Evaluation of her-2/neu as a marker of progression of Barrett's esophagus

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A. Study Purpose and Rationale

Esophageal adenocarcinoma previously a rare malignancy, representing only a minority of cases of esophageal carcinoma, has recently exhibited a dramatic increase in incidence. "The incidence of esophageal adenocarcinoma, has risen faster than that of any malignancy in the United States, with an estimated rise of 300% to 350% since the 1970s" (1). The major identified risk factors predisposing to the development of esophageal adenocarcinoma are long-standing gastroesophageal reflux disease and the resultant development of Barrett's esophagus. Despite the rapid increase in the incidence of esophageal adenocarcinoma, the prognosis of this disease remains dismal, with overall 5-year survival <25% (2). The poor outcomes for this cancer have focused clinical interest on Barrett's esophagus, the premalignant lesion of esophageal adenocarcinoma.

Barrett's esophagus describes esophageal metaplasia from the norinal squamous lining to a columnar epithelium in response to gastric acid reflux. Barrett's esophagus is thought to represent the first step in a sequence of tumorigenesis consisting of metaplasia-dysplasia-carcinoma. Only a minority of patients with gastroesophageal reflux disease [GERD] go on to develop the histological changes of Barrett's esophagus and its associated increased cancer risk. "Classic long-segment Barrett's esophagus develops in approximately 15% of patients with GERD, and studies suggest that about 2.6 million Americans are likely to develop Barrett's esophagus" (3). Barrett's esophagus can be identified on endoscopy and patients with this histologic change are followed closely with frequent endoscopies to detect dysplastic transformation early before progression to invasive adenocarcinoma.

Published estimates of the risk of progression from Barrett's mucosa to esophageal adenocarcinoma range from 0.2 to 2.9% (4). Low-grade dysplasia [LGD], the first step in progression towards adenocarcinoma, is usually monitored with frequent endoscopic surveillance. A 19-year prospective study of 848 patients with LGD, found that 2.1% of these patients progressed to develop esophageal adenocarcinoma (5). However, once high-grade dysplasia [HGD] is identified, the risk of progression to adenocarcinoma appears high, ranging in reports between 16 and 59% (6, 7). In fact, 30-50% of patients who undergo prophylactic esophagectomy for HGD actually have invasive adenocarcinoma detected in the resected section (4). To date little is known about the molecular steps underlying the transformation of Barrett's metaplasia to a dysplastic lesion, and why only a small subgroup of patients run through the sequence of Barrett's esophagus-LGD-HGD-invasive cancer.

Her-21neu encodes a transmembrane tyrosine kinase receptor with substantial homology to epidermal-growth factor receptor (2). Overexpression of *her-2/neu has* been shown to cause malignant transformation of cells, stimulating cellular proliferation in the absence of growth factor stimulation (8). Her-2/neu is amplified in some cases of breast cancer, where it is associated with increased aggressiveness of disease. Trastuzumab (herceptin), a monoclonal antibody to HER-2, is clinically available and is used in the treatment of breast cancer with her-2/neu amplification. A small pilot study - published in August in Gastrointestinal Endoscopy - has described a high association of amplification of the her-2/neu gene with esophageal adenocarcinoma. In this pilot study, there was no amplification of this gene in 8 samples with metaplasia alone, but amplification was detected in 4 of 8 samples with adenocarcinoma (9). Two other small studies have used FISH [fluorescence in-situ hybridization] analysis to look at amplification of *her-2/neu in* Barrett's esophagus or esophageal dysplasia. Walch et al examined pathologic specimen from 25 patients with esophageal adenocarcinoma for her2/neu amplification, evaluating an archived series of specimen from these patients that included normal mucosa through metaplasia and finally adenocarcinoma (10). They report her-2/neu amplification in 0/13 samples

with normal mucosa, 0/4 samples with metaplasia, 0/8 with LGD, 2/12 (17%) with HGD, and 8/23 (35%) with adenocarcinoma. Brien et al examined 63 cases of esophageal adenocarcinoma for her-21neu amplification and found amplification in 12/63 (19%) (2).

In this study, we will perform a large cross-sectional analysis that assesses her2/neu amplification in LGD and HGD that develop& within Barrett's esophagus. This study will determine the frequency of her-2/neu amplification in LGD and HGD. In comparing the frequency of this genetic change, we hope to determine if her-2/neu amplification acts as a marker of progression of dysplasia or occurs as a step in turnorigenesis and advancement of disease towards adenocarcinoma.

,Our null hypothesis is there is no difference in the frequency of her-2/neu amplification in LGD and HGD. The primary alternative hypothesis is that her-2/neu amplification is preferentially seen with progression of dysplasia and occurs more frequently in HGD than in LGD.

