H. PYLORI-NEGATIVE GASTRITIS
WHAT TO DO WHEN HELICOBACTERS AREN'T THERE

Robert M Genta
Caris Diagnostics
and
University of Texas Southwestern Medical Center
Dallas, Texas

INTRODUCTION
H. pylori infection was recognized as the main cause of chronic active gastritis and peptic ulcer disease just over two decades ago, and shortly thereafter the first effective treatments were introduced. Since then, and likely even before its discovery, the prevalence of H. pylori infection has been steadily declining, particularly in industrialized and emerging economy countries; this is probably a reflection of improved sanitary conditions, widespread treatment of infected patients, and the pervasive use of antibiotics. In parallel with this decline, an increased proportion of H. pylori-negative ulcers has been reported, both in the United States and elsewhere. Furthermore, although no studies documenting this phenomenon have been published to date, there is a common perception amongst pathologists practicing in the Western world that chronic active gastritis with no detectable H. pylori organisms ("H. pylori–negative chronic active gastritis") is on the rise. The often cited 1990s axiom that even a small number of polymorphonuclear neutrophils in a gastric biopsy is an almost certain indication of current H. pylori infection is no longer applicable. While no formal hypothesis has been put forward and tested, commonly offered explanations include antibiotic therapy administered to treat other infections, the masking effect of proton-pump inhibitors (PPIs), and failure to detect organisms because of inadequate sampling or sub-optimal staining techniques.

The purpose of this presentation is to help the practicing pathologist confronted with a gastric biopsy that "looks like H. pylori should be there, but is not."

WHEN AND HOW TO SEARCH FOR H. PYLORI
Just as we used to say that active gastritis means H. pylori infection, we also said that there is no H. pylori infection without active gastritis. As it turns out, we were wrong on both accounts. As any experienced gastrointestinal pathologist will know, when an anti-H. pylori immunohistochemical stain (HP/IHC) is used in all gastric biopsies, one will occasionally find organisms in unexpected backgrounds, such as a virtually normal mucosa, an antral mucosa with reactive gastropathy and no active inflammation, or a fundic mucosa with a minimal subepithelial rim of lymphocytes and plasma cells and no neutrophils. Having been fooled by a few such cases, I advocate the preemptive use of HP/IHC in all gastric biopsies.
Those who cannot or choose not to routinely use the HP/IHC or other appropriate special stains for the detection of *H. pylori* must decide when to request such stains. When *H. pylori* are not detected by whatever means one uses routinely, a more sensitive stain (ideally the HP/IHC) should be used in the following circumstances:

1. Chronic active gastritis (CAG)
2. Focal active gastritis ("focally enhanced")
3. Chronic inactive gastritis with lymphoid follicles
4. Atrophic corpus gastritis, to exclude *H. pylori* before suggesting autoimmune gastritis
5. When a duodenal or gastric ulcer is described in the endoscopy report, irrespective of the appearance of the gastric mucosa in the biopsy
6. When the biopsies are obtained to confirm the success of eradication therapy
7. Whenever suspicious speckles that could be *H. pylori* are seen
8. If a MALT lymphoma is either seen or reported to have been treated in the past

In circumstances 2 through 7 the expected (and desirable) finding is the absence of *H. pylori*, as in the case of a treated MALT lymphoma. *H. pylori*-negative MALT lymphomas do exist, but they are a distinct minority (<10%); therefore, a diligent search for the organisms is necessary. If they are not found, a suggestion for more representative sampling is usually well received by clinicians, who also need to determine the extent of the lymphoma. The true dilemma occurs in the case of *H. pylori*-negative CAG.

**The 3R Approach to *H. pylori*-Negative CAG**

**Reconsider.** When *H. pylori* is not found by HP/IHC one should first reconsider the initial diagnosis of CAG. After critically reviewing ~400 gastric biopsy specimens that had been diagnosed as "CAG - No *H. pylori* detected by the *H. pylori* Blue stain" we found that only approximately 120 cases represented true CAG; most of the other cases were reactive gastropathy with small erosions (where neutrophils are usually abundant; chronic inactive gastritis (neutrophils may have been present in the lamina propria, but were not found in the epithelium); and specimens from the cardia (where active inflammation is usually associated with reflux and not with *H. pylori* infection). Of the real CAG cases, the HP/IHC yielded a little over another 25% new positives. If going back to one's own dubious diagnoses seems futile or painful, it may be helpful to consider that each previously undetected case is a patient who will be treated and will not develop peptic ulcers, and whose risk of gastric cancer will be reduced by more than 70%. Table 1 shows possible sources of inaccurate diagnoses of CAG.

**Re-stain.** If one is convinced that the histologic appearance is unequivocally that of CAG, a more sensitive stain should be examined. Thus, if only H&E-stained slides were prepared, a Giemsa (modified to Blue or Yellow) or a silver stain should be done; if still negative, the HP/IHC (which ought to be done in first place) should then be ordered. If the HP/IHC is negative and the impression of *H. pylori* gastritis is overwhelming, a second HP/IHC may be appropriate, particularly if the control was not perfect.

This sequential strategy will eventually yield cases of unequivocal CAG with no *H. pylori*. This is the time to
Retreat. After reviewing the slides, restaining, and perhaps staining once more, those who adhere to the precept "You are not obliged to finish the task, but neither are you free to neglect it" will be probably satisfied that they have not neglected the task and will feel free to desist from further action. They will issue a well-worded report stating that, in spite of the characteristic histologic appearance, no *H. pylori* was found after a meticulous search (and a long list of CPT codes). Such reports, however, are unlikely to gratify an inquisitive clinician, who will be left wondering what should be done. Therefore, striving to finish the task is a better alternative.

After a diagnosis of *H. pylori*-negative CAG
Calling the clinician and explaining the circumstances that lead to an equivocal diagnosis is probably the best way to avoid being perceived as a timid pathologist. Before discussing a case, however, it is necessary to be maximally informed.

Two common clinical circumstances may decrease the gastric *H. pylori* load: recent use of antibiotics and proton-pump inhibitors. Other causes for the failure to detect organisms in a gastric biopsy are listed in table 2.

Antibiotic regimens not specifically prescribed for the treatment of *H. pylori* can temporarily attenuate or eradicate the infection in a proportion of patients, depending on the type of antibiotic used, the length of the treatment, and whether or not the patient was coincidentally using PPIs. In most patients intentional or unintentional eradication causes the disappearance of polymorphonuclear neutrophils from the gastric mucosa within days of the start of the therapy\(^{20, 21}\), leaving the histopathologic impression of a chronic inactive gastritis. Incomplete and unsuccessful eradication, however, may greatly reduce the bacterial load (thus making them undetectable in certain parts of the stomach) with little or no decrease of active inflammation. If one can elicit a history of recent antibiotic treatment, a significant step is made in finding an explanation for *H. pylori* CAG. Furthermore, the pathologist can predict that the infection, inadequately treated, will soon reemerge, and a new set of biopsies in a few weeks will likely show organisms.

A much more common reason for the apparent disappearance of *H. pylori* from some compartments of the stomach (particularly the antrum) is the use of proton pump inhibitors (PPIs).\(^{10}\) Now available in more than ten different preparations, some of which can be obtained over the counter, PPIs alter the gastric acid environment and induce shifts in the *H. pylori* populations within the stomach, usually reducing the bacterial burden in the antrum\(^ {22}\) while increasing the inflammation in the corpus\(^ {23-25, 26-28}\). Not only do these changes cause false negative urea breath tests\(^ {29-31}\), but they have also been shown to hinder the histopathologic detection of *H. pylori* in gastric biopsies\(^ {28}\). Thus, many diagnoses of *H. pylori*-negative CAG in antral biopsies could be avoided if samples from the gastric corpus were also available.

These explanations may convince the clinician to perform a non-invasive test (serology, urea breath test, fecal antigen detection) or to repeat the endoscopy and take more representative biopsies. Nothing could be worse than leaving the impression that *H. pylori*-negative CAG is a final diagnosis and the patient does not need further workup.
Table 1 - Causes of *Helicobacter pylori*-negative chronic active gastritis

<table>
<thead>
<tr>
<th>Causes</th>
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<tr>
<td>Crohn’s Disease</td>
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<tr>
<td>Focally enhanced gastritis</td>
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<tr>
<td>Lymphocytic gastritis with a strong active component</td>
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<tr>
<td>Autoimmune gastritis with a strong active component</td>
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<tr>
<td>Reflux carditis (activity limited to the cardia)</td>
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<td>Drug-related (e.g. NSAIDs)</td>
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<td>Biopsy near an erosion or ulcer</td>
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<td>Granulomatous gastritis</td>
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<td>Infectious (CMV, staphylococcus, syphilis)</td>
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Table 2 - Possible reasons for failure to detect *H. pylori*

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<tr>
<th>Reason</th>
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<tr>
<td>Diagnostic error</td>
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<tr>
<td>Bacteria were missed</td>
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<tr>
<td>Condition is not chronic active gastritis</td>
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<tr>
<td>Sampling error</td>
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<tr>
<td>Proton pump inhibitors shift bacteria from antrum into body</td>
</tr>
<tr>
<td>Factors causing an unfavorable local environment for bacteria</td>
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<tr>
<td>Intestinal metaplasia</td>
</tr>
<tr>
<td>Ulcer</td>
</tr>
<tr>
<td>Suppression resulting from incidental antibiotic or proton pump inhibitors use</td>
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REFERENCES


Gastric Lumps and Bumps: A tale of two polyps
Henry D. Appelman, M.D.
Department of Pathology
University of Michigan
appelman@umich.edu

General Comments

The stomach is far less rich in polyps than is the colon. As a result, we know much less about gastric polyps than colonic polyps, and the literature tends to be much more recent and sparser. About 5 to 10% of biopsies of endoscopic gastric polyps are normal mucosa. The reasons for this include the biopsy forceps missing the lesion, an intramural lesion that is not available to endoscopic biopsy, normal mucosa that somehow turned into an endoscopic bump and finally the pathologist doesn’t know a polyp when he or she sees one. There are about 10 named gastric polyps involving the mucosa including two surface adenomas, a deep adenoma, two heterotopias, juvenile and Peutz-Jeghers polyps, polyps with no names and no literatures and finally the two that will be discussed here, fundic gland polyps and hyperplastic polyps. From a clinician’s standpoint, the main issue is whether the polyp is neoplastic, and if it is, then whether it is benign or malignant. If it is not neoplastic, then in general, there does not seem to be a great interest in polyp type, but we type it anyway. There are a few exceptions to this. It appears from our practice that there is so little interest on the part of the endoscopists in the mucosa adjacent to a polyp or in the mucosa in the rest of the stomach, that it is only biopsied infrequently.

