery (6) since the risk of progression to adenocarcinoma is much higher with an incidence of 6.6 cases/100 patient years (6). Given the wide variability of cancer progression from HGD and the low rate of progression of Barrett's, clearly dysplasia alone is not entirely reliable for establishing risk. If better methods were available, personalized surveillance protocols as well as individualized therapies could be implemented. Given the above limitations of histology, the use of molecular biomarkers has generated great enthusiasm for their potential to improve patient care.

Nuclear Biomarkers for Barrett's Esophagus and Esophageal Adenocarcinoma

Many biomarkers have been identified to predict neoplastic progression of Barrett's esophagus. The best characterized include aneuploidy/tetraploidy and 17p loss of heterozygosity (LOH) (22). Detection of these biomarkers has been performed using flow cytometry of biopsy specimens. Although both biomarkers have shown utility to predict cancer progression, alone and in conjunction with histology, flow cytometry is not widely available for routine clinical use, preventing widespread acceptance by the medical community. Furthermore, these biomarkers are limited by sampling error on endoscopy. If gastroenterologists were given an imaging tool that provides a "red flag" capability for visually detecting and localizing dysplasia, they could employ a more intelligent biopsy strategy that would lead to improved risk stratification, fewer procedures, and better follow-up.

Cell Surface Biomarkers

Ideal molecular targets for endoscopic imaging of Barrett's esophagus, dysplasia, and esophageal adenocarcinoma must have the following characteristics: 1) location near the luminal surface of esophagus, 2) highly specific for dysplasia, and 3) increased in prevalence with histological progression (23). New candidate biomarkers have been identified from studies in genomics that appear to increase in frequency in the progression to adenocarcinoma, thus may prove useful as biomarkers for early detection of esophageal cancer (24). Several of these potential targets are cell surface proteins. These targets are preferred to those in the nucleus for clinical imaging. The pharmacokinetics of molecular probes that bind to the cell surface is significantly more predictable since molecular probes that internalize will be broken down by proteolytic enzymes within the cell. The ability to acquire real time images of molecular expression patterns of esophageal mucosa allows for visualization of changes in target expression over time, thus allowing physicians to monitor the effectiveness of therapy. In addition, changes in molecular expression occur well before that of structural features, thus real time molecular imaging would allow for earlier detection and intervention.

Identification of Potential Cell Surface Targets

Carcinogenesis is a multi-step process that results in numerous mutations and alterations in gene expression. Studying these patterns can reveal key genes and their subsequent proteins that play an important role in the development of cancer. DNA microarrays can be employed to identify unique patterns of dysregulated gene expression in esophageal cancer, and the proteins that correspond to these over-expressed genes can be found by a database search. Upregulated cell surface proteins can then be identified and developed as specific targets for molecular imaging. Because early detection is the key to effective management of cancer, cell surface proteins that are over expressed in the *progression* to adenocarcinoma are the most useful, as they reveal disease at a stage when effective treatment can still be administered.

Gene amplification plays an important role in promoting neoplastic progression in many tumor types (25). This process results in highly increased mRNA and protein expression, providing a powerful growth advantage. Gene amplification can be detected via quantitative PCR analysis of tissue or by using single nucleotide polymorphism (SNP) microarrays, as shown in Fig. 3. A SNP is a single nucleotide in the DNA sequence of a gene that differs among individuals or among paired chromosomes in an individual. Using the SNPs as a marker is one method of revealing gene amplification. SNP arrays are similar to DNA microarrays but instead contain DNA representing all possible variations of one or several genes in a grid pattern.

Use of gene amplification for early detection of esophageal adenocarcinoma

Genes that are amplified in esophageal adenocarcinoma include ERBB2, EGFR, CCND1, PIK3C and GATA4 (26, 27). Miller et al looked at amplification of these genes as well as 8 others (CCNE1, KRAS, HNF3 α , C-MYC, DYRK2, A1B1, AKT1 and IGF1R) and found no significant increase in amplification frequency in advanced esophageal adenocarcinoma as compared to the earlier stage I tumors. This result suggests that amplification is involved early in cancer progression rather than late (24). In addition, the same study demonstrated that the levels of DNA amplification were much higher in adenocarcinoma (3–22 fold) as compared to HGD (2–4 fold), suggesting that gene amplification may initiate during HGD and impel malignant growth (24). These findings have important implications for the

surveillance of HGD. If gene amplification occurs before malignant transformation, one can use molecular probes to detect the products of amplified genes on endoscopy to identify disease before malignant transformation occurs and allow for early intervention.

