At the Bedside: Helicobacter pylori, dysregulated host responses, DNA damage, and gastric cancer

Rahul S. Dalal and Steven F. Moss

Department of Medicine, Warren Alpert Medical School of Brown University, Providence, Rhode Island, USA

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ABSTRACT
Clinical trials performed in populations at high GC risk have demonstrated that eradication of Helicobacter pylori from the stomach with a course of combination antimicrobials helps prevent gastric carcinogenesis when they are administered before precancerous lesions have yet developed. In this review, we consider the insights into H. pylori-associated gastric carcinogenesis that have been gained from these and many other clinical studies in the field to highlight priority areas for basic research and clinical investigation. Among these are defining the magnitude of the risk reduction that may be achieved in clinical practice and at a population level by H. pylori eradication and investigating when, during the slow multistep progression to GC, intervention will be of the most benefit. Additional strategies to prevent GC induced by H. pylori, including chemoprevention, dietary modification, and close endoscopic surveillance, may also have value in augmenting the risk reduction. Why only a small subpopulation of those infected by H. pylori go on to develop GC may be partially explained by genetic susceptibility related to SNPs in several genes regulating the intensity of the gastric inflammatory response to H. pylori. Investigation of the basic mechanisms underlying the promotion of GC by H. pylori and the associated inflammatory response will likely continue to improve clinical strategies for the prevention of one of the most common causes of cancer death globally. See related review, At the Bench: H. pylori, dysregulated host responses, DNA damage, and gastric cancer. J. Leukoc. Biol. 96: 213–224; 2014.

Introduction
H. pylori colonizes the gastric mucosa of approximately one-half of the human population worldwide, leading to chronic gastric inflammation in all and clinically important adverse outcomes in a sizable minority [1]. Most infections with this gram-negative bacterium occur in early childhood and are not associated with symptoms. However, ~10% of colonized individuals ultimately develop gastric or duodenal ulcers. In such cases, H. pylori is sought and if present, treated with H. pylori eradication therapy. Gastric noncardia adenocarcinoma or the even rarer mucosal-associated lymphoid tissue lymphoma occur in ~1% of those harboring H. pylori, usually several decades after the initial infection (Table 1). H. pylori is unevenly distributed throughout the world and is most prevalent in resource-poor countries (in the range of 70–90%) and in as few as 10% or less of some Western populations [1]. GC, which is largely attributable to H. pylori [2], is one of the most common causes of death from cancer worldwide, responsible for ~10,900 deaths/year in the United States [3] and ~660,000 annual deaths globally [4].

H. pylori eradication therapy: Generally consists of a proton pump inhibitor, such as omeprazole, and at least two antibiotics, such as amoxicillin and clarithromycin. Amoxicillin may be replaced with metronidazole in those allergic to penicillin. A common alternative is a quadruple therapy consisting of omeprazole, bismuth, metronidazole, and tetracycline, especially if the first treatment course was not successful. After successful eradication, recolonization is rare.

Gastric cardia: First portion of the stomach, immediately below the gastroesophageal junction. H. pylori has a higher association with malignancies in gastric regions outside of the cardia, i.e., the “noncardia.”

Even before the role of H. pylori in gastric carcinogenesis was recognized, clinicians had been aware that GC typically developed in stomachs that had been chronically inflamed [5]. Furthermore, it was appreciated that the slow progression from gastritis to cancer occurred over decades and was often accompanied by a predictable series of histological intermediary steps, including the development of gastric glandular atrophy, IM, and dysplasia [6]. The concept of chronic inflammation as a precursor and risk factor for carcinogenesis dates...
TABLE 1. Conditions Positively or Negatively Associated with *H. pylori* Infection in Epidemiological Studies

<table>
<thead>
<tr>
<th>Positively associated</th>
<th>Negatively associated</th>
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<tbody>
<tr>
<td>Chronic gastritis</td>
<td>Childhood-onset asthma</td>
</tr>
<tr>
<td>Peptic ulcer disease</td>
<td>Inflammatory bowel disease</td>
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<tr>
<td>Gastric noncardia adenocarcinoma</td>
<td>Celiac disease</td>
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<tr>
<td>Gastric mucosal-associated lymphoid tissue lymphoma</td>
<td>Eosinophilic esophagitis</td>
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<tr>
<td>Idiopathic thrombocytopenic purpura</td>
<td>Esophageal adenocarcinoma</td>
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<tr>
<td>Iron-deficiency anemia</td>
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See text for details.

back over 150 years to Rudolf Virchow, and it is now appreciated that chronic inflammation is to be important in the pathogenesis of many human malignancies, including cancers of the esophagus, liver, breast, cervix, pancreas, and colon [7].

**Gastric atrophy:** Loss of glandular cells with subsequent replacement by fibrous tissue.

**IM:** Transformation of normal gastric epithelium to resemble that of the small intestine and in later stages, the colon.

**Dysplasia:** An abnormality of cell development often consisting of high numbers of immature cells with a decrease in the number of mature cells, an early indicator of a neoplastic process.

Improved, recent understanding of the basic mechanisms by which *H. pylori* promotes inflammation, DNA damage, and carcinogenesis has been coupled over the last 20 years to multiple epidemiological and clinical studies linking *H. pylori* and/or the *H. pylori*-associated gastric inflammatory response to GC. In this “bedside-to-bench” clinical research review, we summarize findings from the clinical trials of *H. pylori* eradication for the prevention of GC and attempt to highlight knowledge gaps in the field that require ongoing basic science research to help fine-tune and complement *H. pylori* eradication efforts for the prevention of GC and attempt to highlight knowledge gaps in the field that require ongoing basic science research to help fine-tune and complement "bedside-to-bench" clinical research reviews. As described in the accompanying bench-to-bedside review, *H. pylori* has evolved mechanisms to evade its own elimination by the innate immune system. For example, *H. pylori* bears a LPS composed primarily of tetra-acylated lipid A, which demonstrates 1000-fold reduced immunogenicity compared with *Escherichia coli* LPS [8]. Additionally, the bacterium’s flagellin harbors mutations in the TLR5-binding site, making it a poor PRR ligand as well [9].

