The Role of Polyamines in Gastric Cancer

Kara M. McNamara[1,2], **Alain P. Gobert**[1,3], **Keith T. Wilson**[1,2,3,4,5]

[1]Division of Gastroenterology, Hepatology, and Nutrition, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA

[2]Program in Cancer Biology, Vanderbilt University Medical Center, Nashville, TN, USA

[3]Center for Mucosal Inflammation and Cancer, Vanderbilt University Medical Center, Nashville, TN, USA

[4]Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, TN, USA

[5]Veterans Affairs Tennessee Valley Healthcare System, Nashville, TN, USA

Abstract

Advancements in our understanding of polyamine molecular and cellular functions has led to increased interest in targeting polyamine metabolism for anticancer therapeutic benefits. The polyamines putrescine, spermidine, and spermine are polycationic alkylamines commonly found in all living cells and are essential for cellular growth and survival. This review summarizes the existing research on polyamine metabolism and function, specifically the role of polyamines in gastric immune cell and epithelial cell function. Polyamines have been implicated in a multitude of cancers, but in this review, we focus on the role of polyamine dysregulation in the context of *Helicobacter pylori*-induced gastritis and subsequent progression to gastric cancer. Due to the emerging implication of polyamines in cancer development, there is an increasing number of promising clinical trials using agents to target the polyamine metabolic pathway for potential chemoprevention and anticancer therapy.

Introduction

Gastric cancer (GC) is the fifth most common cancer and the third most common cause of cancer-related death worldwide (1). The highest incidence of GC cases occurs in Latin America, and Eastern and Central Asia, while the lowest incidence rates are seen in North America, and North and East Africa. GC disproportionately affects older individuals, frequently men, and has a 5-year survival rate of 31% in the United States (1). There are two types of gastric carcinoma: 1) The intestinal type progresses through distinct histological steps of the "Correa Cascade" from non-atrophic gastritis (NAG), to multifocal atrophic gastritis (MAG), intestinal metaplasia (IM), dysplasia, and carcinoma (2–5). 2) The diffuse

type of GC occurs when infiltrating neoplastic cells do not form glandular structures and although the precancerous pathway is less well-defined, it frequently arises in a background of IM (6).

Infection with *Helicobacter pylori* and genetic mutations are etiologic agents of GC. *H. pylori* is a Gram-negative bacterium classified as a type I carcinogen that infects approximately 50% of the world's population (7). This pathogen specifically colonizes the mucosa of the human stomach and colonization typically correlates with development of chronic inflammation (8). Most colonized individuals do not exhibit symptoms, however long-term *H. pylori* carriers have increased risk of developing site-specific disease. Among those infected, approximately 10% develop peptic ulcer disease, less than 0.1% develop mucosa-associated lymphoid tissue lymphoma, and 1–3% develop gastric adenocarcinoma (8). Of importance, *H. pylori* infection is associated with both intestinal and diffuse GC (9). Several studies have shown that eradication of the bacterium results in attenuated progression to GC (9,10), but the treatment for *H. pylori* does not consistently reduce cancer risk, particularly once precancerous lesions are present (11,12). Moreover, since half of humans are infected (7), universal eradication is not practical and antibiotics are often ineffective, notably in underdeveloped regions (13,14). Lastly, *H. pylori* infection is indolent, mimicking a commensal (15), and therefore its presence cannot reliably predict who will develop disease progression. Stomach cancers in *H. pylori*-infected patients tend to develop slowly through pre-cancerous changes and rarely cause symptoms until late in the disease course, and thus often go undetected resulting in a low 5-year survival rate (8). Recently, two studies from our group have shown in a long-term cohort study in subjects in a high GC risk region of the Andean mountains of Colombia that *H. pylori* treatment is associated with reduced histological progression of precancerous lesions and development of GC, if subjects remained free of infection over a 16-year (16) and 20-year follow-up period (5). These findings indicate that detailed follow-up after *H. pylori* treatment may be helpful in determining long-term outcome.

Notably, while *H. pylori* is an important risk factor, a small percentage of diffuse GC arises from inherited cancer predisposition. Hereditary diffuse GC, a family GC syndrome, is commonly caused by variants of the *CDH1* gene that encodes for cadherin-1 (17). Molecular consequences of *CDH1* loss include: *i*) disruption of cell-cell adhesion, *ii*) induction of WNT/β-catenin oncogenic signaling, and *iii*) activation of EGFR signaling (18,19).