The two common polyps, hyperplastic and fundic gland, have not been rigidly defined in the literature or in the textbooks, and as a result, we do not know their histologic limits.

Hyperplastic polyps

Historical perspective: During the 1960’s and 1970’s, the most common gastric polyp had three names from 3 different years, regenerative polyp in 1965, hyperplastic polyp in 1971 and hyperplaseogenous in 1973. The hyperplastic designation won over the other two. In the early literature, these polyps were described as mainly single, although a few patients made multiples. Histologically, they had a complex surface architecture, deep cysts, and excess stroma. The architecture included coarse villiform changes on the surface, ulcers both in the flat and villiform areas, an edematous inflamed lamina propria, striking distortion of the pits, and glands did not participate. There were a variety of different types of epithelium including normal pit or foveolar epithelium, hypertrophic pit epithelium with huge distended goblet cells, and regenerative epithelium with the typical syncytial undifferentiated cytoplasm and vesicular nuclei that characterize regenerative epithelium everywhere. In some polyps, the epithelium is more atypical with elongated stratified nuclei, more frequent mitoses, and less cytoplasmic mucin. Intestinal metaplasia occurs, but is not a dominant component, and in one study from Japan, it was mentioned that the likelihood of finding intestinal metaplasia in the polyp was less than
finding it in the surrounding mucosa$^{11}$. Of course this comment was based on Japanese stomachs which commonly have intestinal metaplasia. I cannot find comparable information on intestinal metaplasia in United States stomachs with these polyps.

Hyperplastic polyps are said to occur in three settings. The first, the sporadic polyp is the most common. The second and third settings are based on the findings of somewhat similar changes in polypoid mucosa on the gastric side of a gastroenteric anastomosis, and on the top of the proximal-most gastric folds, originally described in refluxers, but also found in non-refluxers. It turns out that the anastomosis and proximal fold polyps never quite achieve the distortion, exuberance and stroma of the sporadic polyps, so it is questionable whether they are all the same.

Elster in 1976 stated that “hyperplastic polyps of the stomach have no counterpart in other parts of the gastrointestinal tract and are thereby organotypical”$^{4}$. This was reemphasized by Hattori from Japan nine years later, and it is clear that this is a true analysis$^{11}$. We see occasional polyps in other sites, especially the colon, that have somewhat similar architecture and stroma, but they never quite look the same.

**Is there a universally accepted, clear-cut definition of the hyperplastic polyp?** In the literature and in the textbooks it becomes hardly any author ever actually defines it, but the authors do describe it, although the descriptions are not necessarily comparable. In one description from Stolte et al from Germany in 1995$^{12}$, a hyperplastic polyp was characterized by lengthening, tortuosity, and variable cystic dilatation of foveolae, widening of the stroma and edema, increased numbers and dilatation of the capillaries and apical erosions. However, this is a description and not a definition, and it does not tell us what features are required for the diagnosis of hyperplastic polyp and to what extent they must be present. As a result, whenever a study on hyperplastic polyps was published, it was not clear if the authors of those studies actually analyzed the same lesions. We also have no idea how the full-blown polyp develops. Does it start from a localized focus of pit expansion and stromal edema and inflammation or simply from a small polyp containing only elongated foveolae, a lesion which has been designated as focal or polypoid foveolar hyperplasia? Not only don’t we know how this lesion evolves, but we have no clue as to what is the stimulus for its development. We don’t know if it is inflammatory or neoplastic or a mixture. If we look at small lesions that have all of the architectural and stromal features, they appear to arise in the pit or foveolar compartment and look like they are tacked onto the surface. We must be aware of the possibility that since there are no minimal requirements for the diagnosis of hyperplastic polyp, a lot of things are thrown into that category that may not belong there, and some of these are presumably included in published studies of hyperplastic polyps. This is a common situation throughout not just pathology but medicine in general, namely a tendency to try to fit everything into existing categories, rather than separating them into categories that currently are not named.

**There is a fact (or rumor) that hyperplastic polyps occur in inflamed stomachs**$^{13,14}$, as much as 40% with Helicobacter pylori, and others with atrophic gastritis including the autoimmune type. Reports also indicate that there may be additional chemical or reactive gastropathy, but this is so common in stomachs these days, that its significance is questionable. Data such as this suggests that these are inflammatory lesions, but we don’t know that for a fact.
It is also stated that dysplasia occurs in anywhere from 1 to 20% of hyperplastic polyps, and this is size related with more dysplasia in larger lesions. Cancers have even been described in a very, very few polyps. There is clearly something wrong with data that puts anything between 1 and 20%. The inflammatory and dysplastic data had been based on studies of a polyp that had and still has no minimal diagnostic criteria and also on publications some of which are based on studies including only whole intact polyps and on other studies that included biopsies as well which potentially introduced sampling problems.

What separates low-grade dysplasia from regenerative epithelium in these polyps is exactly the same thing that separates low-grade dysplasia from regenerative epithelium everywhere else in the gastrointestinal tract. It is sometimes impossible to tell which kind of epithelium is present. These polyps are sitting in stomachs that contain a lot of solid material and which have churning and mixing motility activities, so these polyps are probably banged around a lot. Therefore, whatever the authors call this epithelium is what it gets published as, and perhaps this accounts for the dysplasia rate that varies from 1 to 20%. Furthermore, is there anything that separates a hyperplastic polyp with dysplasia from an adenoma with secondary hyperplastic polyp changes? We have seen some adenomas in which fairly extensive hyperplastic polyp-like changes involve the surface. Biopsies may exacerbate this distinction because of sampling.

The neoplastic associations, regardless of how we interpret the data, color surveillance recommendations. For instance, the American Society for Gastrointestinal Endoscopy has published guidelines in 2006 that are available on its website (www.ASGE.org). These guidelines state that the polyps should be endoscopically excised wherever feasible and clinically appropriate. No surveillance endoscopy is necessary after adequate sampling (there is no definition of adequate sampling) or removal of nondysplastic polyps. Topographical biopsy “mapping” may be useful to detect the presence of gastritis and intestinal metaplasia.

Topographical biopsy mapping is not done at our institution. I am sure that there are institutions where it is the rule, but I don’t even know that for a fact.

Summary: we do not know what hyperplastic polyps are, what causes them, what their precursors are, and there are no minimal criteria for diagnosis. It is not clear that all hyperplastic polyp studies have actually studied the same polyps, so the results of these studied probably should not be pooled for analysis. The dysplasia/carcinoma risk is not settled. Nevertheless, we will keep trying!

**Fundic gland polyps**

**General Comments:** Beginning in the 1970’s we started seeing another gastric polyp which also had little published information. This is what we now call a fundic gland polyp (FGP). These days it is the most common of all gastric polyps, probably 7 to 1 over its closest competitor, the hyperplastic polyp. Sometimes these are part of a syndrome, familial adenomatous polyposis, that includes cancers, but cancers for all intents and purposes don’t occur in fundic gland polyps. They also tend to carpet the fundus in the body, especially in the patients with FAP. As with hyperplastic polyps, they also appear to be tacked onto the top of the normal oxyntic mucosa indicating that they have developed within the pit compartment.
Historical perspective: It appears that the first recognition or name for fundic gland polyp was by Elster in 1976. He referred to these as “cysts of gastric glands”, and then a year later he changes the name to “fundic gland cysts”. These were described as “fundic glandular cysts in otherwise normal gastric mucosa”. However, an analysis of the illustrations in his papers indicates that this otherwise normal gastric mucosa is really not normal but the superficial glands are disorganized, clustered, often budding and branching. Watanabe from Japan in an analysis of gastric lesions in FAP may have been the first to use the fundic gland polyp designation. In his paper, there was no definition of fundic gland polyp, just many illustrations and descriptions such as “simple hyperplasia of fundic glands”. Actually, it is difficult to recognize any hyperplasia, since there are fewer glands per unit area in FGPs than in normal oxyntic mucosa. What was mentioned in Watanabe’s paper was the fact that the glands were irregular, tortuous, sometimes branching; that is, they were disorganized. Before the Elster and Watanabe papers, we only recognized two types of gastric polyps, adenomas and hyperplastic polyps. Fundic gland polyps were probably included in the mix. We did not recognize them as separate, so we probably called them hyperplastic, since they did not look like adenomas. They don’t have adenoma-like dysplasia.

Diagnostic criteria: In the twelve months from August, 2005 through July, 2006, four pathologists in the gastrointestinal subspecialty sign-out service at the University of Michigan made the diagnosis of fundic gland polyps in 306 patients, 16 of whom had familial adenomatous polyposis. The age distribution of patients with these polyps corresponds to the age of the patients who were biopsied during upper endoscopy. There were twice as many women as men, but this has been found in other studies. Regardless, when I asked my colleagues, and even myself how to define a fundic gland polyp and to list the minimal diagnostic criteria, there was no consensus. Nevertheless, I showed them several polyps which had a variety of changes including short pits, long pits, clusters of glands beneath the surface that varied from area to area, cysts, some of which were gland cysts, some pit cysts and some mixed cysts, and expanded lamina propria which often had a lot of smooth muscle. Everybody diagnosed them as fundic gland polyps, and the basic reasons for diagnosis boiled down to “because they looks like it”. I showed the same polyps to a bunch of our house officers and they came up with the same diagnosis for the same reason. Therefore, we seem to know what a fundic gland polyp looks like, and we can teach other people how to recognize it, but we have a great deal of difficulty defining what it is leads us to that diagnosis.

As was true for hyperplastic polyps, there are no published minimal criteria that will allow a bump in the gastric oxyntic mucosa to be called a FGP. A look at the literature and in the textbooks and even in the World Health Organization Classification of Tumors published in 2000, the definitions are anything but uniform.