B. Study Design and Statistical Analysis

This will be a cross-sectional study that uses FISH analysis to quantify the incidence of her-2/neu amplification in LGD and HGD. 100 consecutive patients with LGD and 100 consecutive patients with HGD who have recently presented to Dr. Charles Lightdale for endoscopy will be studied for her-2/neu amplification. Chi-square analysis based on the categorical outcome of her-2/neu amplification was used to determine the sample size for this study. This analysis was performed using conservative estimates of her-2/neu amplification from published data of 5% amplification among LGD samples and 20% arnplification among HGD samples. The number of specimen calculated to have 80% power to detect this 15% difference in her-2/neu amplification between the two groups was 89. A sample size of 100 specimens in each group will give us more than 80% power to detect a 15% difference between the two groups.

The chi-square test will be used to evaluate the difference in frequency of her*21neu amplification* between LGD and HGD. If there are fewer than five specimens with her-2/neu amplification, then the Fisher Exact test of proportion will be used in place of the chi-square test. Statistical significance will be set at $p<0.05$.

C. Study Procedure

Archived endoscopy specimens will be used for analysis of her-2/neu amplification. DNA will be isolated from these archived specimens collected at endoscopy. Once DNA is obtained, FISH analysis will be used to detect her-2/neu amplification. In FISH analysis, a fluorescent probe that is specific to the her-2/neu gene locus and a fluorescent probe specific for the centromere of chromosome 17 where her-2/neu is located are hybridized to DNA. The two probes have different colors: usually the her-2/neu locus lights up orange with hybridization and the centromere lights up green. The presence of more orange signals than green signals indicates amplification of the her-2/neu gene. Samples will be scored either as positive or negative for her-2/neu amplification based on the amount of her-2 fluorescence relative to the control provided by centromere fluorescence. The hybridized probes required for FISH analysis are available commercially and also in the pathology lab of New York Presbyterian Hospital where FISH analysis is used to assess breast cancer tumor specimens for her-2/neu amplification.

D. Study Drugs

No drugs will be used in this study.

E. Medical Devices

No medical devices will be used in this study.

F. Study Questionnaires

No questionnaires will be used in this study.

G. Study Subjects

Consecutive patients evaluated by Dr. Lightdale with endoscopy and found to have LGD or HGD will be included in this study. Archived endoscopy specimens will be used to obtain DNA for analysis. Patients who have undergone endoscopic therapies for HGD or adenocarcinoma and then on repeat biopsy down-graded to LGD or HGD will be excluded from this study. Patient demographics, such as male:female ratio and mean age, will be reported.

H. Recruitment of Subjects

Patients with Barrett's esophagus who have been evaluated by Dr. Lightdale and found on esophageal biopsy to have LGD and HGD within Barrett's esophagus will be recruited to this study.

I. Confidentiality of Study Data

All study data will be de-identified, maintaining the confidentiality of all patients.

J. Potential Conflict of Interest

There are no conflicts of interest in this study.

K. Location of the Study

The study' will be conducted in Columbia Presbyterian Medical Center. Endoscopy specimens are archived in the pathology department of CPMC. FISH analysis will be done in the research labs of CPMC. Analysis will be done in the office of Dr. Charles Lightdale.

L. Potential Risks

There are no potential risks to subjects in this study.

M. Potential Benefits

There are no immediate benefits to subjects in this study. Potential benefits include expanding our understanding of the molecular mechanisms underlying the development of esophageal adenocarcinoma. See Future Implications section below for detailed description of possible benefits of this study.

N. Alternative Therapies

No therapies will be given in this study.

O. Compensation to Subjects

There will be no compensation given to study subjects.

P. Costs to Subjects

There will be no costs for participation for study subjects.

Q. Minors as Research Subjects

There will be no minors recruited to this study.

R. Radiation or Radioactive Substances

No radiation or radioactive substances will be used in this study.

S. Future Implications

We hope to use our results in this study to evaluate whether her-2/neu can act as a marker of aggressive disease in early dysplasia, identifying patients with LGD likely to progress to adenocarcinoma, or whether this gene is involved in tumorigenesis. If there is no difference in the frequency of her-2/neu amplification between LGD and HGD, then her-2/neu amplification may identify samples with LGD that will progress to HGD. This has two key clinical implications: (1) evaluation of patients -with LGD for her-2/neu amplification will allow identification of high-risk patients who would need more intensive surveillance and possible early intervention and (2) trastuzumab, an anti-HER-2 antibody, may be used to treat patients with esophageal dysplasia with her-2/neu amplification, possibly as early as LGD to prevent further disease progression.

If we find little amplification of her-2/neu at the stage of LGD, as has been previously reported, and significant amplification at the stage of HGD, then her-2/neu amplification may represent a step in esophageal adenocarcinorna tumorigenesis. Her*2/neu gene* amplification may be one important molecular step underlying the transformation of early dysplasia to HGD and carcinoma. In this circumstance, trastuzumab would represent a rational, targeted therapy against esophageal adenocarcinoma that develops, in part, by amplifying the her-2/neu gene. As trastuzumab is widely used for treating her-2/neu positive breast cancer, it may be a new therapy that can be applied easily to the treatment of esophageal cancer to improve the outcome of patients with this cancer.

T. References

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