Environmental factors can increase the risk of developing GC. These include a diet high in salty or smoked food, a diet lacking fruits and vegetables, obesity, and smoking (20,21). In addition, host factors can directly, through their pro-oncogenic or anti-tumoral properties, or indirectly, by affecting the persistence of *H. pylori* and/or chronic gastritis, regulate *H. pylori*-induced GC (22). Polyamines are ubiquitous molecules found in microbes, plants, and animals (23,24). In mammals, they are produced by numerous different cells, including epithelial and immune cells, and play many important biological roles, from embryogenesis to aging (25). Due to their cationic nature, polyamines can interact with macromolecules, e.g., DNA, RNA, ATP, phospholipids, and proteins, and can thus contribute to gene regulation via epigenetic and chromatin structure modifications, translation, cell growth, proliferation, and differentiation (25,26). Therefore, it has been proposed that they have a

major effect on cancer. In this context, we summarize herein the findings about the importance of polyamine metabolism in gastric carcinogenesis.

1. The metabolism of polyamines

1.1. Polyamine synthesis

1.1.   Polyamine synthesis—In mammals, there are three major polyamines– putrescine, spermidine, and spermine, which are sequentially synthesized from the catabolism of the amino acids L-arginine and L-methionine (23,24), as depicted in Fig. 1. First, arginase (ARGI), an enzyme involved in the urea cycle, catalyzes the hydrolysis of L-arginine to L-ornithine and urea (27). Two isoforms of ARGI exist: the cytosolic ARGI1, which is expressed predominantly in the liver and to a lesser extent in the bone marrow, and the mitochondrial ARGI2, which is present in a variety of peripheral mammalian tissues including the digestive and gastrointestinal tract, kidney, and prostate. While the two isoforms catalyze the same biochemical reaction, they differ in cellular expression, regulation, and localization (28). Second, L-ornithine is converted by the rate-limiting enzyme ornithine decarboxylase (ODC), encoded by the gene *ODC1* (called *ODC* in this review to be consistent with the name of the gene in published studies), into the first polyamine putrescine (29,30). Alternatively, putrescine can also be generated from L-arginine by the arginine-agmatine-polyamine pathway (31). Arginine is metabolized to agmatine via arginine decarboxylase (ADC), followed by conversion of agmatine to putrescine by agmatinase (SPEB). Third, putrescine is sequentially converted into spermidine and spermine via spermidine synthase (SRM) and spermine synthase (SMS) respectively; both reactions require the donation of an aminopropyl group from decarboxylated S-adenosylmethionine (dcSAM) (23,25), which is synthesized by the sequential conversion of *i*) methionine to S-adenosylmethionine (SAM) by S-adenosylmethionine synthase (METK) and *ii*) SAM to dcSAM by the rate-limiting enzyme S-adenosylmethionine decarboxylase (SAMDC). The steady state levels of dcSAM are generally kept low to limit polyamine synthesis (25). It should be noted that the irreversible ODC inhibitor α-difluoromethylornithine (DFMO), which was synthesized over 40 years ago, is used to treat African trypanosomiasis, excessive hair growth on the face of women, and is under development as a chemotherapy for hyperproliferative diseases including cancer (32).

Importantly, intracellular polyamine homeostasis is also maintained by the back conversion of spermine to spermidine, and spermidine to putrescine (Fig. 1). This occurs through two distinct pathways. The first pathway involves the enzymes diamine acetyltransferase 1 (SAT1) and peroxisomal N^I-acetyl-spermine/spermidine oxidase (PAOX): SAT1 catalyzes the transfer of an acetyl group from acetyl-coenzyme A (acetyl-CoA) to either spermidine or spermine at the N^I position, then PAOX catalyzes the cleavage of acetylated polyamines, leading to the generation of spermidine or putrescine, 3-acetamidopropanal, and H_2O_2 (33,34). Note that only acetylated polyamines are exported outside of cells (35). The second pathway for polyamine back-conversion corresponds to the direct cleavage of spermine into spermidine, 3-aminopropanal, and H_2O_2 by spermine oxidase (SMOX) (36). SMOX is a major source of spermidine in tissues since it has been shown that deletion of the *Smox* gene in mice results in the reduction of spermidine content and accumulation of spermine (37). In addition, putrescine is also catabolized by the copper containing amiloride-sensitive amine

oxidase (AOC1; Fig. 1), also known as diamine oxidase or amiloride binding protein, to produce 4-aminobutanal, NH_3, and H_2O_2 (38).