In addition to immune evasion, *H. pylori* can directly suppress the host immune response to thrive in the gastric mucosa. VacA, an exotoxin and virulence factor that was originally shown to induce vacuolization of epithelial cells [10], was later discovered to inhibit T cell signaling also, resulting in IL-2 down-regulation and reduced cell proliferation [11].

Other groups have studied the effects of *H. pylori* on T<sub>reg</sub> to induce host tolerance to infection, demonstrating elevated numbers of T<sub>reg</sub> in the gastric mucosa of infected patients [12, 13], driven by specific chemokine/cytokine/homing receptor interactions between the gastric mucosa and peripheral blood T<sub>reg</sub> [14]. Lundgren and colleagues [15] demonstrated that circulating memory T cells in infected human hosts have diminished IFN-γ production and proliferation when exposed to *H. pylori*-pulsed dendritic cells compared with memory T cells from uninfected donors; this effect was eliminated after depletion of CD4<sup>+</sup> CD25<sup>high</sup> T<sub>reg</sub>. Their findings indicate that T<sub>reg</sub> suppress memory T cell responses to *H. pylori* infection and contribute to the organism’s persistence in the host. Furthermore, a mucosal T<sub>reg</sub> number has been shown to increase progressively with worsening stages of inflammation through GC development [16], and elevated T<sub>reg</sub> counts in peripheral blood have also been associated with various malignancies [17].

The ability of *H. pylori* to evade the immune system likely plays an important role in the tendency of this bacterium to persist in the stomach over decades, inciting a low-level inflammatory response that insidiously promotes gastric carcinogenesis in certain susceptible hosts. Intriguingly, some recent epidemiological studies have led to the hypothesis that there may sometimes be “beneficial” effects of persistent *H. pylori* infection as a result of a consistent relationship between the loss of gastric *H. pylori* colonization in fully industrialized nations and the emergence of certain conditions of increasing prevalence there, including some characterized by unrestrained inflammation outside of the stomach. These conditions include childhood-onset asthma, inflammatory bowel disease, celiac disease, eosinophilic esophagitis, and even esophageal adenocarcinoma [18–22] (Table 1). Experimental evidence in mice suggests that the mechanisms involved in this inverse relationship between *H. pylori* and extragastric inflammation may relate to the acquisition of *H. pylori* infection early in life, reprogramming mucosal and systemic immunity in the direction of reduced T<sub>reg</sub> function and increased inflammation outside of the stomach [23, 24]. It remains to be determined whether similar events are operative in naturally infected humans.

**Research questions:** How does loss of *H. pylori* promote inflammation outside of the stomach in humans? Can peripheral or gastric mucosal T<sub>reg</sub> profiles and cell markers be used to risk-stratify individuals for GC?

**H. PYLORI AND MODULATION OF THE IMMUNE RESPONSE**

As described in the accompanying bench-to-bedside review, *H. pylori* has evolved mechanisms to evade its own elimination by the innate immune system. For example, *H. pylori* bears a LPS composed primarily of tetra-acylated lipid A, which demonstrates 1000-fold reduced immunogenicity compared with *Escherichia coli* LPS [8]. Additionally, the bacterium’s flagellin harbors mutations in the TLR5-binding site, making it a poor PRR ligand as well [9].

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**SNPs AND GENETIC SUSCEPTIBILITY TO H. PYLORI-ASSOCIATED GASTRIC CARCINOGENESIS**

Whereas *H. pylori* infection remains the best-known established risk factor for noncardia GC, ~1% of those infected by *H. pylori* develops the condition. This underscores the role of host genetics, in addition to environmental and bacterial virulence factors, in predisposing individuals to disease. *H. pylori* infection initiates a precancerous cascade that is driven by inflammation (Fig. 1). As the severity of the gastric inflammatory response may vary, depending on host genetic makeup, much
research has focused on host SNPs of cytokine and other genes that mediate the inflammatory response [25–27].

Polymorphisms in innate immune system genes

Upon colonization, *H. pylori* encounters the innate immune system, which may influence the subsequent direction of the host inflammatory response. *H. pylori* binds to gastric epithelial cells and activates TLR4, the PRR for LPS on gram-negative bacteria [28, 29]. Whereas *H. pylori* LPS is a relatively weak TLR4 agonist compared with the LPS of other gram-negative bacteria [30], several polymorphisms in the TLR4 gene have been identified that are associated with an up-regulated immune response to *H. pylori* and the development of gastric carcinogenesis [31]. Hold et al. [32] described the TLR4 896 A > G SNP in association with an increased risk of noncardia GC in patients infected with *H. pylori* and more severe gastric inflammation, hypochlorhydria, and atrophy, all of which contribute to the precancerous cascade that is initiated by *H. pylori* infection. In further studies, the TLR4 299 D > G SNP was reported to confer a twofold, increased risk for gastric malignancy, and the 399 T > I SNP was associated with a hazard ratio of 5.38 for intestinal-type GC [33].