The Appelman Approach: FGPs for dummies (for what it is worth): FGPs are architecturally complex but cytologically simple lesions. They are architecturally complex in that the entire mucosa is structurally altered when compared to normal, and they are cytologically simple because all cells are mature gastric epithelial cells. Perhaps because of this combination, they have been referred to as hamartomas. There are two sets of architectural abnormalities, epithelial and stromal. The intensity of these changes varies greatly from one polyp to another. The epithelial architectural alterations involve both the pits and the glands. The pit changes involve length. In most areas of these
polyps, the pits are shorter than normal, but in some polyps, they are longer. Pits also extend deeply into the mucosa and form cysts. The gland changes are more complex and include clusters of glands beneath the surface, parietal cells in the upper parts of the pits or even on the surface, glands with irregular branches and buds, and cystic glands toward the middle and deeper parts of the polyps. Actually, there are many cysts that have a mixture of pit and gland epithelium. The stromal changes include an increase in the amount of lamina propria when compared to normal oxyntic mucosa. This bonus stroma may be edematous, may have inflammation, and often has smooth muscle bundles, probably not extending from the muscularis mucosae, considering that these polyps are tacked onto the surface. The bigger the polyp, the larger the number and size of the cysts. This suggests that these polyps enlarge by an increase in number and size of cysts.

**Fundic gland polyps are said to occur in three settings.** First are those that are associated with familial adenomatous polyposis, in other words in patients with a germline mutation in the FAP gene. Second are sporadic polyps, that is, not in FAP patients, but occurring in people who are not taking proton pump inhibitors. Third are sporadic polyps occurring in patients who have been treated with proton pump inhibitors.

**Genetic Changes:** The polyps in different patients with FAP do not look the same, but neither do the polyps from single FAP patients. However, they all have variations on the same abnormalities including pit and gland architecture and lamina propria changes. In one study on FAP patients with known germline APC gene mutations, APC gene alterations were found in at least one polyp in 9 of these 11 patients, but not all polyps from individual patients had the detectable gene alterations. Conceivably, this variation may be due to the fact that this is a 9-year-old study, and there may be better detection systems now. Furthermore, no individual patient had more than one APC gene alteration in the polyps.

Genetic changes were also analyzed in sporadic fundic gland polyps that have no APC gene defects. In one study from the USA, 52 of 57 polyps from 40 patients have beta-catenin mutations. In this study, there was no mention if the patients were taking proton pump inhibitors. In another study from Japan, 29 of 45 such polyps from 35 patients had the beta-catenin mutation, and none of these patients were on long term proton pump inhibitors. In both these studies, the mutations were found in both the pit epithelium and in the gland cyst epithelium in almost all polyps, and different mutations were found in different polyps from the same patients, in contrast to the findings of the FAP patients. In another study of patients taking proton pump inhibitors, CpG island methylation was found to be more common in sporadic polyps, but we have not had any data since the publication of that study 6 years ago.

**Do proton pump inhibitors cause FGPs?** Twenty years ago these polyps were curiosities, but there has been a striking increase in incidence that seems to coincide with the increased use of PPIs. The data regarding cause and effect are conflicting. In one study from the USA, two groups of patients were compared, a larger group not taking proton pump inhibitors who only had a single upper endoscopy, and a smaller group taking PPIs who had both an initial exam and a follow-up exam if they had no polyps at the first exam. Comparing these two groups, FGPs were much more common in the PPI group, but so were other polyps, and in this study, there was no second endoscopy for the group not taking PPIs. Also, FGPs were described simply as composed of cystic fundic glands, nothing more and nothing less.
The second study from Germany compared over 28,000 patients not taking PPIs with over 2200 patients on the drugs, and the prevalence of FGPs was identical\textsuperscript{22}. We do know that PPIs induce hypertrophy of parietal cells with formation of apical snouts, and they also induce fundic gland cysts. Both of these changes appear to increase with increasing length of time the patients are taking PPIs. Both of these changes appear to be secondary to the hypergastrinemia that results from inhibition of gastric acid production by the drugs.

FGPs contain many parietal cells. The parietal cells in the polyps seem to respond to PPIs as do the native parietal cells, namely they undergo hypertrophy and develop snouts. Perhaps PPIs do not cause FGPs, but they may make tiny ones bigger and endoscopically apparent as a result of the parietal cell hypertrophy and increase in gland cysts\textsuperscript{21}.

**Finally, there is some literature suggesting that in patients with fundic gland polyps, there is an increased risk for colonic adenomas and carcinomas.** However, the studies do not all come to the same conclusion\textsuperscript{9, 30}. In a recent study, FGPs were associated with increased prevalence of hyperplastic colonic polyps in men and colonic adenomas in women mainly over 60 years of age, but there was no increased association with adenocarcinoma.

**Summary:** Fundic gland polyps occur in familial adenomatous polyposis but also spontaneously, and their incidence has increased at the same time as has the use of proton pump inhibitors. They are architecturally complex and cytologically simple polyps. There is no proof that PPIs induce polyps with the same architectural complexity that occurs in FAP patients. The parietal cells in FGPs respond to PPIs exactly like parietal cells in flat mucosa. This may make some tiny FGPs enlarge and become endoscopic polyps. Genetic abnormalities have been found in both FAP and sporadic FGPs, and there may be some alteration in PPI associated polyps. The association between FGPs and colonic neoplasia is not clearly established. Finally, until we have a rigid definition of the minimal criteria for a fundic gland polyp, we will have no clue as to whether PPIs or anything else cause them.

**References for Stomach Polyps**

**General Polyp References**


Hyperplastic Polyp References

Fundic Gland Polyp References
Despite a marked decline in incidence in the West and some decrease in the East, gastric cancer remains a significant cause of morbidity and cancer related deaths worldwide. The prevalence of gastric cancer is closely related to prevalence of *Helicobacter pylori* infection and shows wide geographic variation. Subsequent chronic gastritis, atrophy, and intestinal metaplasia are lesions that confer a high risk for the development of gastric cancer, while gastric dysplasia, the penultimate stage of the carcinogenetic cascade, is a direct neoplastic precursor lesion. Evidence for gastric dysplasia as a direct precursor of adenocarcinoma stems from observational studies reporting high-grade dysplasia (HGD) in close proximity to 40-100% of early gastric cancers and 5-80% of advanced adenocarcinomas. Moreover, dysplasia is also a marker of increased risk for cancer elsewhere in the gastric mucosa.

As with gastric cancer, the prevalence of dysplasia shows wide geographic variations. The difference is likely related to variations in the genetic makeup of the population, as well as variations in environmental factors, such as the prevalence of *Helicobacter pylori* infection (and subtype) and the age at which the infection is acquired. The frequency of dysplasia also varies with the underlying etiology. For instance, prevalence rates of up to 40% have been reported in patients with pernicious anemia but the disease confers only a moderate increase in risk of developing gastric cancer. In the setting of familial adenomatous polyposis (FAP), flat or polypoid dysplasias, typically antral in location, are frequently multiple and may be seen in 2-50% of patients. Patients with a gastric remnant status post gastrectomy, Menetrier's disease, or Peutz-Jegher's syndrome also are at increased risk.
Classification of Gastric Epithelial Dysplasia

The classification of dysplastic lesions has been controversial, with various diagnostic criteria used across the world. Japanese authors refer to these as borderline (Group 3 or 4) lesions, while the terms gastric adenoma (for raised lesions) and gastric dysplasia (for flat/depressed lesions) have been widely used in the Western literature.\textsuperscript{32, 67, 77}

Earlier guidelines for the diagnosis and grading of gastric dysplasia embraced a three-tiered system of mild, moderate and severe dysplasia. As in any segment of the gastrointestinal tract, dysplasia was defined as "unequivocally neoplastic epithelium that may be associated with or give rise to invasive adenocarcinoma."\textsuperscript{54, 57, 66} Later schemes have proposed a two-tiered system of low- and high-grade dysplasia,\textsuperscript{48, 69, 70, 78} which has proven to be more reproducible and provides a clinically meaningful risk stratification.\textsuperscript{32, 47} The WHO recommends the terminology of non-invasive low-grade and high-grade intraepithelial neoplasia, and defines carcinoma as invasion into the lamina propria or beyond.\textsuperscript{34} However, the terminology of adenoma/dysplasia is widely entrenched, and continues to be used, particularly in North America.

A significant debate has occurred over differentiating adenocarcinoma from high-grade dysplasia. Furthermore, the complexity of cyto-architectural features has been considered to be of paramount importance for the diagnosis of carcinoma in Japan, while breach of the basement membrane and invasion into the lamina propria has been considered the \textit{sine qua non} of malignancy and hence a prerequisite for the diagnosis of cancer in the West.\textsuperscript{47, 76} As an attempt to bridge differences, the Vienna classification was developed as a consensus between Western and Asian investigators.\textsuperscript{78} This consensus view takes into account the discrepancies in the reporting of dysplasia between Japanese and Western pathologists. For example, non-invasive intramucosal neoplastic lesions with high-grade cellular and/or architectural atypia are classified as "intramucosal carcinoma" in Japan, whereas similar lesions are diagnosed as high-grade dysplasia by most Western pathologists. In the Vienna classification, high-grade lesions without invasion of the lamina propria and adenocarcinomas with invasion confined to the lamina propria, are now placed into a single diagnostic category, a rationale supported by current endoscopic management.
**Phenotypic variants of gastric dysplasia**

Most examples of dysplasia have an "intestinal" phenotype, i.e., resembling colonic adenomas. These lesions are commonly referred to as adenomatous (or type I) dysplasia. The histologic characteristics include crowded glands lined by tall columnar cells with pencillate, overlapping, and hyperchromatic nuclei which show pseudostratification and inconspicuous nucleoli. Other less common histologic variants include foveolar (type II or non-adenomatous) dysplasia and pyloric type dysplasia. The distinctive feature of Type II dysplasia is the presence of glands lined by either low cuboidal or columnar epithelium with pale-clear cytoplasm, round-oval, vesicular nuclei and variably prominent nucleoli. Although prior studies have suggested that this form of dysplasia is almost always low-grade, more recent studies indicate that Type II dysplasia may be associated with distinct clinico-pathological characteristics and is more often high-grade when evaluated in a high-risk population. Some authors have indicated that Type II dysplasia is more commonly associated with poorly-differentiated adenocarcinoma.