Specific Genes Warranting Further Investigation for use in Esophageal Adenocarcinoma Surveillance and Treatment

The most promising protein targets for detection during endoscopy are those that are over-expressed in HGD and located on the epithelial cell surface. In the search for these cell surface targets, researchers first looked to other cancers. For example, amplification and over-expression of EGFR and the structurally related ERBB2 have been established as indicators of poor prognosis in several human cancers, such as breast and ovarian cancer (28, 29). Oncogenic activation of MET represents another mechanism of carcinogenesis in other cancers, including papillary carcinoma of the thyroid as well as carcinoma of the colon, pancreas, and ovary (30). Al-Kasspoles et al (26) studied EGFR and ERBB2 expression in esophageal adenocarcinoma and found that 30.8% had multiple copies of EGFR and 15.4% had multiple copies of ERBB2 (26). Miller et al (31) showed that MET over-expression in esophageal adenocarcinoma is a consequence of gene amplification. In addition, tissue microarrays demonstrated a uniformly high abundance of MET activation in primary esophageal adenocarcinoma and cells metastatic to lymph nodes, but to a lesser extent in a subset of metaplastic and dysplastic Barrett's samples. High compared to low MET expression has also been found to be associated with significantly reduced overall and disease specific 5-year survival rates as well as an increased risk of developing distant metastases (32). These candidate targets all belong to the receptor tyrosine kinase family, whose normal function is to serve as the link between the extracellular milieu and intracellular signaling. Mutations of receptor tyrosine kinases often lead to developmental abnormalities, metabolic defects, or oncogenesis (33).

Molecular Imaging Probes

Fluorescent probes specific for these target proteins are being developed for endoscopic visualization. Different classes of probe technologies have been evaluated for performing targeted imaging and are summarized in Fig. 4. Monoclonal antibodies were first investigated for tumor detection and drug delivery because of their high specificity (33, 34). However, their use *in vivo* has been limited by delivery challenges, immunogenicity and cost for reagent development. These difficulties have prompted the investigation of other classes of potential probes, including peptides, DNA and RNA aptamers, single domain proteins (affibodies), nanoparticles, and small molecules. Peptides are short chains of amino acids linked by amide bonds. Their advantageous features include high clonal diversity, very small size, flexibility for labeling with a variety of fluorescent dyes, rapid binding kinetics, and minimal concern for immunogenicity. A heptapeptide has been isolated, labeled with fluorescein, then topically applied to $n = 18$ dysplastic lesions during colonoscopy. Using probe-based confocal endomicroscopy, these pre-malignant lesions were visualized with a sensitivity of 81% and specificity of 82% (35). Similarly, Phase 1 and 2 clinical studies are ongoing for early detection of esophageal cancer as well.

Aptamers are single stranded nuclease-resistant DNA or RNA molecules that can tightly bind a variety of targets including small molecules and proteins. They are easily modified for labeling purposes and have very high binding affinities, comparable to antibodies, but have the advantage of small size. In addition, aptamers can readily penetrate tumors, reach peak levels quickly and clear from the body rapidly, resulting in low toxicity and immunoreactivity (36). Affibodies are small single domain proteins that are based on the B-domain scaffold of staphylococcal protein A. Like aptamers and peptides, they are desirable

because of their small size, leading to rapid tumor localization and fast clearance from non-specific compartments (37). Their utility has already been demonstrated for in vivo targeting and detection of tumors using SPECT or PET imaging. Baum, et al (38) used a HER2-specific affibody molecule to detect the HER2 status of lesions not amenable for biopsy on PET or SPECT in patients with breast cancer. This study resulted in high quality images and even small metastases were clearly detected as HER2 positive.

Nanoparticles are small probes with size between 1 and 100 nanometers. Quantum dots are one type of nanoparticle made from semiconductor materials that release bright fluorescence over a wide range of wavelengths. Nanoparticles are used in cancer detection after conjugation to specific targeting agents such as antibodies, aptamers, and folic acid (39). The main advantages of nanoparticles over organic fluorophores include: 1) more narrow emission spectra allowing for investigation of a larger number of fluorescent probes simultaneously and 2) resistance to chemical and metabolic degradation (40). The main disadvantage for in vivo use, however, is the low penetration depth of light through tissues and concerns about toxicity. (41). Small molecules have also been developed as a class of molecular probe. Their main advantage over the protein based technologies is their ability to diffuse past the cell membrane for use to image intracellular function (42). However, because they are highly lipophilic, a property needed to cross the cell membrane, these probes have a high rate of hepatobiliary excretion (43).