Other innate immune system modulators with known polymorphisms associated with GC include NOD2 and MBL. NOD2 is overexpressed in the gastric mucosa of patients infected with *H. pylori* and is involved with recognition of muramyl dipeptide, a breakdown product of peptidoglycan [34]. Like LPS, NOD2 induces a signal transduction cascade that activates NF-κB, a transcription factor that up-regulates proinflammatory cytokines [35]. Recently, Companioni et al. [36] identified two NOD2 SNPs in patients from 10 European countries that were significantly and inversely associated with noncardia GC (OR 0.60 and 0.58), the subtype specifically associated with *H. pylori* infection. These SNPs may therefore protect against GC in *H. pylori*-infected patients. In contrast, Hnatsyszyn et al. [37] previously reported an 802 C > T NOD2 SNP in Western Poland, where the T allele frequency correlated with severity of inflammation in the gastric mucosa, reaching its highest level in patients with noncardia GC. MBL is also involved in innate immune recognition and binds a variety of microbial pathogens, resulting in their elimination via activation of complement. Baccarelli and coworkers [38] reported that the codon 52 D variant of the MBL2 gene is associated with a 1.94-fold risk for GC (OR 1.9).

**IL-1 gene cluster polymorphisms**

IL-1β, a cytokine expressed from the IL-1 gene cluster on chromosome 2q, is known to play a central role in initiating and augmenting the host inflammatory response that leads to GC development [39]. It also inhibits acid secretion from gastric epithelial cells [40]. El-Omair and colleagues [41] first studied the relationship between IL-1β polymorphisms and precancerous lesions in GC first-degree relatives from Scotland, a cohort with a particularly elevated GC risk in the presence of *H. pylori* infection. IL-1β polymorphisms causing high IL-1β production were associated with an increased risk for the precancerous conditions of hypochlorhydria and gastric atrophy. Two subsequent case-control studies reported an increased risk for noncardia GC but not for cardia GC or esophageal cancer, consistent with a state of reduced acid exposure from the antisecretory effects of IL-1β on the gastric mucosa [41, 42]. In a recent meta-analysis of IL-1 gene-cluster polymorphisms, a significantly increased risk for GC was associated with the IL-1β-511*T allele and IL-1RN*2 variable number tandem repeat polymorphisms for intestinal-type GC in Caucasians [43].

An interesting study by Figueiredo et al. [44] examined not only the effects of IL-1 polymorphisms but also *H. pylori* virulence strains and combined host/bacterial genotypes on GC development. They initially identified a significantly higher association of glandular atrophy and IM with *H. pylori* strains positive for vacA*St*, vacAm1, and cagA, a marker for the cag pathogenicity island that encodes a type IV secretion system and genes that increase production of IL-8 in the gastric epithelium. These strains were also seen more frequently in GC patients than those with gastritis alone. Both IL-1β-511*T allele carriers and IL-1RN*2 homozygotes had more severe gastritis and glandular atrophy. In a combined host/bacterial genotype analysis, IL-1β-511*T carriers infected with cagA, vacA*St*, and vacAm1-positive *H. pylori* strains had greatly increased GC risk (OR 25, 87, 7.4, respectively), as did IL-1RN*2 homozygotes infected with these same strains (OR 23, 32, 8.8, respectively). Furthermore, their statistical analysis determined that the risks conferred by bacterial and host genotypes were independent, establishing a potential role for combining host with bacterial genotyping to screen individuals at high risk for GC.

Although there is ample evidence for IL-1β SNPs being associated with GC in several Western populations, results have not been generalizable to Asian populations. Recently, however, Wang et al. [45] reported that IL-1β-511*T carriers were at a significantly increased risk for GC in a Chinese population. In addition, the allelic and genotypic distributions of the IL-1RN*2 allele were strongly associated with GC risk in an Asian population. The IL-1RN*2 allele was also associated with severe gastritis, indicating that the IL-1RN*2 allele has a potent role in increasing the risk for GC in Asian populations. These findings suggest that the IL-1RN*2 allele may play a significant role in the development of GC in Asian populations.
been consistent, particularly across ethnic groups. Of four comprehensive meta-analyses on the IL-1β gene, three found a significant positive correlation between the IL-1β-511*T allele and intestinal or noncardia GC risk [44–47]. The meta-analyses by Xue et al. [43] and Camargo et al. [45] found correlations among Caucasians but not among Asian populations. These discrepancies may be attributed to differences in study design, inclusion criteria, sample size, or other genetic influences not yet described. The lack of associations for many of the published IL-1 SNPs and GC in Asians may be related to the higher prevalence of GC in these populations, making such an association more difficult to demonstrate compared with low-incidence regions [48]. Another potential explanation considers the role of diet in modifying genetic risk for GC. Shen et al. [49] found that IL-1β variation correlated with chronic inflammation and risk for metabolic syndrome, an association that became even more pronounced in subjects consuming a diet low in polyunsaturated fatty acids. As diets across ethnic groups vary in consumption of certain macronutrients, this may be partly responsible for the reported discrepancies in genetic association studies for IL-1β.

Despite the inconsistent associations of IL-1β with GC across ethnic groups, studies in mice have confirmed a role for IL-1β [51]. The well-studied TNF-α, IL-8, IL-10, and IFN-γ gene polymorphisms

After extensive studies on IL-1 gene cluster polymorphisms, SNPs in other cytokines known to be involved in H. pylori pathogenesis have also been identified as GC risk factors, including TNF-α, IL-8, IL-10, and IFN-γ. TNF-α is produced in the gastric mucosa and like IL-1β, suppresses gastric acid secretion [51]. The well-studied TNF-α 308-aa genotype was shown to have a twofold increased risk for GC [42], a result that was also supported by a meta-analysis (OR 1.49) [52]. IL-8 is a mediator of chronic inflammation that acts as a neutrophil and lymphocyte chemoattractant. The IL-8 251T > A SNP may increase GC risk, according to three separate studies performed in populations in Southeast Asia [53–55]. In contrast to the cytokines described above, IL-10 serves an anti-inflammatory role by down-regulating various proinflammatory cytokines, including IL-1β and TNF-α. Most studies on the relationship between IL-10 genotype and GC have investigated haplotypes rather than individual polymorphisms. In a Thai population, Wu et al. [56] demonstrated that the IL-10 promoter haplotype GCC (−1082/+819/+592) occurred with higher frequency in GC patients relative to healthy controls but with a stronger association with cardia (OR 3.21) than noncardia (OR 1.96) GC. The GCC haplotype was also associated with greater cytokine production than the ATA haplotype. In contrast, El-Omar and coworkers [42] identified an increased noncardia GC risk (OR 2.5) with homozygosity for the low-producing ATA haplotype. Individuals harboring this haplotype likely had a more vigorous inflammatory response to H. pylori infection that promoted malignancy. However, it remains unclear how the IL-10 GCC haplotype influences GC development.