**Pyloric** type dysplasia is a recently recognized type of dysplasia. Frequently observed in the body fundus, it is commonly seen in the older population. Some series indicate that it is commonly shows high grade dysplasia.

**Tubule neck (or globoid)** dysplasia is exceedingly rare and is believed to be a precursor of diffuse-type gastric carcinoma. It occurs in non-metaplastic gastric epithelium and appears as enlarged, clear cells occupying the gland neck region and confined within the basement membrane.

In the setting of inherited germline E-cadherin/CDH1 gene mutation, prophylactic gastrectomies have shown examples of "signet ring cell carcinoma in situ," often with a "pagetoid" spread between the gastric foveolar and glandular epithelium within the basement membrane. These changes are often multifocal and have a predilection for the proximal stomach and the body-antral transitional zone.
Grading of gastric dysplasia

The two-tiered scheme of low and high grade is widely used in all classification schemes. Practically, gastric biopsies need to be categorized into one of several categories: negative for dysplasia, indefinite for dysplasia, low grade dysplasia/adenoma, high grade dysplasia/adenoma, intramucosal carcinoma or an invasive adenocarcinoma.

Indefinite for dysplasia

There are cases for which one cannot establish a diagnosis with certainty. Commonly it means differentiating between reactive epithelial changes and dysplasia. It should be seen as a provisional designation that emphasizes the need to follow up the patient and to obtain additional biopsies. Alternatively, it should not be used as a wastebasket term for all cases with reactive atypia which are obviously in response to inflammatory or direct mucosal injury. Clues to the reactive nature of the epithelial changes includes the presence of vascular congestion and a gradual rather then abrupt transition between the atypical and adjacent normal cells.\(^5^4, ^6^6\)

Low-grade dysplasia (Adenoma; Non-invasive intraepithelial neoplasia-low grade)

We used the term "adenoma" for elevated mucosal lesions and low grade dysplasia for flat lesions that show minimal architectural disarray and cytological atypia.\(^3^2, ^4^7, ^5^4, ^6^6, ^9^5\) As mentioned earlier, in most cases, the morphological appearance is reminiscent of colonic adenomas and the lesions often occur in a background of intestinal metaplasia. The criteria for separating Type II dysplasia into low and high grade categories are not well established. The presence of gastric foveolar type epithelium with elongated, hyperchromatic nuclei that show some degree of pseudostratification is categorized as low-grade Type II dysplasia. Although a designation of low-grade implies a comparatively reduced risk of malignant transformation, it must be recognized that low-grade dysplasia occurring in a background of extensive intestinal metaplasia may be associated with a higher risk of malignancy.\(^7^1\)
High-grade dysplasia (Adenoma with high-grade dysplasia; Non-invasive intraepithelial neoplasia-high grade)

Marked cytological atypia or architectural complexity deserve a diagnosis of high-grade dysplasia. High grade dysplastic glands are commonly lined by rounded, pleomorphic nuclei that show prominent nucleoli and loss of polarity. Marked irregularities of the nuclear membrane and clumping of chromatin are also features often associated with high grade dysplasia. However, marked glandular crowding, budding, and intra-luminal bridges should raise the question of early gastric cancer. Typical or atypical mitoses may be present in either low grade or high grade dysplasia, but are more often and more easily discernible in the latter category.\textsuperscript{32, 47, 69}

Intramucosal adenocarcinoma

The controversy and disparity in literature regarding separation of "dysplasia" from "carcinoma" has already been alluded to above. The current approach to this problem is based on two facts: 1) "invasion," particularly when limited to the lamina propria, is difficult to identify on routine histology, and 2) intramucosal adenocarcinomas have a less than 10% risk of nodal metastases\textsuperscript{24} and neoplastic lesions with invasion of the lamina propria but confined to the mucosa are, therefore, amenable to a conservative approach through endoscopic resection. Currently, lesions which show marked architectural atypia in the form of fused glandular pattern, cribriforming or intra-luminal necrosis, as well as those that show definite evidence of invasion into the lamina propria in the form of single cells or small clusters of cells, are categorized as intramucosal adenocarcinoma.

Characteristics of intramucosal of adenocarcinomas

Intramucosal adenocarcinoma belong to the category of early gastric cancers (EGC), which are defined as invasive adenocarcinomas confined to the mucosa or submucosa, whether lymph node metastasis is present or not.\textsuperscript{29, 49} In Western series, EGCs represent between 15% and 21% of all newly diagnosed cancers, while in Japan, they account for over 50% of the cases.\textsuperscript{23, 28, 37, 83} The higher prevalence of gastric cancer, a more liberal use of upper endoscopy, perhaps a better technique including chromoendoscopy, and a difference in diagnostic criteria may explain the difference.
Most EGCs are small, measuring between 2 cm and 5 cm and localized on the lesser curvature and around the angulus.\textsuperscript{49, 55} Multiple tumors are seen in 3\% to 13\% of the patients, and are associated with a worse prognosis.\textsuperscript{23, 53}

The Paris classification divides EGCs into 3 types based on the endoscopic macroscopic appearance (figure 2): Protruded (type I), superficial (type II), and excavated (type III).\textsuperscript{40} Type II is further subdivided into IIa (elevated type), IIb (flat type), and IIc (depressed type). Superficial (type II) EGCs account for about 80\% of the cases, with type IIc being the most common subtype.\textsuperscript{90} Type IIb accounts for 58\% of small tumors measuring less than 5 mm.\textsuperscript{45} Notably, this endoscopic classification has shown to be a good indicator of the risk for nodal metastasis, reportedly low in type Ia or IIa EGC.\textsuperscript{19}

The majority of EGCs are well differentiated glandular carcinomas. Tubular and papillary variants represent 52\% and 37\% of cases, respectively, and can be difficult to differentiate from dysplasia (see above). Signet ring cell carcinoma and poorly differentiated carcinoma represent 26\% and 14\% of the cases, and are usually depressed or ulcerated (types IIc and III).\textsuperscript{23, 49, 90} Diffuse type EGCs tend to have a greater depth of invasion.\textsuperscript{19}

**Progression and outcome of early gastric neoplasms**

**A) Low-grade dysplasia**

Although assessing regression of low-grade dysplasia is difficult because of sampling issues and inter-observer variation in the diagnosis, it has been reported in 38-75\%, while persistence is seen in 19-50\% of cases.\textsuperscript{6, 9, 46}

Historical data have reported progression to adenocarcinoma in 0-23\% of patients with LGD within a span of 1-4 years, but recent studies indicated a lower risk of progression (0-9\%), while there is a significant risk of malignant transformation associated in high-grade (10-100\%).\textsuperscript{68, 91}
B) High-grade dysplasia

This diagnosis is more ominous, since HGD has been noted to persist in 14-58% of the cases and to progress to cancer in 60-85% of patients over a median interval of 4-48 months.\(^{20, 27, 43, 46, 70, 73, 91}\) Regression, though, also has been reported, and varies from 0-16%.

C) Intramucosal adenocarcinoma

In a series of patients diagnosed with EGC and followed without surgery, 63% of the tumors progressed to advanced carcinomas over a span of 6 to 88 months.\(^{84}\) However, when resected, the prognosis of EGCs is excellent, with a five-year survival rate greater than 90% in most series.\(^{17, 23, 26, 39, 83}\) The size and depth of invasion are the two major prognostic indicators, with the larger the diameter, the greater the risk of submucosal infiltration.\(^{28, 52, 93}\) Notably, the risk of invasion should not be overlooked even in very small tumors. In one series, 15.5% of 3-5 mm EGCs invaded the submucosa.\(^{62}\) Even for intramucosal EGCs, lymph node metastases have been reported in up to 7% of cases. However, the five-year survival remains close to 100%.\(^{23, 52, 93}\) For EGCs extending into the submucosa, the rate of lymph node metastases is between 8% and 25%, and the five-year survival is 80% to 90%.\(^{23, 93}\)

**Management of early gastric neoplasms**

Given the demonstrated low rate of malignant transformation of low grade dysplasia and the development of newer endoscopic imaging techniques, such as chromoendoscopy, annual endoscopic surveillance with re-biopsy is typically performed and surgical resection is not necessary.\(^{72, 88}\) Similarly, a diagnosis of indefinite for dysplasia should also prompt endoscopic surveillance and biopsy.

Although a diagnosis of high-grade dysplasia in years past was the indication for surgery, nowadays, this diagnosis as well as a diagnosis of intramucosal adenocarcinoma (provided deep submucosal invasion is ruled out with certainty with endoscopic ultrasound) will be managed endoscopically since *endoscopic mucosal resection* and *submucosal dissection* offer definitive therapy.
Endoscopic mucosal resection has rapidly become the treatment of choice in association with endoscopic ultrasound for staging. The primary criteria of EGC amenable to EMR are elevated lesions less than 2 cm in size, depressed lesions less than 1 cm in size without ulceration, and the absence of lymph node metastasis. Endoscopic submucosal dissection (ESD) is a more recent method developed in order to increase the en bloc and R0 resection rate, especially for lesions larger than 20 mm in diameter. Drawbacks of endoscopic submucosal dissection include the fact that it is technically a substantially more difficult procedure and that it is associated with a higher perforation rate.\textsuperscript{35, 59, 65, 92} Finally, the eradication of H. pylori improves prognosis of patients with early neoplasms. In a study of 132 patients with EGC who underwent EMR, no new cases of gastric cancer were observed after resection when H. pylori was eradicated; in contrast, 13.5\% of untreated patients had new early-stage intestinal-type gastric cancer.\textsuperscript{85}
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Update On Gastric Cancer: Molecular Pathology and Targeted Therapies
Antonia R. Sepulveda MD., PhD,
Department of Pathology and Laboratory Medicine,
University of Pennsylvania, Philadelphia, PA

Gastric carcinoma is the fourth most frequent cancer worldwide, representing the second most common cause of death from cancer (approximately 700,000/year)\(^1\). In the United States 21,259 new cases of stomach cancer were estimated in 2007, remaining stable at 21,130 new cases in 2009\(^2\). The incidence of gastric cancers (GC) involving the distal stomach, body and fundus, which have been associated with Helicobacter infection have declined over past decades, while adenocarcinomas of the cardia and gastroesophageal GE-junction (GEJ) is increasing. Combined figures for GE junction and gastric cancer indicate 1.4 million new cases diagnosed annually with 1.1 million attributed deaths\(^3\).