Molecular Imaging and its implications for surveillance

For improved surveillance, molecular imaging of targets that are over-expressed early in the progression to adenocarcinoma can be used for early detection. As mentioned previously, progression to adenocarcinoma often goes undetected because dysplasia is not visible on routine endoscopy and the practice of random biopsies of the entire esophagus is prone to sampling error. Molecular imaging would be advantageous here because it provides a wide area view of the esophageal mucosa that can be quantified on fluorescence as a measure of progression. A wide area view is critical for effective surveillance of cancer in the esophagus where a large mucosal surface area is at risk. Current endoscopic strategies have been reported to miss a significant proportion of lesions since screening requires meticulous inspection over such very large area.

Current technologies developed for obtaining a wide area view include autofluorescence imaging, narrow band imaging, and light-scattering spectroscopy. Autofluorescence imaging provides optical contrast by exciting endogenous fluorophores and measures differences in the concentration of metabolites as well as in hemoglobin content and collagen thickness (44). Narrow band imaging assesses differences in the tissue penetration of light at various wavelengths to enhance the visualization of glandular structures in the mucosa (45). In both of these techniques, neoplastic mucosa produces a reduction in signal intensity compared with that of adjacent mucosa. This relationship limits the dynamic range of detection, and neoplasia may be difficult to distinguish from artifacts caused by mucus, inflammation and shadows. In light-scattering spectroscopy, backscattered light from the nuclei in epithelial cells reveals nuclear size and density. The presence of enlarged, dense and crowded nuclei are used as pathological indicators of neoplasia, but these features can be caused by regeneration in the esophageal mucosa as well (46).

Molecular imaging using probe-based technologies, on the other hand, identifies targets that are highly specific to neoplasia. For example, by comparison to conventional white light imaging (Fig. 5A), the use of a fluorescent-labeled peptide may improve our ability to detect HGD (Fig. 5B). Here, the FITC-labeled peptide ASYNYDA-GGSK-FITC has been topically applied during endoscopy to bind to cells found to be HGD on histology (47). On

the contrary, labeled peptide probes produce increased fluorescence intensity at the site of neoplastic mucosa, making them more desirable for in vivo use (48).

Molecular Imaging and its Implications for treatment

Protein targets that are over-expressed late in the progression of adenocarcinoma can also be used by molecular imaging to guide therapy. Current treatments for esophageal adenocarcinoma result in only a modest survival advantage, and even post-operatively, only 25% of patients survive after 5 years (49). Conventional chemotherapy, radiation and surgical treatments have not resulted in markedly improved prognosis and are associated with significant side effects. In comparison, targeted agents against EGFR and VEGF have improved outcome in other GI malignancies, such as colorectal cancer (49), increasing the interest for expanded use of targeted therapies as adjuncts to current treatment strategies for esophageal adenocarcinoma.

Targeted Therapies for Esophagogastric Cancer

Many recent studies have focused on targeted treatments for esophagogastric cancers, including agents against EGFR, Her2/neu and VEGF. Most of the targeted treatments are still in early phase clinical trials; however some recent studies have shown promising results, a summary is shown in Figure 6. Knowledge of the relative levels of target expression in individual patients is needed to select the correct agent. Currently, testing for target expression is done using multiple laboratory-based methods including: immunohistochemistry, DNA microarray, quantitative PCR, and fluorescence in-situ hybridization (FISH). However, testing for receptor positivity with these methods is limited by tissue sampling error. Target protein expression can be varied within the tumor and even within individual glands (50). Thus, when the tissue is processed, positive and negative regions can easily be combined or missed, depending on where the sample was taken. Again, molecular imaging can overcome this limitation by providing highly localized spatial information about target locations during endoscopy.

Anti-EGFR treatment

Current anti-EGFR therapies that have been evaluated in esophagogastric cancers include the monoclonal antibodies: cetuximab, panitumumab and matuzumab and the tyrosine kinase inhibitors: erlotinib and gefitinib (51).