IFN-γ is a cytokine critical for innate and adaptive immunity, as well as tumor surveillance. It is also a key player among the Th1 cytokines, which have been shown to promote H. pylori gastritis [57]. Thye et al. [58] conducted a genome-wide linkage analysis that revealed an association between a polymorphism at the −56 position of human IFN-γ with an increased host susceptibility to H. pylori infection. More recent studies have demonstrated associations between polymorphisms in IFN-γR1 (−56C/T) and -2 (C allele) and increased risk for GC, the former, more specific for early onset disease (<40 years of age at diagnosis) [59, 60]. Genotypic variation in the Th1-mediated immune response may therefore play an important role in GC risk.

Host genetic makeup and the pathogenesis of GC

Host polymorphisms in several individual cytokines described above have been shown to associate strongly with GC risk. But, in addition to their independent effects, El-Omar et al. [42] have identified interactions among multiple at-risk cytokine SNPs, including IL-1β-511*T, IL-1RN*2*2, TNF-α-308*A, and IL-10 ATA/ATA genotypes, such that patients carrying three to four of these polymorphisms had a greatly enhanced 27-fold, increased GC risk. Furthermore, H. pylori infection was necessary for these effects, suggesting that the interaction between infection and host genetic predisposition contributes to GC development.

Genome-wide association studies are powerful tools to probe novel, underlying genetic susceptibility, as demonstrated recently by Mayerle et al. [61] regarding TLR1 and the low-affinity Fc IgG IIA receptor gene FCGR2A as susceptibility loci for H. pylori infection in Northern Europe. Recently reported genome-wide association studies in Southeast Asia have suggested some hitherto-underinvestigated polymorphisms as GC risk factors. The candidate genes in the Chinese population studies include mucin 1, the PGER4 and a phospholipase C isoform, each of which can be functionally linked to gastric inflammatory pathways [62]. In contrast, in the Japanese population, the novel candidate gene identified, prostate stem cell antigen [63], has no obvious role in the regulation of gastric inflammation. It is noteworthy that each candidate identified in these genome-wide association studies was associated with a relatively modest, increased GC risk (OR 4 or less), even after factoring in an interaction with H. pylori infection per se, as shown in the Chinese population [62]. From these and other studies, one can conclude that in patients infected with H. pylori, an elevated risk for GC can be linked to host genotypic variations in the innate immune system and in certain cytokines that interact with bacteria to promote an exaggerated inflammatory response.

The resulting mucosal damage and risk for GC are more pronounced when genetically susceptible hosts are infected with more virulent strains of H. pylori, particularly those that are cagA-positive. Additionally, the chronically inflamed and achlorhydric gastric environment may enhance the growth of other microbes that may contribute to gastric mucosal oxidative stress and onco-
genosis. Unfortunately, despite the identification of some host and bacterial factors that increase GC risk, this has not yet translated to clinically applicable tools to identify those individuals for whom H. pylori eradication or GC screening may be especially warranted. The difficulty is, in part, a result of the lack of consistency in the identified high-risk polymorphisms across ethnic groups. More studies are therefore necessary to identify and validate the specific sets of genotypes that predispose different populations to GC. Additionally, large studies that combine host with bacterial genotyping are important to determine whether the very high OR reported by Figueiredo et al. [44] can be confirmed across other populations.

Clinical and research questions: Can knowledge of susceptible SNPs be used clinically to identify H. pylori-positive patients or sub-populations at greatest risk for GC, in whom to concentrate H. pylori eradication efforts? Can H. pylori genotyping be used in concert with the above strategy to focus further H. pylori elimination? Is there a common SNP profile of high risk among different populations?

EFFECTS OF H. PYLORI ERADICATION ON REDUCING GC RISK

Whereas GC incidence is declining worldwide, it remains a significant public health burden, particularly in Asia [64, 65]. Early investigation of H. pylori by Marshall and Warren [66] showed this organism to be a causal agent of chronic gastritis, the first step in the progression to GC, and H. pylori was officially classified a group I (definite) carcinogen by the World Health Organization two decades ago [67]. Several cohort studies and RCTs have subsequently been performed to support this designation and provide an estimate of the magnitude of the GC risk reduction achievable by H. pylori eradication.

Cohort studies

Three retrospective cohort studies investigated the effect of H. pylori eradication on GC incidence [68–70]. The largest followed 80,000 Taiwanese subjects hospitalized with peptic ulcer disease who underwent H. pylori eradication. Those who received early eradication (within 1 year of hospitalization) had a GC risk similar to the general population, whereas those with delayed eradication had an elevated risk (standardized incidence ratio 1.36, 95% CI, 1.24–1.49). This study, however, was limited by variation in patient follow-up between 2 and 10 years, as well as a lack of confirmation of H. pylori eradication. The other retrospective studies, conducted in Japanese populations, also demonstrated an effect of H. pylori eradication on reduced GC incidence.

Three prospective interventional studies demonstrated a similar effect of H. pylori eradication on GC incidence in Japan [71–73]. In the landmark study by Uemura et al. [71], a subgroup of 253 patients received eradication therapy, none of whom developed GC, compared with 36 GC cases in the untreated group (n=993). The major limitations of these studies relate to their relatively brief follow-up period of less than one decade. However, an important, common theme emerging from them is that for eradication therapy to confer a GC risk reduction, it needed to be given before the development of significant gastric atrophy—the apparent “point of no return”, beyond which eradication therapy may be futile.