Clinical trials of targeted therapies for advanced gastric cancer have generally included gastric and gastroesophageal junction adenocarcinomas. Additionally, some trials used the classification of GE junction adenocarcinomas as described by Siewert, classifying GE junction carcinomas into types I, II and III depending on the relative extent of involvement of the esophagus and stomach\(^4\).

Histopathologically and genetically, gastric and gastro-esophageal junction cancers are heterogeneous and are influenced by gene-environment interactions resulting in activation of multiple molecular pathways. The molecular subtypes of gastric cancer include three main groups of tumors characterized by either the chromosomal instability pathway (CIN), the microsatellite instability pathway (MSI), and the CpG island methylator phenotype pathway (reviewed in \(^5\)). Currently, it is not clear whether and how these subtypes of gastric and GEJ carcinomas can be useful in clinical practice to predict specific pathways with mutational and regulatory alterations that may interfere with targeted therapies.

Surgical resection is the only potentially curative option for gastric cancer and is recommended for stages Tis-T3N0-N2M0 or T4N0M0\(^6\). For tumors not amenable to surgical curative resection, including locally advanced, recurrent, or metastatic cancers, a number of chemotherapy regimens can be used, albeit with limited success, such that 5 year survival rates for advanced gastric and GE junction cancers remain extremely poor at 20-50% for stages II-III and 5-10% for stage IV tumors. Recently, a number of agents that target specific molecules in cancer related pathways have become available and are being tested in patients with gastric and GE junction carcinomas. Here we will review the targeted therapies that have advanced to phase II or III clinical trials and offer promise in the treatment of these cancers. In addition, the specific roles of pathology and molecular testing as it relates to specific targeted therapies will be discussed.

**Cell surface receptor inhibitors**

**EGFR Family Inhibitors**

Current available therapies target the EGFR pathways through inhibition of the EGFR using two different mechanisms: 1) inhibition of the EGFR via monoclonal antibodies (i.e. cetuximab, matuzumab, panitumumab, trastuzumab) or 2) tyrosine kinase inhibitors (i.e. gefitinib, erlotinib).
The EGFR family of transmembrane receptor tyrosine kinases is composed of four members: HER1 (also known as the EGFR and erbB1), HER2 (p185, HER2/neu, ErbB-2), HER3 (also termed ErbB-3), and HER4 (also termed ErbB-4). The molecular structures of EGFRs include an extracellular ligand-binding domain, a short transmembrane domain, and an intracellular domain with tyrosine kinase (TK) activity (except HER3). The binding of different ligands, including epidermal growth factor (EGF) and TGF-alpha to the extracellular domain initiates a signal transduction cascade that contributes to neoplastic behavior including cell proliferation, apoptosis, adhesion, migration, and differentiation. Ligand binding induces EGFR homodimerization as well as heterodimerization with other types of HER proteins. HER2 does not bind to any known ligand, but it is the preferred heterodimerization partner for other members of the HER family. Ligand binding to the EGFR extracellular domain leads to EGFR activation followed by dimerization, resulting in phosphorylation of the intracellular tyrosine kinase which results in a series of intracellular signals including the activation of Ras/Raf/mitogen activated kinase (MAPK) or the AKT/mTOR pathways (reviewed in 7).

Table 1: Cell surface receptor inhibitors: Ongoing phase II and III trials for gastric and GEJ cancers (adapted from 7)

<table>
<thead>
<tr>
<th>Inhibitor-type</th>
<th>Drug</th>
<th>Clinical trials phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR Antibody</td>
<td>Cetuximab</td>
<td>III</td>
</tr>
<tr>
<td></td>
<td>Panitumumab</td>
<td>III</td>
</tr>
<tr>
<td></td>
<td>Matuzumab</td>
<td>I-II</td>
</tr>
<tr>
<td>EGFR tyrosine kinase inhibitors</td>
<td>Gefitinib</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td>Erlotinib</td>
<td>II</td>
</tr>
<tr>
<td>HER2-R Antibody</td>
<td>Trastuzumab</td>
<td>III</td>
</tr>
<tr>
<td>EGF/HER2-R</td>
<td>Lapatinib</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td>BIBW 2992</td>
<td>II</td>
</tr>
<tr>
<td>VEGF-R antibody</td>
<td>Bevacizumab</td>
<td>III</td>
</tr>
<tr>
<td>VEGF-R tyrosine kinase inhibitors</td>
<td>Vatalanib</td>
<td>II</td>
</tr>
</tbody>
</table>

**EGFR (HER1; erbB1) targeted therapy**

Cetuximab is a recombinant chimeric IgG1 monoclonal antibody that binds specifically to the extracellular domain of EGFR and competitively inhibits the binding of EGF and other ligands such as TGF-alpha. Cetuximab also mediates antibody-dependent cell cycle toxicity. Cetuximab is currently approved for patients with advanced colorectal and head and neck cancer 8. Currently there are several ongoing phase III trials evaluating cetuximab in combination with other chemotherapy agents for patients with advanced gastric and GE junction cancers.

Whether the molecular testing considerations regarding mutations in the Kras pathway will be used for treatment of gastric and GE junction cancers with cetuximab, as they are for colorectal cancer is not established 9, 10. A recent study 11 presented at the 2009 ASCO annual meeting assessed whether the mutational profile of KRAS and BRAF genes affected the response to cetuximab combination therapy in GC. In this study, the frequency of mutations in KRAS and
BRAF was lower as compared to colorectal cancer, and the mutational status of KRAS and BRAF genes did not correlate with the response to cetuximab-based therapy in advanced gastric cancer patients. Forty four tumor samples were collected from patients with locally advanced or metastatic GC undergoing cetuximab combination therapy as first-line treatment in two consecutive phase II studies, FOLCETUX Study and DOCETUX Study. The mutational status of KRAS (exon 2) and BRAF (exon 15) was detected by PCR amplification followed by direct sequencing. KRAS and BRAF mutations were detected in 5 (11.4%) and 1 (2.3%), respectively, of the 44 tumors analyzed. These frequencies are consistent with those previously reported in GC. The only BRAF mutation found in 1 sample was the classic V600E substitution. As a whole, 13.6% of the analyzed tumors carried a mutation in either KRAS or BRAF genes. KRAS and BRAF mutations were, as expected, mutually exclusive. As this study examined a small cohort, additional studies are warranted before clinical guidelines can be established.

Panitumumab is the first fully human monoclonal antibody (IgG2) specific to EGFR. It has been used successfully in patients with advanced colorectal cancer who failed standard therapies. A phase III trial (REAL III) is due to start investigating the role of panitumumab in combination therapy for locally advanced or metastatic gastric or GEJ cancer.

The EGFR tyrosine kinase inhibitors have proved minimal evidence of efficacy in gastric carcinomas, but studies are ongoing.

HER2 (HER2/neu, ErbB-2) targeted therapies
HER2 has no known ligand (orphan receptor), and preferentially heterodimerizes with HER3 which lacks intrinsic tyrosine kinase activity. The HER2, and the HER2/HER3 heterodimer is likely to be the most effective complex for activating pathways downstream of EGF receptors.

Overexpression and amplification of HER2 has been described in 6-35% of gastric and GEJ adenocarcinomas (Table 2).
Trastuzumab (Herceptin) is a monoclonal antibody which specifically targets HER2 protein by directly binding the extracellular domain of the receptor. Trastuzumab enhances survival rates in both primary and metastatic HER2-positive breast cancer patients. The efficacy of trastuzumab in breast cancer patients has led to investigate its antitumor activity in patients with HER2-positive cancers, including gastric adenocarcinomas.
HER2/neu positivity rates have been reported to be more frequent in intestinal type gastric cancer (21.5%) than in diffuse gastric cancer (2%) or mixed types (5%) (Table 2). These findings were confirmed in the ToGA trial with HER2 positivity being more frequent in intestinal than diffuse/mixed cancer (32.2% vs 6.1%/20.4%), respectively. In addition, HER-2/neu amplification in gastric carcinoma is associated with poor outcome and has been shown to be an independent prognostic factor.

Results from the largest study to date (ToGA trial) evaluating the addition of trastuzumab (Herceptin) to chemotherapy in HER2-positive advanced gastric cancer were reported at the 2009 ASCO meeting. The ToGA trial is the first randomized Phase III trial providing prospective information on HER2-positivity rates in GC (Table 2). The trial enrolled 3,883 patients from 24 countries. A HER2-scoring system modified from the protocol in breast cancer was used: a score of IHC 3+ and/or FISH positive was defined as HER2 positive. The modified HER2-scoring system showed concordance between IHC and FISH results of 87.5%. In breast cancer most IHC 0/1 samples are FISH negative but, in the ToGA cohort, the frequency of IHC
0/1 samples testing FISH positive was almost as high as IHC 2/FISH-positive samples (23% vs. 26%). The study reported an overall HER2-positivity rate of 22.1% evaluated from 3807 patients.