Cetuximab

For patients with unresectable or metastatic esophagogastric cancer (which account for more than 50% of new cases) the prognosis is poor with a median survival of less than one year (51). Currently, eight trials have evaluated the use of cetuximab in various combination chemotherapy regimens for patients with metastatic disease. Cetuximab works primarily by blocking EGFR as well as stimulating EGFR internalization by endocytosis ((52,53) Overall, the trials reported encouraging objective response rates of 40–69% and median overall survival of 9.5–17 months (45).

Matuzumab

The efficacy of matuzumab was recently evaluated in a study by Rao et al (54) in previously untreated patients with advanced esophagogastric cancer. Patients were given matuzumab in combination with ECX chemotherapy (epirubicin, cisplatin and capecitabine). The regimen was associated with clinically meaningful tumor response and the pharmacodynamic markers in skin reflected inhibition of EGFR signaling at all dose levels.

Anti-Her2/neu (ERBB2) Therapies

HER-2/neu oncogene amplification on FISH has been shown to correlate with reduced patient survival and appears to independently predict poor outcome in patients with Barrett's associated adenocarcinoma (55). Anti-Her-2/neu therapies under investigation include the monoclonal antibody trastuzumab and the tyrosine kinase inhibitor lapatinib.

Trastuzumab

Trastuzumab is thought to work via the following mechanisms: preventing Her2/neu receptor dimerization, increasing endocytosis and destruction of the receptor, inhibiting shedding of the extracellular domain and inducing antibody dependent cytotoxicity (56). Trastuzumab was recently evaluated in the ToGA trial in which patients with HER2-positive esophageal and gastric adenocarcinoma (locally advanced, recurrent, or metastatic) were randomized to receive trastuzumab + chemotherapy (5-fluorouracil or capecitabine and cisplatin) every 3 weeks for 6 cycles or chemotherapy alone. Results showed that the median overall survival was significantly improved with trastuzumab + chemotherapy compared to chemotherapy alone: 13.8 vs. 11.1 months, respectively ($p=0.0046$). The response rate was 47% in the trastuzumab + chemotherapy arm and 35% in the chemotherapy arm ($p=0.00178$) (51). These results were ground-breaking as they represent the first phase III evaluation of a targeted therapy in esophagogastric cancers with a positive response (51).

Anti-VEGF Therapies

Anti-VEGF agents are the focus of targeted therapy for many solid tumors. In esophageal adenocarcinoma, increased expression of VEGF correlates with the transition from Barrett's esophagus to HGD and from microinvasive to locally advanced cancer (56, 58). Anti-VEGF therapies under investigation include the monoclonal antibody bevacizumab and the multipletarget tyrosine kinase inhibitor, sunitinib.

Bevacizumab

Bevacizumab is thought to work via a direct anti-angiogenic effect as well as possibly exerting its anti-tumor effect by normalizing the "leaky" and disorganized vasculature within tumors which decreases interstitial fluid pressure and thus increases chemotherapy drug delivery (59,60). In a recent trial of patients with previously untreated metastatic gastroesophageal cancer, bevacizumab was added to the mDCF regimen (modified docetaxel, cisplatin, fluorouracil). The addition of bevacizumab was found to be tolerable and had notable long term patient outcomes: 6-month progression free survival of 79%, median overall survival of 16.8 months and 2 year overall survival of 37% (61).

Conclusion

Because of the focal and patchy nature of HGD in Barrett's esophagus, there is great need for novel imaging methods to improve the sensitivity of detection. Molecular imaging is an exciting new modality that visualizes the entire mucosal surface of the esophagus in real time. Novel probes such as peptides that target cell-surface receptors have vast potential to guide clinical use of targeted therapies and to reveal the unique protein expression patterns leading to early detection of cancer. Unlike all other current techniques, this one can provide details about molecular expression in the epithelium of the distal esophagus. The use of peptides as probes offers the advantages of specific detection, easy delivery, low cost and minimal concern for immunogenicity. Finally, the technique is not limited to known targets, and can be applied to new targets as they become identified on comprehensive genomic assays. Molecular imaging would allow clinicians and researchers to better stratify the risk of individual patients with Barrett's metaplasia, dysplasia and adenocarcinoma and to treat

patients based not only on the spot samples of histology, but also on a personal level formulated on the specific molecular changes of each lesion.