1.2.   Synthesis of hypusine—Spermidine serves an essential role as the substrate for the synthesis of hypusine [N^ε-(4-amino-2-hydroxybutyl) lysine], a unique amino acid found only in the protein eukaryotic translation initiation factor 5A (EIF5A) (39–41). Spermidine is cleaved by deoxyhypusine synthase (DHPS; Fig. 1), resulting in the transfer of the 4-aminobutyl moiety to the ε-amino group on a specific lysine (Lys50 in mice and humans) found on EIF5A (40,42). Then, deoxyhypusine hydroxylase (DOHH) hydroxylates this intermediate to form hypusine, thus activating EIF5A (43). This post-translational modification of EIF5A is called hypusination. Two isoforms of EIF5A have been characterized, EIF5A1, which is expressed in all cells and tissues, and EIF5A2, which is mainly present in the testes, brain, and tumor cells (44,45).

Hypusinated EIF5A (EIF5A^{Hyp}) can bind specific mRNAs that contain an AAAUGU consensus sequence (46–48). The EIF5A^{Hyp}/mRNA complex (Fig. 2) is translocated to the cytoplasm by the nuclear transporter exportin-1 (49), reaches ribosomes, and facilities translation (50). In addition, it has been shown that EIF5A^{Hyp} alleviates ribosome stalling at proline repeats during translation elongation of other non-polyproline motifs, such as peptides enriched in diglycyl motifs and in basic and acidic amino acids (51–53).

Substitution of the critical lysine residue of EIF5A at the hypusination modification site with alanine, aspartic acid, or isoleucine inhibits yeast growth, revealing an essential role for EIF5A and its hypusine modification in yeast cell viability (54). Similarly, deletion of both EIF5A genes (55) or a single DHPS gene (56) causes loss of viability in yeast. Conversely, DOHH is not required for yeast growth (57). Moreover, knockout of *Eif5a1*, *Dhps*, or *Dohh* in mice leads to embryonic lethality, whereas heterozygous mice are viable and fertile (58,59). Interestingly however, homozygous *Eif5a2* knockout mice are viable and fertile (60). These data clearly demonstrate the essential role for hypusination in embryogenesis, and cell growth and development. Furthermore, because EIF5A^{Hyp} is required for proliferation, it has also been proposed that the spermidine/hypusine pathway can play a major role in carcinogenesis. Thus, it has been shown that *EIF5A*, *DHPS*, and/or *DOHH* knockdown reduces tumor cell growth and proliferation *ex vivo* in glioblastoma, neuroblastoma, vulvar high grade intraepithelial neoplasia, hepatocellular carcinoma, and pancreatic ductal, cervical, prostate, and ovarian cancer, as well as GC (44).

Hypusination regulates the growth and proliferation of numerous cancerous cell lines (44). Coni *et al.* demonstrated that hypusination promotes colorectal cancer (CRC) cell growth through direct regulation of MYC biosynthesis (61). Silencing and inhibition of DHPS resulted in reduced proliferation and cell viability of CRC cell line HCT116 *in vitro* and *in vivo* in xenografts in nude mice (61). Similarly, treatment with the DHPS inhibitor, N^1-guanyl-1,7-diaminoheptane (GC7) decreased EIF5A^{Hyp} levels, MYC protein levels, and polyp size in $Apc^{Min/+}$ mice treated with azoxymethane (61). Altogether, these data suggest that hypusination may play a critical role in tumor growth.

1.3. Regulation of ODC by antizyme—Most cellular proteins are tagged for proteasomal degradation through ubiquitination. However, the non-covalent association of ODC with antizyme (OAZ; Fig. 1) enhances its interaction with the proteasome leading to increased ubiquitin-independent degradation of ODC (29). OAZ production is induced by polyamines, particularly spermidine and spermine, in a mechanism that involves frameshifting to stimulate translation of *OAZ* mRNA. The protein OAZ inhibitor (AZIN) binds OAZ with a higher affinity than ODC, resulting in the release of ODC from OAZ-ODC complexes (62). Thus, AZIN increases ODC activity and subsequent intracellular polyamine formation, promoting the development of cancerous cells (62).