In the ToGA trial, patients with HER2-positive gastroesophageal and gastric adenocarcinoma (locally advanced, recurrent, or metastatic) were randomized to receive Trastuzumab (H; Herceptin) H+CT (5-fluorouracil or capecitabine and cisplatin) q3w for 6 cycles or CT alone. The primary end point was overall survival (OS); secondary end points included overall response rate (ORR), progression-free survival, time to progression, duration of response, and safety. Median OS was significantly improved with H+CT compared to CT alone: 13.5 vs. 11.1 months, respectively (p=0.0048; HR 0.74; 95% CI 0.60, 0.91). ORR was 47.3% in the H+CT arm and 34.5% in the CT arm (p=0.0017). This first randomized trial investigating anti-HER2 therapy in advanced GC showed that H+CT is superior to CT alone. The OS benefit indicates that H is a new, effective, and well-tolerated treatment for HER2-positive GC.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Number</th>
<th>Region</th>
<th>Over-expression (%)</th>
<th>IHC method</th>
<th>Amplification (%)</th>
<th>Method</th>
</tr>
</thead>
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<tr>
<td>Yano et al.</td>
<td>200</td>
<td>Japan</td>
<td>23</td>
<td>HercepTest</td>
<td>27</td>
<td>FISH</td>
</tr>
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<td>Gravalos et al.</td>
<td>166</td>
<td>Europe</td>
<td>13</td>
<td>HercepTest</td>
<td>if IHC 2+</td>
<td>FISH</td>
</tr>
<tr>
<td>Allgayer et al.</td>
<td>203</td>
<td>Europe</td>
<td>91</td>
<td>Elite kit</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Park et al.</td>
<td>Gast: 182</td>
<td>Korea</td>
<td>16</td>
<td>HercepTest</td>
<td>7 patients</td>
<td>FISH/CISH</td>
</tr>
<tr>
<td>Lordick et al.</td>
<td>1527</td>
<td>Europe; Asia; Latin America</td>
<td>22*</td>
<td>HercepTest</td>
<td>-</td>
<td>FISH</td>
</tr>
<tr>
<td>Tanner et al.</td>
<td>Gast: 131 GEJ: 100</td>
<td>Europe</td>
<td>-</td>
<td>-</td>
<td>12.2 24</td>
<td>CISH</td>
</tr>
<tr>
<td>Bang et al.</td>
<td>Gast; GEJ</td>
<td>14 countries</td>
<td>Gast: 20.9 GEJ: 32.2</td>
<td>HercepTest</td>
<td>-</td>
<td>FISH**</td>
</tr>
</tbody>
</table>

Table 2. HER2 expression and amplification in gastric and GEJ cancers (modified from Gravalos et al. 16) * HER2 overexpression by IHC or FISH** (PharmDx). IHC (immunohistochemistry); CISH (chromogenic in situ hybridization).

Antiangiogenic agents

The current status of anti-angiogenic agents for gastric and GE junction tumors has been recently reviewed. Tumor associated angiogenesis requires a number of pro-angiogenic factors, among which vascular endothelial growth factors (VEGF family A-D) play a key role in vasculogenesis and angiogenesis. A number of anti-angiogenic agents has been investigated or are undergoing clinical trials.
Bevacizumab is a chimeric monoclonal antibody that binds the VEGF-receptor and prevents the interaction of VEGF to its receptors (Flt1 and KDR) on the surface of endothelial cells. Clinical data of bevacizumab therapy in patients with advanced gastric or GEJ cancer is promising but is limited to phase II trials.

Another approach to inhibit the VEGF pathway uses tyrosine kinase inhibitors directed against the receptors of VEGF (Flt1 and KDR). There are several compounds available, some specifically targeting VEGF receptors, such as PTK787/ZK222584 (Vatalanib) and others that inhibit both the VEGF receptors and other tyrosine kinase receptors such as sunitinib and sorafenib. Phase II results are available for sorafenib, in which 44 patients with advanced gastric or GE junction cancers were treated with a combination of docetaxel, cisplatin and sorafenib, with an objective response rate of 38.6%. The median PFS was 5.8 months and the median OS was 14.9 months.

**Other targeted therapies for gastric and GEJ cancers**

A large number of specific inhibitors of molecular targets in gastric and GE junction cancer pathways are in the early stages of clinical trials, while supporting data for others is limited to pre-clinical studies. Among these agents are the insulin-like growth factor-I (IGF-IR) receptor inhibitors, fibroblast growth factor (FGF) receptor inhibitors and c-Met signaling pathway inhibitors. A number of cell cycle associated drug targets including Aurora kinase inhibitors, polo-like kinase inhibitors and cyclin dependent kinase (cdk) inhibitors are being tested.

Another approach has involved the use of inhibitors of cancer associated epigenetic changes. Histone deacetylase (HDAC) inhibitors are under considerable research given the potential to re-express tumor suppressor genes silenced by hypermethylation in cancer cells. A phase II clinical trial is ongoing.

Other agents under current trials include heat shock protein 90 (HS90) inhibitors, ubiquitin-proteasome pathway inhibitors, PI3K/Akt/mTOR pathway inhibitors and matrix metalloproteinase (MMP) inhibitors.
Bibliography


GASTRIC LYMPHOMAS:
25+ Years of Revolution/Evolution

Jerome S. Burke, MD
Department of Pathology
Alta Bates Summit Medical Center
Berkeley, California

The gastrointestinal tract is the most common location of extranodal lymphomas and the stomach in particular serves as an excellent model to illustrate many of the general issues concerning extranodal lymphomas. The stomach is the most frequent site of involvement of extranodal gastrointestinal lymphomas with small intestine second in frequency. Large intestinal and rectal lymphomas are less common, but are found in patients with AIDS and occasionally as a complication of ulcerative colitis and Crohn disease. In a study of 371 patients with primary gastrointestinal lymphomas registered in a German Multicenter Study, stomach accounted for 277 of cases (74.8%). Curiously, the incidence of primary gastric lymphomas in the United States is increasing, specifically in patients over 60 years of age; this increase appears independent of the AIDS epidemic and the now common diagnosis of extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT) type.

The concept of extranodal marginal zone lymphoma of MALT type has revolutionized the criteria for the morphologic diagnosis of an extranodal lymphoma, specifically those lymphomas dominated by small lymphocytes. Moreover, since the initial descriptions of lymphomas of MALT in 1983 and 1984 by Drs. Peter Isaacson and Dennis Wright, the criteria have evolved and expanded to encompass not only morphologic standards, specifically a more precise definition of various cell types in MALT lymphoma, the proposals for “lymphoepithelial lesions” and “follicular colonization,” the relationship between MALT lymphomas and marginal zone B cells, and a scoring system for diagnosis of gastric MALT lymphoma, but also to embrace immunophenotypic, biologic, molecular genetic and clinical discoveries. Such developments include refinement of the immunophenotype, the association of gastric MALT lymphoma and Helicobacter pylori infection, the notion of acquired MALT, the biology of auto-antigen activation and continuous somatic mutations, genetic aberrations, as for example the discovery of the t(11;18)(q21;q21) chromosomal translocation in lymphomas of MALT, and the clinical implications of specific cytogenetic alterations.

Revolution – Florid Gastric Lymphoid Hyperplasia (“Pseudolymphoma”) is Likely Malignant Lymphoma!
In establishing a histologic diagnosis of an extranodal lymphoma, pathologists are aware that various reactive lymphoid hyperplasias exist that mimic extranodal lymphomas clinically and pathologically, such as gastric lymphoid hyperplasia and chronic lymphocytic gastritis. To complicate matters, malignant lymphoma of extranodal marginal zone (MALT) type may develop in association with these reactive lymphoid infiltrates in the stomach.

In the 1960’s and 1970’s, the morphologic criteria to distinguish extranodal lymphoma from extranodal lymphoid hyperplasia were extrapolated from those used traditionally to distinguish malignant lymphoma from lymphoid hyperplasia in lymph nodes. The major criteria for this distinction included monomorphic lymphocytic infiltrates, cellular atypia, germinal centers and architectural disruption (Table 1).

<table>
<thead>
<tr>
<th><strong>Extranodal Lymphomas</strong></th>
<th><strong>Lymphoid Hyperplasias</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Infiltrate monomorphous</td>
<td>Infiltrate polymorphous</td>
</tr>
<tr>
<td></td>
<td>(lymphocytes in stages of transformation)</td>
</tr>
<tr>
<td>Cytologic atypia</td>
<td>Cytologic maturity</td>
</tr>
<tr>
<td></td>
<td>(lymphocytes, plasma cells, and immunoblasts)</td>
</tr>
<tr>
<td>Germinal centers uncommon</td>
<td>Germinal centers common</td>
</tr>
<tr>
<td></td>
<td>(usually in center of infiltrate)</td>
</tr>
<tr>
<td>Massive infiltration with architectural destruction</td>
<td>Random infiltration with architectural retention</td>
</tr>
</tbody>
</table>

Because more than 50% of gastric lymphomas are large B cell lymphomas with associated monomorphism, cellular atypia, and architectural destruction (e.g., obliteration of glands), these traditional criteria generally have proven to be reliable and applicable. Reactive lymphoid conditions that are at the opposite end of the spectrum equally do not pose diagnostic problems. Lymphocytic infiltrates that exhibit polymorphism, that display a range of mature lymphocytes (including plasma cells and immunoblasts), that are associated with well-defined germinal centers and that do not destroy completely the architectural landmarks of the stomach usually can be diagnosed confidently as benign and reactive.

The main difficulty in the separation of gastric extranodal lymphomas from
lymphoid hyperplasias concerns MALT lymphomas that are composed of small lymphocytes and that frequently are associated with germinal center formation. For these cases, the traditional histological criteria are not applicable. Multiparameter studies have revealed that many histologically ambiguous extranodal small lymphocytic proliferations in stomach are, in fact, monoclonal and, therefore, are presumed to be malignant lymphomas especially of MALT type. The application of immunologic and molecular genetic analyses to gastric small lymphocytic proliferations has served to vividly alter the traditional histologic criteria and has revealed myriad inconsistencies in these criteria (Table 2). For example, in a review of 97 cases originally diagnosed as gastric pseudolymphoma between 1970 and 1985 at the AFIP, 79% were reclassified as malignant lymphoma, with fully two-thirds of the newly classified lymphomatous cases interpreted as lymphomas of MALT type. The remaining cases were regarded as examples of lymphoid hyperplasia or atypical lymphocytic infiltrates. Consequently, the term “pseudolymphoma” is regarded as imprecise and anachronistic and no longer is acceptable as a diagnostic category.