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References

1. Pohl H, Welch HG. The role of over diagnosis and reclassification in the marked increase of esophageal adenocarcinoma incidence. *J Natl Cancer Inst.* 2005; 97:142–46. [PubMed: 15657344]
2. Provenziale D, Schmitt C, Wong JB. Barrett's esophagus: a new look at surveillance based on emerging estimates of cancer risk. *Am J Gastroenterol.* 1999; 94:2043–2053. [PubMed: 10445526]
3. Spechler SJ. Columnar-lined esophagus. *Definitions Chest Surg Clin N Am.* 2002; 12:1–13.
4. Sampliner RE. Practice Guidelines on the diagnosis, surveillance, and therapy of Barrett's esophagus. The Practice Parameters Committee of the American College of Gastroenterology. *Am J Gastroenterol.* 1998; 93:1028–1032. [PubMed: 9672324]
5. Ronkainen J, Aro P, Storskrubb T, et al. Prevalence of Barrett's esophagus in the general population: an endoscopy study. *Gastroenterology.* 2005; 129:1825–1831. [PubMed: 16344051]
6. Sharma P. Barrett's Esophagus. *NEJM.* 2009; 361:2548–2556. [PubMed: 20032324]
7. Shaheen NJ, Crosby MA, Bozyski EM, et al. Is there publication bias in the reporting of cancer risk in Barrett's esophagus? *Gastroenterology.* 2000; 119:333–8. [PubMed: 10930368]
8. Bresalier RD. Barrett's esophagus and esophageal adenocarcinoma. *Annu Rev Med.* 2008 Sep 10.
9. Sarr MG, Hamilton SR, Marrone GC, Cameron JL. Barrett's esophagus: It's prevalence and association with adenocarcinoma in patients with symptoms of gastroesophageal reflux. *Am J Surg.* 1985; 149:187–93. [PubMed: 3966636]
10. Falk GW, Skacel M, Gramlich TL, et al. Fluorescence in situ hybridization of cytologic specimens from Barrett's esophagus: a pilot feasibility study. *Gastrointest Endosc.* 2004; 60(1):280–84. [PubMed: 15278064]
11. Canto M, Setrakian S, Willis J, et al. Methylene blue directed biopsies improve detection of intestinal metaplasia and dysplasia in Barrett's esophagus. *Gastrointest Endosc.* 2000; 51:560–8. [PubMed: 10805842]
12. Georgakoudi I, Jacobson BC, Van Dam J, Backman V, Wallace MB, Müller MG, Zhang Q, Badizadegan K, Sun D, Thomas GA, Perelman LT, Feld MS. Fluorescence, reflectance, and light-scattering spectroscopy for evaluating dysplasia in patients with Barrett's esophagus. *Gastroenterology.* 2001 Jun; 120(7):1620–9. [PubMed: 11375944]
13. Georgakoudi I, Feld MS. The combined use of fluorescence, reflectance, and light-scattering spectroscopy for evaluating dysplasia in Barrett's esophagus. *Gastrointest Endosc Clin N Am.* 2004 Jul; 14(3):519–37. ix. [PubMed: 15261200]
14. DaCosta R, Wilson B, Marcon N. Spectroscopy and fluorescence in esophageal diseases. *Best Practice & Research Clinical Gastroenterology.* February; 2006 20(1):41–57. [PubMed: 16473800]
15. Chen Y, Aguirre AD, Hsiung PL, et al. Ultrahigh resolution optical coherence tomography of Barrett's esophagus: preliminary descriptive clinical study correlating images with histology. *Endoscopy.* 2007 Jul; 39(7):599–605. [PubMed: 17611914]
16. Hamamoto Y, Endo T, Noshō K, Arimura Y, Sato M, Imai K. Usefulness of narrow-band imaging endoscopy for diagnosis of Barrett's esophagus. *J Gastroenterol.* 2004 Jan; 39(1):14–20. [PubMed: 14767729]
17. Rabinowitch PS, Longton G, Blount PL, et al. Predictors of progression in Barrett's esophagus III: baseline flow cytometric variables. *Am J Gastroenterol.* 2001; 96:3071–3083. [PubMed: 11721752]
18. Western AP, Sharma P, Topalovski M, et al. Long term follow-up of Barretts high grade dysplasia. *Am J Gastroenterol.* 2000; 95:1888–93. [PubMed: 10950031]