Recently, Lee and coworkers [74] published a longitudinal cohort study to assess the benefit of mass population-wide H. pylori eradication. A community-based eradication program was launched in 2004 on Matsu Island, Taiwan, and the prevalence of H. pylori infection and premalignant gastric pathology, as well as changes in GC incidence, was compared between the pre- and postchemoprevention periods (1999–2003 and 2004–2008, respectively). A significant reduction in gastric atrophy but not IM was observed, supporting the point of no return hypothesis. Up to this time, a nonsignificant 25% reduction in GC incidence has been reported, but the ultimate benefit in GC incidence and mortality is likely to rise progressively over the years. Longer-term follow-up of this cohort is keenly awaited. Proving whether all of the changes that may be observed are a result of the single intervention of H. pylori eradication is of course impossible in cohort studies such as this where diet and other lifestyle issues are likely also changing rapidly in parallel.

RCTs

The strongest evidence supporting H. pylori eradication for GC risk reduction comes from the RCTs performed in high-risk populations in China, Japan, and Colombia. A meta-analysis published in 2009 that included several of these studies concluded that the reduction in GC incidence in patients that received H. pylori eradication was statistically significant (RR 0.65, 95% CI, 0.43–0.98) [75]. However, two of the data sets included in this analysis were published only in abstract form, and one of the studies included drew concerns as a result of a reduction in GC reported at 10 years relative to 5 years of follow-up. The individual studies therefore merit their own discussion and evaluation.

In the only placebo-controlled RCT that addressed GC incidence as the primary outcome, seven cases of GC developed in the eradication group compared with 11 in the placebo arm during the 7.5-year follow-up period [76]. Whereas this reduction in GC was not statistically significant (P=0.33), a subgroup analysis of patients without precancerous pathology did show a reduction in GC incidence after eradication (P=0.02). Their results again support a point of no return and a potentially protective role for H. pylori eradication in patients without precancerous lesions, such as gastric atrophy, IM, or dysplasia.

You and colleagues [77] evaluated GC incidence as a secondary outcome. In their factorial design, 3365 Chinese subjects were randomized to H. pylori eradication, vitamins C/E and selenium, garlic and/or placebo. Whereas fewer cases of GC developed in the H. pylori treatment group (1.7%) compared with the placebo (2.4%) at 7.3 years of follow-up, the findings were again not statistically significant (P=0.14). However, a recent extended follow-up concluded that eradication treatment significantly reduced GC incidence by 39% and mortality by 33%, 15 years after therapy [78]. One concern for this study is that only 46% of patients receiving eradication treatment remained H. pylori-negative after 7 years. This surprisingly high level of treatment failure would diminish the
estimate of benefit of *H. pylori* eradication in this study. This also suggests that other bacteria within the gastrointestinal tract may contribute to GC risk in collaboration with *H. pylori*.

Mera and coworkers [79] conducted the only additional RCT with >10 years’ follow-up, evaluating GC incidence as a secondary outcome. After 12 years, no difference in GC incidence was evident between eradication and control groups. However, longer-term eradication was associated with greater regression of precancerous lesions, and eradication therapy was more effective for less-advanced lesions, consistent with the point of no return hypothesis. The complex study design was limited by a protocol change; after 6 years of follow-up, the trial was unblinded, and patients not treated previously were offered eradication therapy. For this reason, Fuccio and coworkers [75] excluded the most recent version of this study in their meta-analysis and instead included an earlier publication of the trial [80].

Unlike other RCTs, Fukase et al. [81] evaluated *H. pylori*-positive patients with early (endoscopically resectable) GC and the risk for developing metachronous GC after eradication. In their multicenter trial in Japan, they reported an OR of 0.35 for metachronous GC (*P* = 0.009). Whereas widely considered one of the best-executed trials on this topic, the study was limited by its open-labeled design and the lack of blinding during follow-up endoscopy. Additional studies of metachronous GC after endoscopic resection from Korea and Japan have shown a similar trend, although not always attaining statistical significance [82–84].

**Metachronous cancer**: A new primary cancer occurring at a different time than the initial cancer.

### The case for *H. pylori* eradication programs in high-risk regions

Whereas the Fuccio et al. [75] meta-analysis concluded a statistically marginally beneficial effect of *H. pylori* eradication on GC incidence, individual, albeit underpowered, studies do appear to demonstrate a benefit for therapy in high-risk populations when administered before the development of advanced preneoplastic changes. The clinical data also show a benefit for eradication therapy after resection of early GC. An important consideration is the complex and poorly understood interaction between *H. pylori* and the gastric microbiome, as described in the accompanying review by Hardbower, Peek, and Wilson, and the effects of eradication antibiotics on this interaction and gastric homeostasis are worthy of investigation. The case for eradication could be strengthened further with more RCTs with larger populations and long-term follow-up to investigate the effect of eradication on GC incidence as a primary outcome. However, with the designation of *H. pylori* as a definite carcinogen in 1994 [67] and the acceptance of this designation by public health authorities in high GC regions, ethical considerations now preclude recruitment of a comparator “placebo” arm of subjects who are not offered eradication therapy. Still, a recent systematic review of cost-effectiveness studies from Asia, North America, and Europe concluded that *H. pylori* serology, with treatment of positive cases and endoscopic population screening, is cost-effective, especially for regions with high GC incidence [85]. This is of particular interest for the Asia-Pacific region, where Japan, Korea, and China account for 60% of new GC cases [86].