**Table 2**

*Modifications of the Traditional Histologic Criteria for Distinguishing Extranodal Lymphomas From Lymphoid Hyperplasia*

- Extranodal lymphomas may be polymorphous (e.g., peripheral T cell lymphomas)

- Extranodal lymphomas may be composed of cytologically mature-appearing lymphocytes (marginal zone lymphoma of MALT type and other low-grade lymphomas with or without plasma cell differentiation)

- Germinal centers may be observed at the periphery of extranodal lymphomas and in the centers of many low-grade extranodal lymphomas, especially those of MALT type

- Degree of infiltration, architectural and epithelial destruction is highly variable in benign and malignant extranodal lymphocytic infiltrates

The conventional view was that extranodal lymphomas are characterized by nuclear membrane irregularities or atypia. Marginal zone lymphomas of MALT type, however, are cytologically mutable. Although MALT lymphomas typically are composed of marginal zone or centrocyte-like cells in the marginal zones, they also may have a monocytoid appearance, or they be composed of plasma cells, or they may have no, or only subtle, nuclear membrane irregularities similar to the cells of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL); the latter variant often is readily confused with extranodal lymphoid hyperplasia. A small lymphocytic proliferation that appears mature is the histologic norm of most cases of gastric lymphoid hyperplasia, yet cytologic maturity or minimal atypia is equally the histologic hallmark of most low-grade malignant lymphomas including those of MALT. How then
can these two groups be distinguished in a small biopsy specimen? In many cases, it is impossible to do when applying only histological criteria. This histologic dilemma prompted the use of the noncommittal term “extranodal small lymphocytic proliferation” to describe histologically ambiguous or indeterminate extranodal lymphocytic infiltrates. Current morphologic criteria used to separate a benign small lymphocytic infiltrate from a malignant small lymphocytic infiltrate includes the designations of whether the infiltrate is monomorphous, dense, or exhibits cytologic atypia or Dutcher bodies and whether this results in destruction of glands, follicles, or other structures indigenous to the stomach. If these morphologic features are unequivocally present, then the lymphocytic infiltrate likely is malignant lymphoma (see Table 3).

Our perspective of the small extranodal lymphocytic proliferations that appear mature has revised dramatically with the application of immunologic markers to analyze the clonality of these lesions. Correlative immunopathologic studies have now reduced the number of cases regarded as atypical or indeterminate. Naturally, cases remain, especially small biopsy specimens, in which the histologic features or immunologic findings remain equivocal; these cases should receive not only a descriptive diagnosis but also a request for a repeat biopsy with reservation of fresh tissues to determine clonality, whether by immunohistochemistry, flow cytometry, molecular techniques, or a combination thereof. When only fixed, paraffin-embedded tissues are available, a consistent determination of clonality in a biopsy specimen dominated by small lymphocytes usually is not possible in most laboratories. In some cases, however, immunologic studies of paraffin-embedded tissues can reveal an aberrant phenotype in the suspicious small lymphocytic population, such as the coexpression of B cell antigen CD20 and T cell antigen CD43. In most instances, the aberrant immunophenotype supports the diagnosis of an extranodal gastric B cell lymphoma. Prudence is necessary in the evaluation of immunoglobulin gene rearrangement studies employing polymerase chain reaction (PCR) techniques in fixed paraffin-embedded tissues; small monoclonal bands may occur in extranodal reactive lymphoid hyperplasias, as for example in chronic active gastritis associated with *Helicobacter pylori*.

As well as diagnostic problems encountered with the gastric lesions dominated by small lymphocytes, benign reactive germinal centers (lymphoid follicles) pose another risk in diagnosis. The presence of germinal centers is a commonly accepted histologic attribute of extranodal lymphoid hyperplasias. Germinal centers, however, are regular constituents of most gastric lymphomas of MALT. The essential morphologic characteristic of MALT lymphomas is their emulation of normal MALT as typified by Peyer patches found in the terminal ileum. The neoplastic B cells of MALT lymphomas are found in marginal-type zones surrounding reactive follicles and frequently attenuated rims of mantle zone lymphocytes. The germinal centers vary in appearance but commonly appear atrophic as a result of impingement by the
surrounding small lymphocytes. In MALT lymphomas of the stomach associated with peptic ulceration, germinal centers occur at the base of the ulcer and in the adjacent mucosa where they seem encroached upon and entrapped by the monotonous marginal zone lymphocytes. In some cases, the neoplastic B cells in the marginal zone invade the germinal centers in a process referred to as “follicular colonization.” Follicular colonization may simulate follicular lymphoma in cases where there are numerous follicles. At times, the lymphomatous proliferation may be so extensive as to result in architectural obliteration with masking of any residual germinal centers; however, the presence of former germinal centers can be highlighted by the immunohistochemical demonstration of follicular dendritic cells employing an antibody against CD21 or CD23.

**Evolution – Refinement of Morphologic Criteria, Immunophenotype, Biology, Molecular Genetics and Clinical Therapy of Gastric MALT Lymphomas**

The morphologic features of extranodal marginal zone lymphoma of MALT type have witnessed gradual changes since the initial descriptions by Isaacason and Wright. Although originally thought to be of follicular center cell origin, the lymphoma cells of MALT were later designated as “centrocyte-like” and currently are termed as “marginal zone cells.” Gastric lymphomas of MALT type typically are characterized by an expansion of the marginal-like zones surrounding benign germinal centers. As described, marginal zone cells constitute a variety of cell types ranging from small, to intermediate-sized lymphocytes, to large cells resembling immunoblasts. The marginal zone or centrocyte-like cells are small to medium-sized lymphocytes with variable nuclear membrane irregularities resembling a centrocyte or small cleaved cell. The most common form has abundant pale-staining cytoplasm and is referred to as monocytoid. Others forms of gastric MALT exhibit plasmacytoid features or resemble small lymphocytes. Occasionally, signet ring-type cells are observed in gastric MALT lymphomas and appear to represent a peculiar type of lymphoepithelial lesion in which the foveolar cells, disaggregated by the lymphomatous infiltrate, acquire a globoid, signet ring-type appearance. Regardless of cytologic composition, the marginal zone cells of MALT cells share immunophenotypic characteristics. Gastric MALT lymphomas are B cell derived with CD20 expression and frequently containing numerous admixed CD3-positive reactive T cells. Up to 50% of cases exhibit aberrant coexpression of CD43 which can prove helpful in diagnosis. Unlike CLL/SLL and mantle cell lymphomas, the lymphomas of MALT origin lack CD5 and are without bcl-1 gene rearrangements. MALT lymphomas differ from follicular lymphomas in that they are CD10 negative and do not exhibit rearrangements of the bcl-2 proto-oncogene. The absence of CD10 and bcl-2 positivity in gastric lymphoma with an apparent follicular architecture has been rationalized by the concept of “follicular colonization”. As discussed above, follicular colonization refers to the simulation of a follicular lymphoma as a result of invasion by the marginal zone cells of MALT into pre-existing reactive follicles.
The marginal zone cells of MALT not only are thought to invade residual reactive follicles, but also epithelium. As mentioned previously, epithelial invasion by the centrocyte-like cells of MALT has been referred to as a “lymphoepithelial lesion” and is an important morphologic feature in the diagnosis of gastric MALT-derived lymphomas. Such lesions may be accentuated by an immunohistochemical stain for cytokeratin. Lymphoepithelial lesions are most significant as a diagnostic characteristic in the stomach, but are less relevant in other extranodal sites since they may be observed in non-lymphomatous extranodal lymphocytic infiltrates. In stomach, lymphoepithelial lesions usually are defined as invasion of gastric epithelium by three or more lymphomatous cells. A decade following the primary proposal for a lymphoma of MALT, a histologic scoring system for gastric MALT lymphoma was proposed in which invasion of epithelial structures or lymphoepithelial lesions is considered paramount to the diagnosis (Table 3).

### Table 3
**Gastric Marginal Zone (MALT) Lymphomas: Histologic Scoring**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
<th>Histologic Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal</td>
<td>Plasma cells in lamina propria, no lymphoid follicles</td>
</tr>
<tr>
<td>1</td>
<td>Chronic active gastritis</td>
<td>Lymphocyte clusters in lamina propria, no follicles, lymphoepithelial lesions</td>
</tr>
<tr>
<td>2</td>
<td>Chronic active gastritis with florid lymphoid follicle formation</td>
<td>Prominent follicles with surrounding mantle zone &amp; plasma cells, no LELs</td>
</tr>
<tr>
<td>3</td>
<td>Suspicious lymphoid infiltrate in LP, probably reactive</td>
<td>Follicles surrounded by lymphocytes that infiltrate diffusely in LP and +/- epithelium</td>
</tr>
<tr>
<td>4</td>
<td>Suspicious lymphoid infiltrate in LP, probably lymphoma</td>
<td>Follicles surrounded by CCL cells (MZC) that infiltrate diffusely in LP &amp; epithelium</td>
</tr>
<tr>
<td>5</td>
<td>MZ (MALT) lymphoma</td>
<td>Dense diffuse infiltrate of CCL cells in LP with prominent LELs</td>
</tr>
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</table>


Employing the scoring system, a definite diagnosis of low-grade B cell lymphoma of MALT is based on the presence of a dense diffuse infiltrate of centrocyte-like cells/marginal zone cells in the lamina propria with prominent lymphoepithelial lesions. Cases regarded as suspicious lymphocytic infiltrates are those in which reactive follicles are surrounded by marginal zone cells that diffusely infiltrate into the lamina propria and into epithelium in small groups. However, caution is required as there may
be dense infiltrates, slight cytologic atypia, and also lymphoepithelial-like lesions in cases of lymphoid hyperplasia in the stomach. As well, the criteria may be difficult to apply. In an interobserver study, 41 H&E sections of stomach that ranged from simple gastritis to lymphoma were reviewed by 17 pathologists, including hematopathologists, GI pathologists and general pathologists. Interobserver reproducibility was suboptimal and the degree of disagreement was directly related to the pathologist’s experience in evaluating gastric biopsies for MALT lesions. The study recommended that diagnostic accuracy would be enhanced by clinical information, extensive sampling, recognition of lymphoepithelial lesions, immunophenotypic information and cytogenetic results. This recommendation was supported by another report stating that a combination of the morphologic scoring system and B cell clonality analysis by an advanced PCR method accurately discriminated chronic gastritis from covert gastric marginal zone lymphoma. PCR was particularly valuable in the interpretation of cases that exhibited an ambiguous score of grade 3 or 4.