19. Buttar NS, Wong KK, Sebo TJ, et al. Extent of HGD in Barrett's esophagus correlates with risk of adenocarcinoma. *Gastroenterology*. 2001; 120:1630–9.
20. Schnell TG, Sontad SJ, Chejfec G, et al. Long term nonsurgical management of Barrett's esophagus with high grade dysplasia. *Gastroenterology*. 2001; 120:1607–1619.
21. Sharma P. Barretts esophagus: from process to outcomes. *Am J Gastroenterol*. 2001; 96:924–626. [PubMed: 11280587]
22. Souza R. Biomarkers in Barrett's esophagus. *Technique in Gastrointestinal Endoscopy*. 2010; 12:116–121.
23. Cronin J, McAdam E, Danikas A. Epidermal Growth Factor Receptor is Overexpressed in High Grade Dysplasia and Adenocarcinoma of the Esophagus and May Represent a biomarker of Histological Progression in Barrett's Esophagus. *Am J Gastroenterol*. 2011; 106:46–56. [PubMed: 21157443]
24. Miller C, Moy J, Lin L. Gene Amplification in Esophageal Adenocarcinomas and Barrett's with High-Grade Dysplasia. *Clinical Cancer Research*. Oct 15.2003 9:4819–4825. [PubMed: 14581353]
25. Montesano R, Hollsein M, Hainaul P. Genetci alterations in esophageal cancer and their relevance to etiology and pathogenesis: a review. *Int J Cancer*. 1997; 69:225–235. [PubMed: 8682592]
26. Al-Kasspoles M, Moore JH, Orringer M, Beer D. Amplification and overexpression of the EGFR and ERBB2 genes in human esophageal adenocarcinomas. *Int J Cancer*. 1993; 54:213–219.
27. Morgan RJ, Newcomb PV, Hardwick R, Alderson D. Amplification of cyclin D1 and MDM1 in oesophageal carcinoma. *Eur J Surg Oncol*. 1999; 25:364–367. [PubMed: 10419705]
28. Nicholson S, Richard J, et al. Epidermal-growth factor receptor : results of a 6-year follow-up study in operable breast cancer with emphasis on the node-negative sub-group. *Brit J Cancer*. 1991; 63:146–150. [PubMed: 1846551]
29. Slamon DJ, Godolphin W, et al. Studies of the HER-2neu proto-oncogene in human breast and ovarian cancer. *Science*. 1989; 244:707–712. [PubMed: 2470152]
30. Danilkovitch-Miagkova A, Zbar B. Dysregulation of Met receptor tyrosine kinase activity in invasive tumors. *J Clin Invest*. 2002; 109(7):863–867.10.1172/JCI15418 [PubMed: 11927612]
31. Miller CT, Lin L, Casper AM. Genomic amplification of MET with boundaries within fragile site FRAG7G and upregulation of MET pathways in esophageal adenocarcinoma. *Oncogene*. 2005:1–10.
32. Tuynman JB, Lagarde SM, Ten Kate FJ, Richel DJ, Van Lanschot JJ. Met expression is an independent prognostic risk factor in patients with oesophageal adenocarcinoma. *Br J Cancer*. 2008; 98:1102–8. [PubMed: 18349821]
33. Trusolino L, Comoglio PM. *Nat Rev Cancer*. 2002; 2:289–300. [PubMed: 12001990]
34. Willkomm P, Bender H, Bangard M, et al. FDG PET and immunoscintigraphy with 99mTc-labeled antibody fragments for detection of the recurrence of colorectal carcinoma. *J Nucl Med*. 2000; 41(10):1657–63. [PubMed: 11037995]
35. Hsiung P, Hardy J, Friedland S, Soetikno R, Du CB, Wu AP, Sahbaie P, Crawford JM, Lowe AW, Contag CH, Wang TD. Detection of colonic dysplasia in vivo using a targeted heptapeptide and confocal microendoscopy. *Nature Medicine*. 2008; 14:454–58.
36. Perkins AC, Missailidis S. Radiolabelled aptamers for tumour imaging and therapy. *Q J Nucl Med Mol Imaging*. 2007 Dec; 51(4):292–6. Epub 2007 May 1. [PubMed: 17464274]
37. Tolmachev V, Orlova A, Nilsson FY, et al. Affibody molecules: potential for in vivo imaging of molecular targets for cancer therapy. *Expert Opin Biol Ther*. 2007 Apr; 7(2):555–68. [PubMed: 17373906]
38. Baum R, Orlova A, Tolmachev V, Feldwisch J. Receptor PET/CT and SPECT using an Affibody molecule for targeting and molecular imaging of HER2 positive cancer in animal xenografts and human breast cancer patients. *J Nucl Med*. 2006; 47 (Supplement 1):108P.
39. Santra S, Dutta D, Walter GA, Moudgil BM. Fluorescent nanoparticle probes for cancer imaging. *Technol Cancer Res Treat*. 2005 Dec; 4(6):593–602. [PubMed: 16292879]
40. Jaiswal J, Mattoussi H, Mauro M, Simon S. Long-term multiple color imaging of live cells using quantum dot bioconjugates. *Nature Biotechnology*. 2002; 21:47–51.