No healthcare system currently practices widespread eradication therapy for primary prevention of GC. However, Japan has recently extended insurance coverage for *H. pylori* eradication for indications beyond peptic ulcer disease and gastric mucosa-associated lymphoid tissue lymphoma. Bearing in mind that without further healthcare intervention, the cost of GC treatment in Japan is projected to exceed 500 billion yen annually within a decade [87], a recent report proposes a screening and surveillance plan to reduce the economic burden of GC in Japan with a test-and-treat approach for *H. pylori* infection in patients <20 years old (given the low rates of gastritis in this age group) [88]. For *H. pylori*-positive patients >50 years old (who more commonly have advanced gastric pathology), the recommended approach is endoscopic screening to evaluate for atrophic gastritis, the presence of which would indicate subsequent entry into an endoscopic surveillance program. This is analogous to the coloscopic colon cancer prevention programs established in the United States and many other Western populations. With this strategy, a significant decline in healthcare costs and GC-associated mortality is predicted. Should this Japanese model be successful, it is likely to inspire similar programs in other high-risk GC regions to reduce the global burden of this disease.

**Clinical and research questions**: How much distal GC can be prevented by *H. pylori* eradication? How do eradication antibiotics alter the gastric microbiome and gastric homeostasis? At what age should individuals in populations at high risk for GC be screened to optimize GC prevention?

## ADDITIONAL STRATEGIES TO LOWER THE RISK OF *H. PYLORI*-ASSOCIATED GASTRIC CARCINOGENESIS

### Endoscopic surveillance

As GC can occur even after successful *H. pylori* eradication [89], how should individuals in populations at high risk of GC be managed after eradication therapy? As described above, the approach in Japan is to perform periodic endoscopic surveillance under a recently launched national program [90]. A multinational European group has recently proposed guidelines for the endoscopic follow-up of patients with atrophic gastritis and/or IM after *H. pylori* eradication, with the hope of detecting GC at an early and more treatable stage [91]. In that protocol, endoscopy with multiple gastric biopsies is advised every 3 years for such patients, with more frequent endoscopic evaluations proposed if dysplasia is subsequently detected.

Others have proposed that surveillance of preneoplastic gastric lesions might be targeted more effectively, for example, by focusing specifically on patients with extensive IM or the more advanced “incomplete” type of IM or perhaps by combining frequent endoscopic surveillance with biopsies with the measurement of serum pepsinogen levels as a surrogate marker of atrophic gastritis [92].

It remains puzzling that despite the recognition that preneoplastic gastric lesions are a risk factor for subsequent GC development and despite the potential for early diagnosis of GC to
be associated with improved treatment outcome [93], surveillance for gastric IM is not a standard of care in the Western world. This "hands-off" approach to gastric IM, which is recognized as a preneoplastic condition in the stomach, contrasts with the approach to IM found in the esophagus (Barrett’s esophagus), where routine surveillance is widely practiced and advocated in an attempt to reduce the burden of esophageal cancer—even without compelling evidence of efficacy [94].

**GC chemoprevention**

As an alternative or complementary strategy to reduce the burden of GC, there has been considerable interest in the chronic administration of agents that reduce the risk of gastric carcinogenesis when taken prophylactically and continually. In some cases, rodents experimentally infected with laboratory *H. pylori* strains have been investigated as preclinical models to explore efficacy and confirm the mechanisms suspected from other basic science experiments. But often, the scientific rationale for the particular strategy has been borrowed from chemopreventive approaches to colon cancer, a disease whose pathogenesis likely differs considerably from *H. pylori*-associated gastric carcinogenesis. Most of the clinical trials in this area have investigated aspirin and other NSAIDs, as well as selective or specific COX-2 inhibitors.

**NSAIDs**

COX enzymes are expressed in the gastrointestinal tract as two isoforms. COX-1 is constitutively expressed and serves a cytoprotective role in the gastric mucosa, whereas COX-2 is induced by locally acting proinflammatory molecules and promotes carcinogenesis by down-regulating apoptosis [95]. NSAIDs inhibit COX enzymes and are widely used for their anti-inflammatory and analgesic effects.

*H. pylori* induces COX-2 expression in human gastric mucosa [96], and COX-2 overexpression has been demonstrated in GC [97, 98], where it has been associated with poor overall survival [99]. It is therefore reasonable to suspect that NSAIDs may prevent tumor development in *H. pylori*-infected individuals at risk for GC. Aspirin, other NSAIDs, and the more selective COX-2 inhibitors have all been studied as potential chemopreventive agents for GC, as well as to prevent several other common malignancies.

Retrospective case-control studies evaluating the effects of NSAIDs on GC development have demonstrated up to a 45% reduction in GC incidence in a seemingly dose-dependent manner [100]. Two studies stratified results by *H. pylori* status and found that the GC risk reduction with NSAIDs was confined to *H. pylori* positive individuals [101, 102]. Many of these studies, however, were performed in Caucasian populations with low GC incidence. Additionally, one Swedish study found a reduction in GC odds with aspirin but not other NSAIDs [101], whereas a subsequent report from the United Kingdom study described a reduction in GC odds with other NSAIDs but not aspirin [103].

To date, four prospective cohort studies and one RCT in this field have given mixed results. Thun et al. [104] reported a 36% reduction in the RR of fatal GC with aspirin use, a result that was not replicated by Ratnasighe and coworkers [105]. More recently, Abnet et al. [106] described HR reductions for both aspirin and other NSAIDs, whereas Epplein and colleagues [107] found a GC risk reduction with aspirin but not other NSAIDs. A RCT by Cook and coworkers [108] investigated the effects of aspirin on GC in women but found no reduction in GC deaths. The above studies are all limited by potentially inadequate follow-up periods. Many were not designed to detect GC incidence or mortality as a primary outcome, and the cohort studies are limited by recall bias regarding NSAID intake and dosing variability.