2. Polyamine metabolism during *H. pylori* infection and gastric carcinogenesis

2.1. ODC expression—Several studies have reported an increase in ODC protein and mRNA expression in the gastric tissues and macrophages of *H. pylori*-infected patients. Patchett *et al.* used a [^{14}C]ornithine bioassay to examine gastric mucosal ODC activity in 32 normal patients and 22 confirmed GC patients, which was compared to 47 patients with increased risk of upper gastrointestinal malignancy, specifically patients with partial gastric resection or those with FAP (63). Normal patients exhibited significantly lower ODC activity levels than those with gastric resection or confirmed GC. Importantly, ODC activity was significantly increased in the areas of gastric atrophy or IM regardless of the clinical group (63). Furthermore, in a cohort of patients with chronic superficial gastritis, chronic atrophic gastritis, gastric dysplasia, and GC, Miao *et al.* demonstrated by immunohistochemistry a positive correlation between ODC protein expression and degree of malignancy of the gastric mucosa (64), suggesting that ODC is a potential biomarker for precancerous lesions. These data were confirmed by Chaturvedi *et al.*, who further demonstrated that *ODC* mRNA expression is increased in patients with *H. pylori*-induced gastritis and immunohistochemistry revealed that ODC was primarily expressed in mononuclear cells of the lamina propria in these infected patients (65). Furthermore, Hardbower *et al.* used immunofluorescence to reveal a significant increase in the number of CD68^{+}ODC^{+} cells, indicative of expression in the gastric macrophages, in *H. pylori*-infected patients compared to uninfected individuals (66). These studies contrasted with the observation from Elitsur *et al.*, who observed no significant change in ODC activity in the gastric mucosa of 6 *H. pylori*-infected versus 12 non-infected children along with no reported correlation between gastric inflammation and level of ODC (67). The 6 *H. pylori*-positive children displayed gastritis, specifically 3 cases of chronic mild gastritis and 3 cases of chronic active moderate gastritis, but no presence of gastric atrophy. Both the ODC activity assay and ODC immunohistochemistry analysis revealed no difference in gastric ODC activity between infected and uninfected children (67). This discrepancy between studies could be explained by the age of the patients and/or the lack of gastric atrophy seen in these infected children opposed to the adults assessed in the other studies (65,66).

To investigate the relationship between ODC induction and *H. pylori* presence, Konturek *et al.* administered *H. pylori* eradication therapy (omeprazole, amoxycillin, and clarithromycin) for seven days and vitamin C for three months to 20 patients with atrophic gastritis (68). A decrease in severity of gastritis in addition to a reduction in *ODC* mRNA levels was observed in gastric tissues three months after therapy. This echoed the result from the Sakai

group, who had previously shown that eradication of *H. pylori* with triple therapy in atrophic gastritis and gastric ulcer patients decreased mucosal ODC activity and increased apoptosis in the gastric mucosa (69).

In vitro experiments have also been performed to study the induction of ODC by *H. pylori*. Asim *et al.* demonstrated that *ODC* mRNA is not upregulated in *H. pylori*-infected AGS or MKN28 human gastric carcinoma cells, and conditionally-immortalized mouse stomach cells (30). Further, it has been reported that *H. pylori* extracts inhibited ODC activity, but did not affect *ODC* mRNA expression in human gastric MKN45 cells (70). Although *H. pylori* extracts did not affect the viability of the gastric cells, extracts with proteins greater than 50 kDa significantly inhibited proliferation and ODC activity of the gastric cells. In contrast, ODC protein has been shown by Xu *et al.* to be induced through a phospho-CagA-mediated mechanism involving the Src/MEK/ERK/c-MYC pathway in the gastric epithelial cancer cell lines AGS and BGC-823 and the immortalized epithelial cell line GES-1 (71); it should be noted that the induction of ODC was surprisingly observed as soon as 2 hours post-infection, and *ODC* mRNA was not shown in this study. Overall, these studies suggest that the induction of ODC in transformed gastric epithelial cells (GECs) is not a major hallmark of the response to *H. pylori*.

However, Gobert *et al.* showed an increase in *Odc* mRNA levels in RAW 264.7 macrophages between 2 to 12 hours post co-culture with *H. pylori* SS1 and a significant increase in ODC enzymatic activity, accessed by the conversion of L-[^{14}C]ornithine to [^{14}C]CO$_2$ after 24 hours with *H. pylori* SS1 (72). Furthermore, Cheng *et al.* demonstrated that infection of RAW 264.7 macrophages with *H. pylori* exhibited a time-dependent 12-fold increase in ODC promoter activity that peaked at 6 hours, which was similarly seen with ODC mRNA, protein, and enzyme activity (73). Additionally, to investigate the role of the macrophage-derived ODC seen in *H. pylori*-infected patients, Hardbower *et al.* generated mice with myeloid-specific deletion of *Odc* (*Odc* ^{mye}) by crossing C57BL/6 *Odc$^{fl/fl}$* with a myeloid-specific *LysM$^{cre/cre}$* driver (66). *H. pylori*-induced ODC protein was immunolocalized in CD68$^+$ cells of C57BL/6 *Odc$^{fl/fl}$* mice, but was not detected in gastric macrophages of *Odc* ^{mye} mice, demonstrating the specificity of the immunodetection.