41. Kairemo K, Erba P, Bergstrom K, Pauwels E. Nanoparticles in Cancer. *Current Radiopharmaceuticals*. 2008; 1:30–36.
42. Tolmachev V, Stone-Elander S, Orlova A. Radiolabelled receptor-tyrosine-kinase targeting drugs for patient stratification and monitoring of therapy response: prospects and pitfalls. *Lancet Oncol*. 2010; 11:992–1000. [PubMed: 20667780]
43. Gelovani JG. Molecular imaging of epidermal growth factor receptor expression activity at the kinase level in tumors with positron emission tomography. *Cancer Metastasis Rev*. 2008; 27:645–53. [PubMed: 18626573]
44. Borovicka J, Fischer J, Neuweiler J, et al. Autofluorescence endoscopy in surveillance of Barrett's esophagus: a multicenter randomized trial on diagnostic efficacy. *Endoscopy*. 2006; 38:867–972. [PubMed: 16981102]
45. Wolfsen HC, Crook JE, Krishna M, et al. Prospective, controlled tandem endoscopy study of narrow band imaging for dysplasia detection in Barrett's Esophagus. *Gastroenterology*. 2008; 135:24–31. [PubMed: 18442484]
46. Qiu L, Pleskow DK, Chuttani R, Vitkin E, Leyden J, Ozden N, Itani S, Guo L, Sacks A, Goldsmith JD, Modell MD, Hanlon EB, Itzkan I, Perelman LT. Multispectral scanning during endoscopy guides biopsy of dysplasia in Barrett's esophagus. *Nat Med*. 2010; 16:603–6. [PubMed: 20383155]
47. Goetz M, Wang TD. Molecular imaging in gastrointestinal endoscopy. *Gastroenterology*. 2010; 138:828–33. [PubMed: 20096697]
48. Li M, Anastassiades CP, Joshi B, Komarck CM, Piraka C, Elmunzer BJ, Turgeon DK, Johnson TD, Appelman H, Beer DG, Wang TD. Affinity peptide for targeted detection of dysplasia in Barrett's esophagus. *Gastroenterology*. 2010; 139:1472–80. [PubMed: 20637198]
49. Pande AU, Iyer RV, Rani A, et al. Epidermal Growth Factor Receptor-Directed Therapy in Esophageal Cancer. *Oncology*. 2007; 73:281–289. [PubMed: 18477853]
50. Wilikinson N, Black J, Rouukhadze E. Epidermal Growth Factor Expression Correlates with Histologic Grade in Resected Esophageal Adenocarcinoma. *Journal of Gastrointestinal Surgery*. 2004; 8(4)
51. Ku G, Ilson D. Esophagogastric cancer: Targeted agents. *Cancer Treatment Reviews*. 2010; 36:235–248. [PubMed: 20122806]
52. Goldstein NI, Prewett M, Zuklys K, Rockwell P, Mendelsohn J. Biological efficacy of a chimeric antibody to the epidermal growth factor receptor in a human tumor xenograft model. *Clin Cancer Res*. 1995; 1:1311–8. [PubMed: 9815926]
53. Baselga J, Norton L, Masui H, et al. Antitumor effects of doxorubicin in combination with anti-epidermal growth factor receptor monoclonal antibodies. *J Natl Cancer Inst*. 1993; 85:1327–33. [PubMed: 8340945]
54. Rao S, Starling N, Cunningham D, et al. Phase I study of epirubicin, cisplatin and capecitabine plus matuzumab in previously untreated patients with advanced oesophagogastric cancer. *Br J Cancer*. 2008; 99:868–74. [PubMed: 19238629]
55. Brien TP, Odze RD, Sheehan CE, McKenna BJ, Ross JS. HER-2/neu gene amplification by FISH predicts poor survival in Barrett's esophagus-associated adenocarcinoma. *Hum Pathol*. 2000; 31:35–9. [PubMed: 10665910]
56. Mobius C, Stein HJ, Becker I, et al. The 'angiogenic switch' in the progression from Barrett's metaplasia to esophageal adenocarcinoma. *Eur J Surg Oncol*. 2003; 29:890–4. [PubMed: 14624783]
57. Bang YJ, Van Cutsem E, Feyereislova A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet*. 2010; 376:687–97. 10.1016/S0140-6736(10)61121-X [PubMed: 20728210]
58. Vallbohmer D, Peters JH, Kuramochi H, et al. Molecular determinants in targeted therapy for esophageal adenocarcinoma. *Arch Surg*. 2006; 141:476–81. discussion 481–2. [PubMed: 16702519]
59. Jain RK. Normalizing tumor vasculature with anti-angiogenic therapy: a new paradigm for combination therapy. *Nat Med*. 2001; 7:987–9. [PubMed: 11533692]