Additionally, two RCTs specifically evaluated the effects of selective COX-2 inhibitors on precancerous gastric lesions. Wong and colleagues [109] reported an association between celecoxib treatment and regression of advanced gastric lesions in *H. pylori*-positive patients, but no benefit was noted for the COX-2 inhibitor after *H. pylori* eradication. In the trial by Leung and coworkers [110], there was again no effect of rofecoxib on IM at 5 years in *H. pylori*-negative patients.

Thus far, three meta-analyses have attempted to clarify the effects of NSAIDs on GC development. The first included eight case-control studies and one cohort study, reporting 27% and 22% reductions in the odds of GC development for aspirin and other NSAID use, respectively [111]. The second evaluated 14 studies, including three cohort studies and one RCT, and calculated a 38% reduction in GC development in aspirin users [112]. The most recent meta-analysis included data from RCTs of aspirin for prevention of vascular events that reported cancer death as a secondary endpoint [113]. The authors found a significant preventative role for aspirin in GC with a HR of 0.42.

Thus, overall, there appears to be a protective effect of aspirin and other NSAIDs against noncardia GC, with very limited evidence for COX-2 inhibitors. The few studies that have considered *H. pylori* status found benefit for NSAID use only in *H. pylori*-positive patients. This brings into question any role for NSAID chemoprevention of GC after *H. pylori* eradication, although this requires further study. To justify GC chemoprevention programs with NSAIDs, more RCTs will be needed to determine the dose, duration, and specific drugs in this class that reduce GC incidence and mortality in various populations and will need to weigh carefully the incidence of secondary toxicities.

**Polyamine metabolism**

Another chemopreventive strategy that might be of value in limiting *H. pylori*-induced gastric carcinogenesis involves the inhibition of polyamine pathways. As discussed by Hardbower, Peek, and Wilson in the accompanying bench-to-bedside review, *H. pylori* up-regulates ODC expression in gastric macrophages and increases expression of spermine oxidase, which together, lead to the generation of ROS when the ODC path-
way is activated [114]. Downstream effects include gastric cellular apoptosis and DNA damage [115, 116]. DFMO has been used in clinical trials of colon cancer prevention together with the COX-2-selective NSAID sulindac [117]. The rationale for this combination relates to the separate contribution of each agent to decrease the pool of polyamine metabolites—DFMO via inhibition of polyamine synthesis and sulindac through activating cellular polyamine export. Other clinical trials using DFMO, alone or in combination with other chemopreventive agents, are ongoing for several cancer types. Although none has yet been conducted for GC prevention, stomach cancer may represent a particularly amenable target for DFMO inhibitory agents, based on the known pathogenesis of H. pylori-associated gastric inflammation in gastric carcinogenesis.

Dietary manipulation

Specific dietary constituents have long been suspected of promoting GC [6], based originally on the high-salt, high-nitrosamine content of the diet consumed in Japan, where GC is highly prevalent. Migrant studies documenting a lower risk of GC, as the children and grandchildren of the indigenous Japanese spread westward to Hawaii and the United States mainland, were then used as evidence for the Western diet being less carcinogenic for the stomach [118]. Contemporary reinterpretation of these observations implicates the declining prevalence of H. pylori as the likely responsible environmental-protective element. Nevertheless, the role of diet as a cofactor in gastric carcinogenesis is being explored anew, taking into account the confounding effect of H. pylori infection, as well as novel, mechanistic insights from basic science studies. In the case of dietary salt intake, recent clinical studies from diverse geographical regions have confirmed the relationship between high-salt consumption and GC and have demonstrated synergy between high-salt intake and H. pylori infection [119–121].

The mechanistic basis underpinning this is currently being evaluated in animal models and coculture systems, as described in detail by Hardbower, Peek, and Wilson in the accompanying review. These model systems can be used to identify, for example, that feeding the cancer-prone Mongolian gerbil a high-salt diet can up-regulate the transcription of the CagA oncoprotein of H. pylori in vivo [122]. Although diets low in fresh fruits and vegetables and with a large amount of highly preserved foods seems to be generally associated with a high risk of GC (perhaps explicable on the basis of a high-salt or nitrate intake), studies designed to address the potential relationship between specific micronutrient intakes and gastric carcinogenesis have been inconsistent and unimpressive overall, with the probable exception of a protective effect of vitamin C [123]. The recent identification of low dietary iron intake and/or low iron stores with heightened carcinogenic risk (again related to enhancing cag-associated H. pylori virulence) [124] should now lead to clinical studies specifically focused on interactions between iron and H. pylori infection in GC-risk profiling and exploring whether the mechanisms observed in model systems can be applied to clinical trials.

H. pylori was first declared a definite (class I) carcinogen by the World Health Organization’s International Agency for Cancer Research in 1994 [67] and remains the only bacterium so designated. Since that time, multiple, albeit individually underpowered, clinical trials have confirmed the important role of H. pylori in the development of gastric adenocarcinoma and have demonstrated that eradication of H. pylori is likely to reduce the prevalence of GC significantly. As described in the accompanying review by Hardbower, Peek, and Wilson, the evidence from basic and translational studies is that H. pylori promotes gastric carcinogenesis mainly through indirect mechanisms involving the gastric mucosal inflammatory response and the generation of ROS locally to induce mutations, epigenetic changes, and altered cellular turnover and histology. There has been and will continue to be major clinical research efforts to augment the benefit of H. pylori eradication through endoscopic surveillance and/or chemopreventive approaches, based on the improved knowledge of the pathogenesis of H. pylori-associated gastric carcinogenesis. In Table 2, we highlight the lessons learned so far from studying H. pylori in GC and the questions that still remain to be answered by clinical and basic science research.