Paradoxically, most MALT-type lymphomas arise in extranodal sites without normal MALT, such as the stomach, while non-MALT lymphomas, for example Burkitt lymphoma, arise in MALT sites. In order to resolve this apparent paradox, it was proposed that lymphomas in non-MALT sites arise in a setting of “acquired” MALT. This non-indigenous extranodal lymphoid tissue is thought to be acquired secondary to an infection, such as Helicobacter pylori in the stomach, or an autoimmune disease. The lymphoid infiltrates in stomach associated with Helicobacter pylori seemingly predispose patients to malignant lymphoma. There currently is sufficient histological, clinical, and epidemiologic evidence for the virtually fixed association of gastric MALT lymphomas with Helicobacter pylori infection, particularly the CagA strain. For example, in a collaborative study of more than 230,000 patients whose serum had been stored, 33 cases of gastric lymphoma developed a median of 14 years after serum collection. The patients with gastric lymphoma were significantly more likely than matched controls to have evidence of previous Helicobacter pylori infection. In contrast, no association was discovered among 31 patients who had developed nongastric non-Hodgkin lymphoma and previous Helicobacter pylori infection. In addition, molecular analysis by PCR has documented the clonal progression from Helicobacter pylori-associated chronic gastritis to MALT lymphoma of the stomach and Helicobacter pylori provides the antigenic stimulus for prolonging the clonal expansion of gastric MALT lymphomas. This view has been supported by Isaacson’s group who have exhibited evidence of ongoing Ig gene mutations in most case of MALT-type lymphoma. The discovery of ongoing mutations reinforces the perception that direct antigen stimulation is paramount in the clonal expansion of gastric MALT lymphomas.

Clinically, gastric MALT lymphomas arise in adults with a peak in the seventh decade and with a male:female ratio of approximately 1.5:1. Patients commonly present with nonspecific gastritis and/or a peptic ulcer and at endoscopy, there often are reddened and slightly thickened rugae with superficial spreading of lesions without
formation of a tumor mass. The gastric lesions commonly are multifocal and most patients have stage IE disease. The linkage of *Helicobacter pylori* with gastric MALT lymphoma led to antibiotic therapy for treatment of low-grade gastric lymphomas of MALT and this therapy may induce sustained remissions in about 75% of patients. There are no apparent differences in survival and relapse-free survival between patients treated with antibiotics and with other modalities, such as local treatment, combined treatment, or chemotherapy; in fact, no consensus exists for the optimal treatment of primary gastric lymphoma. In one study of gastric MALT lymphomas treated by various modalities, the five year projected overall survival was 82%. One significant issue for pathologists is the interpretation of stomach biopsy specimens following antibiotic therapy for gastric MALT lymphoma. Clearly, no diagnostic problem exists if the biopsy reveals regression of the lymphoma with loss of lymphocytic aggregates in the lamina propria or if the biopsy exhibits a total absence of histological regression with persistence of lymphoma. Parallel to the histological scoring system employed for the initial diagnosis of gastric marginal zone lymphoma, a histological grading system has been proposed for treated patients. (Table 4).

**Table 4**

**Gastric MZ (MALT) Lymphoma: Post-Therapy Grading** *

<table>
<thead>
<tr>
<th>Score</th>
<th>Lymphoid Infiltrate</th>
<th>LEL</th>
<th>Stromal Changes</th>
</tr>
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<tbody>
<tr>
<td>CR</td>
<td>Absent or scattered plasma cells &amp; small lymphoid cells in the LP</td>
<td>–</td>
<td>Normal or empty LP &amp;/or fibrosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pMRD</td>
<td>Aggregates of lymphoid cells or lymphoid nodules in the LP/MM &amp;/or SM</td>
<td>–</td>
<td>Empty LP &amp;/or fibrosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rRD</td>
<td>Dense, diffuse, or nodular extending around glands in the LP</td>
<td>+/-</td>
<td>Focal empty LP &amp;/or fibrosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC</td>
<td>Dense, diffuse, or nodular</td>
<td>+/-</td>
<td>No changes</td>
</tr>
</tbody>
</table>

CR, complete histologic remission; pMRD, probable minimal residual disease; rRD, responding residual disease; NC, no change; LEL, lymphoepithelial lesions; LP, lamina propria; MM, muscularis mucosa; SM, submucosa

*Gut 2003;52:1656*

The probable minimal disease category (pMRD) does not signify a requirement for further therapy and patients are managed with followup as though they were in remission. Of interest, approximately 50% of patients with histologically negative post-antibiotic therapy gastric biopsies (CR/pMRD) exhibit persistent monoclonality, although in some patients the clone disappears with prolonged followup. In other patients, continued monoclonality may result in a delay in realizing remission.
However, following antibiotic therapy for gastric MALT lymphoma, the association between ongoing monoclonality and risk of relapse is tenuous. One bone of contention is that serial gastric biopsy specimens frequently exhibit an oscillating clonal status, due perhaps to sampling or recurrent *Helicobacter pylori* infection. Therefore, except for clinical investigations, the determination of clonality employing PCR currently is not considered pragmatic or recommended in the evaluation of post-therapy gastric MALT lymphoma biopsy specimens.

The establishment of extranodal gastric marginal zone lymphoma of MALT type as a recognized clinicopathologic entity has progressed to incorporate molecular genetics and specific chromosomal translocations. For MALT lymphomas in general, the genetic abnormalities encompass trisomies 3, 12 and 18, as well as balanced translocations, specifically t(11;18)(q21;q21), t(14;18)(q32;q21), t(1;14)(p22;q32) and t(3;14)(p14;q32). The most common translocation in gastric MALT lymphoma arising in approximately 20-30% of cases (although lower in North America) is t(11;18)(q21;q21) in which t(11;18) fuses with amino terminal of the inhibitor of apoptosis *API2* at 11q21 to the carboxyl terminal of *MALT1* at 18q21 leading to a chimeric fusion product. *MALT1* is involved in antigen receptor-mediated NFκB activation. The *API2-MALT1* fusion product is detectable by FISH. The t(11;18)(q21;q21) is restricted to extranodal MALT lymphomas and has not been reported in other forms of marginal zone lymphoma, such as splenic or nodal, or in chronic gastritis associated with *Helicobacter pylori*. Gastric MALT lymphomas without a t(11;18)(q21;q21) often exhibit aneuploidy, as for example trisomy 3 or 18.

In this decade, the discovery of t(11;18)(q21;q21) in some patients with gastric MALT lymphoma has led to exciting clinical prognostic correlations. Specifically, patients with gastric MALT lymphoma who prove positive for t(11;18)(q21;q21) often fail to respond to *Helicobacter pylori* therapy and this translocation frequently arises in patients who are *Helicobacter pylori* negative. Employing endosonographic staging, such t(11;18)(q21;q21) positive patients who do not respond to *Helicobacter pylori* eradication with antibiotics are found to have disease that has spread beyond the gastric submucosa into muscularis and/or serosa in contrast to patients with lymphoma limited to the mucosa and submucosa who generally are t(11;18)(q21;q21) negative. Moreover, t(11;18)(q21;q21) is uncommon in extranodal diffuse large B cell lymphoma and patients with this translocation rarely metamorphose to diffuse large B cell lymphoma. In contrast, aneuploid t(11;18)(q21;q21) negative patients who are unresponsive to *Helicobacter pylori* treatment are at risk to evolve to diffuse large B cell lymphoma. For example, microsatellite screening of gastric MALT and large B cell lymphomas display alleic imbalances limited to t(11;18)(q21;q21) negative patients that are shared by both MALT and diffuse large B cell lymphomas; this observation indicates that t(11;18)(q21;q21) negative patients are the genesis of MALT lymphomas than convert to one of large B cell type. It is therefore paramount to ascertain as to whether or not the gastric marginal zone lymphoma of MALT type is t(11;18)(q21;q21) positive since this information has prognostic and therapeutic repercussions.
In the 1990’s, many diffuse large B cell lymphoma cases of stomach were designated as “high-grade MALT lymphoma” based on the belief that these cases had transformed from “low-grade MALT lymphoma” especially when both components were present in a single specimen. Despite the likelihood that such transformations occur, the term “high-grade MALT lymphoma” presently is discouraged and all such cases are interpreted as large B cell lymphoma. By current definition, marginal zone lymphoma of MALT type is strictly an indolent or low-grade extranodal lymphoma. Despite the considerable recent emphasis on MALT-type lymphomas, in fact, greater than 50% of gastric lymphomas are high-grade, diffuse large B cell lymphomas. Except in small gastroscopic biopsy specimens, differentiation of large cell lymphoma from carcinoma usually is not a problem in view of the tendency of the lymphomas to be diffuse, massive, and lacking in cohesive cell aggregates. Nonetheless, immunohistochemical verification of diagnosis is recommended and is particularly valuable in delineating cases of large cell lymphoma from gastric adenocarcinoma. The infiltration by lymphoma around, or into, partially intact gastric glands, negative mucin and keratin stains, positive reactivity for CD20, and the lack of syncytial cell aggregates or malignant acinar formation aid in the distinction of large cell lymphoma from poorly differentiated adenocarcinoma, even in small biopsy specimens. Small gastric biopsy specimens, however, are subject to sampling errors and artifactual distortion, and on occasion it may be necessary to request a second biopsy, in order to render a more complete pathologic examination.

One consequential diagnostic issue is the observation of large cells in a background of a marginal zone lymphoma of MALT type in a gastric biopsy specimen. No current consensus exists as to how many large cells are required to establish the evolution from MALT lymphoma to one of diffuse large B cell type. Clearly, the presence of large cells in discrete nodular aggregates or sheets is likely an indication of transformation; however, diagnostic difficulties remain for cases in which the large cells are numerous and diffusely admixed with small marginal zone lymphocytes. In one study of 106 patients with gastric MALT lymphomas, the prognostic impact of a large cell component was assessed by semiquantitative analysis of clusters and diffusely intermingled malignant large cells; in MALT lymphomas, the observation of a diffuse large cell component in the range of 1-10%, with and without non-confluent clusters of large cells, predicted a significantly worse prognosis. In a report from Italy, the presence of scattered large cells that comprised 5–10% of the MALT lymphoma cell population was regarded as prognostically irrelevant, whereas compact clustered large cells that represented more than 10% of the MALT lymphoma proved significant, as they were associated with a worse survival.

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