60. Willett CG, Boucher Y, di Tomaso E, et al. Direct evidence that the VEGF specific antibody bevacizumab has antivasular effects in human rectal cancer. *Nat Med.* 2004; 10:145–7. [PubMed: 14745444]
61. Shah M, Jhaver M, Ilson D, Lefkowitz R, Robinson E, Capanu M, Kelsen D. Phase II Study of Modified Docetaxel, Cisplatin, and Fluorouracil With Bevacizumab in Patient With Metastatic Gastroesophageal Adenocarcinoma. *J Clin Oncol.* 2010; 28 by American Society of Clinical Oncology. 10.1200/JCO.2010.32.0770

Fig 1. Incidence and Disease-Specific Mortality of Esophageal Adenocarcinoma (1975–2001)

The incidence (thin line) of esophageal adenocarcinoma has increased dramatically over the last 30 years. Despite current treatments, disease specific mortality (thick line) has followed a similar trend. Reprint with permission.

Fig 2. Progression of esophageal cancer

The diagnosis of dysplasia in Barrett's esophagus is challenging even for the most experienced pathologists, as shown by histology (H&E) of (A) Barrett's esophagus (intestinal metaplasia) (B) LGD, (C) HGD, and (D) ACA.

Fig 3. DNA microarray

DNA microarrays can be used for determining which genes are upregulated in the progression from Barrett's esophagus to adenocarcinoma. Here, genes that are amplified on the SNP array can be used as candidates for targeted detection.

	Antibody	Small Molecule	Peptide	Aptamer	Nanoparticle	Affibody
Molecular Probe						
Strengths	↑ specificity ↑ diversity ↑ affinity known targets	intracellular imaging	↓ size ↑ diversity ↓ immunogenicity ↓ cost	↓ size ↑ diversity ↓ immunogenicity ↑ affinity	• narrow emission spectra • resistant to degradation	↓ size ↑ clearance ↓ cost
Weaknesses	immunogenicity ↑ cost ↓ quantity	Non-specific binding	↓ affinity degradation in vivo	↑ cost ↓ quantity ? toxicity	↓ light penetration in tissues	↓ detection ability due to lack of Fc fragment for 2° staining

Fig 4. Potential molecular imaging probes

Chart shows a comparison different classes of molecular probes being developed for targeted imaging.

Fig 5. Conventional and fluorescent imaging of high-grade dysplasia

A) Conventional white light endoscopy image of normal squamous esophageal epithelium and HGD. B) Endoscopic image of esophageal epithelium with fluorescent (peptide) probe targeted to HGD. Fluorescence improves image contrast and allows for easier detection of HGD.

Author	Target	Agents	Disease Stage	Histology	No. of Patients	Objective Response Rate	Overall Survival
Multiple (45)	Anti-EGFR	Cetuximab + Combination CT	Metastatic	GEJ and Gastric adenoCA	Range 38-52	40-69%	9.5-17 months
Rao et al (48)	Anti-EGFR	Matuzumab + ECX	Metastatic	GEJ and Gastric adenoCA	21	65%	Not stated
Bang et al. (51)	Anti-Her2/neu	Trastuzumab + Combination CT	Metastatic	GEJ and Gastric adenoCA	594	47%	13.8 months
Shah et al (55)	Anti-VEGF	Bevacizumab + mDCF	Metastatic	GEJ and Gastric adenoCA	44	67%	16.8 months

Fig 6. Summary of Targeted Agent Trials

Chart summarizes study outcomes for several targeted agents currently under investigation for treatment of gastric and gastroesophageal cancer.