Insights into the role of H. pylori in GC development have led to the adoption of H. pylori eradication programs in those areas of the world at high GC risk that possess the resources to intervene at a public health level [87, 88, 125]. In contrast, in some developed countries, such as the United States, there has been a reluctance to advocate widespread H. pylori screening and eradication. This hesitancy is based on some epidemiological studies demonstrating an inverse relationship between H. pylori prevalence and esophageal adenocarcinoma (for which a fairly consistent literature exists) [18] and certain other extragastric inflammatory states, including asthma and inflammatory bowel disease, where the evidence is less clear-cut [20, 22]. Such data have led to the concept that H. pylori may “protect” against some diseases and that the decline of H. pylori in many Western populations may have unexpected, adverse consequences [126]. Further large and well-designed, prospective clinical studies into these relationships, as well as basic and translational research addressing the diverse effects of H. pylori on the immune system, may lead to better insights into the

**Clinical and research questions:** Does DFMO, an inhibitor of polyamine synthesis, reduce GC risk in H. pylori-infected individuals? Can other clinically applicable strategies to reduce reactive oxygen generation in the stomach be identified?

**Summary and future directions**

**Polyamines:** Organic polycationic amino acids that bind DNA and modulate cell growth and apoptosis.

**Clinical and research questions:** Is it feasible to modulate dietary salt and iron intake in population studies? Can diet modulation prevent GC outside of clinical studies? Are there other mechanisms by which low-salt and low-iron diets enhance H. pylori virulence and gastric carcinogenesis?
**TABLE 2. Summary of Current Knowledge and Future Questions to be Addressed for the Prevention of *H. pylori*-Associated GC**

<table>
<thead>
<tr>
<th>Lessons learned</th>
<th>Outstanding questions</th>
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<tr>
<td>There is an inverse relationship between gastric <em>H. pylori</em> colonization and extragastric inflammation. <em>H. pylori</em> modulates the immune system to promote its persistence in the gastric mucosa, where elevated T&lt;sub&gt;reg&lt;/sub&gt; numbers are seen in the presence of infection. Polymorphisms in host cytokine genes, particularly IL-1β, TNF-α, and IL-10, are associated with increased GC risk.</td>
<td>How does loss of <em>H. pylori</em> promote inflammation outside of the stomach in humans? Can peripheral or gastric mucosal T&lt;sub&gt;reg&lt;/sub&gt; profiles and cell markers be used to risk-stratify individuals for GC? Can knowledge of susceptible SNPs be used clinically to identify <em>H. pylori</em>-positive patients or subpopulations at greatest risk for GC, in whom to concentrate <em>H. pylori</em> eradication efforts? Can <em>H. pylori</em> genotyping be used in concert with the above strategy to focus further <em>H. pylori</em> elimination? Is there a common SNP profile of high risk among different populations? How much distal GC can be prevented by <em>H. pylori</em> eradication? How do eradication antibiotics alter the gastric microbiome and gastric homeostasis? At what age should individuals in populations at high risk for GC be screened to optimize GC prevention? What is the cost-effectiveness of endoscopic surveillance of gastric IM after <em>H. pylori</em> eradication? What dose, duration, and specific NSAIDs best reduce GC incidence and mortality with minimal secondary toxicities? How do different NSAIDs differentially affect gastric mucosal COX-1 and COX-2 expression, and how does this correlate with GC incidence? Is there any role for NSAID chemoprevention of GC after <em>H. pylori</em> eradication?</td>
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<tr>
<td><em>H. pylori</em> eradication in high-risk populations reduces the development of GC.</td>
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<tr>
<td>Aspirin and NSAIDs have chemopreventive activity against noncardia GC in patients infected with <em>H. pylori</em>.</td>
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<tr>
<td><em>H. pylori</em> up-regulates ODC expression in gastric macrophages and spermine oxidase in gastric epithelial cells, leading to the generation of ROS, gastric cellular apoptosis, and DNA damage. High-salt and low-iron dietary intake are associated with increased GC risk in preclinical and some clinical studies.</td>
<td>Does DFMO, an inhibitor of polyamine synthesis, reduce GC risk in <em>H. pylori</em>-infected individuals? Can other clinically applicable strategies to reduce reactive oxygen generation in the stomach be identified? Is it feasible to modulate dietary salt and iron intake in population studies? Can diet modulation prevent GC outside of clinical studies? Are there other mechanisms by which low-salt and low-iron diets enhance <em>H. pylori</em> virulence and gastric carcinogenesis?</td>
</tr>
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</table>

seemingly dual effects of *H. pylori* within versus outside of the stomach.

Finally, it is important to acknowledge that GC is a highly heterogeneous disease. Whereas *H. pylori* is strongly linked to gastric adenocarcinoma throughout most of the stomach, *H. pylori* is not associated with the development of cancers of the gastric cardia, a site located just distal to the gastroesophageal junction [127]. Interestingly, cancers of the cardia are still relatively rare, but they are increasing steadily in prevalence in the Western world. Additionally, most of the clinical trials and the concepts that have emerged from them have focused on the “Correa cascade” [6, 26] of progressive histopathologic changes, with IM as an important intermediate and resulting in the more common “intestinal” morphological subtype of GC. In contrast, the “diffuse” GC subtype, which rarely results from a germ-line mutation in an E-cadherin-encoding gene (leading to hereditary diffuse GC), is a more undifferentiated type of cancer, in which tumor cells are dispersed and not organized into glandular structures. Sporadic diffuse GC is, like the intestinal GC subtype, also linked to prior *H. pylori* infection, although perhaps less tightly [128], and it is preceded by a less well-defined, histological progression without recognizable, histological precursor lesions other than chronic gastritis. Whether and how *H. pylori* eradication impacts the development of sporadic GC of the diffuse subtype have been much less studied and are also worthy of future attention.

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KEYWORDS:
- gastric carcinogenesis
- eradication therapy
- chemoprevention
- intestinal metaplasia
- single nucleotide polymorphisms
At the Bedside: Helicobacter pylori, dysregulated host responses, DNA damage, and gastric cancer

Rahul S. Dalal and Steven F. Moss

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