Thus, *H. pylori* infection is associated with an increase of ODC expression, notably in macrophages (Fig. 2).

2.2.   Induction of SMOX—SMOX is an essential enzyme in polyamine metabolism in the GI tract due to its efficient regulation of spermidine concentration (37). To examine the relationship between SMOX expression and *H. pylori*-associated GC risk, Chaturvedi *et al.* evaluated biopsies and *H. pylori* clinical isolates from Colombian patients living in either the Andean mountain region, in which the risk for developing GC is high, or in the Pacific coast region that is associated with a lower risk of GC in *H. pylori*-infected patients (74). Immunostaining of the gastric biopsies revealed that SMOX protein was more abundant in the epithelium of *H. pylori*-infected patients from the high-risk region compared to the low-risk population; this was observed for all stages of *H. pylori*-mediated disease, i.e., NAG, MAG, and IM, and SMOX levels increased progressively from NAG to MAG to IM (74). Furthermore, *in vitro* co-culture of the human gastric adenocarcinoma cell line AGS with

clinical *H. pylori* isolates showed that *SMOX* mRNA expression was significantly more induced by high-risk strains compared to low-risk strains, establishing the essential role of *H. pylori* in SMOX induction (74). Additionally, Murray-Stewart *et al.* examined the epigenetic silencing of the tumor suppressor miR-124 from both the high-risk and low-risk gastric biopsies and its association with SMOX levels (75). *H. pylori*-infected AGS cells transfected with miR-124 maintained basal levels of *SMOX* mRNA, while the negative control and wild-type AGS cells exhibited a 6–8-fold increase in *SMOX* mRNA levels. Subsequent analysis of DNA methylation via pyrosequencing of gastric mucosa DNA from 90 patients in Columbia revealed a significantly elevated level of DNA methylation of three *mir-124* gene loci in the high-risk population compared to the low-risk population, which was correlated with increased SMOX protein expression by immunohistochemistry (75).

Additionally, we have shown that Mongolian gerbils infected for 12 weeks with the PZ5056 strain of *H. pylori* isolated from a subject from the high-risk region of Colombia exhibited more dysplasia and SMOX staining localized to the dysplastic glands than animals infected with a low-risk clinical isolate (74). In contrast, our group did not observe a significant increase in *Smox* mRNA expression nor an increase of spermidine concentration in the gastric tissue of C57BL/6 mice infected with *H. pylori* PMSS1 for 4 weeks compared to control animals; there was no *Smox* expression and a dramatic reduction of spermidine concentration in mice with *Smox* deletion, demonstrating that basal SMOX activity affects polyamine concentrations in stomach tissues (76). Additionally, *in vitro* experiments on RAW 264.7 macrophages infected with *H. pylori* showed a significant increase in *Smox* mRNA expression and subsequent spermine catabolism (77,78). These data demonstrate that SMOX is induced in GECs and macrophages in *H. pylori* gastritis (Fig. 2).

2.3. Other enzymes—*AOC1* mRNA and protein expression is significantly increased in human stomach adenocarcinoma tissues compared to normal tissues (79), suggesting a role for this enzyme in the progression of human GC. In addition, the gene encoding for AZIN1, which supports ODC activity, has been identified as one of the 18 genes differentially expressed in human gastric tumors (80), and this was confirmed in a targeted analysis performed on 140 Japanese patients (81).

2.4. Polyamine concentrations in humans during H. pylori infection and GC
—Because polyamines support proliferation, it could be considered that their levels may be a marker of tumorigenesis. Thus, Lundell & Rosengren analyzed gastric neoplastic tissue from patients undergoing a total or subtotal gastrectomy (82). The putrescine and spermidine concentrations were significantly increased compared to the normal gastric mucosa from the same individuals in all 16 patients; however, the spermine levels did not differ between the two groups. Subsequent analysis of ODC activity in 14 patients revealed an increase, although not statistically significant, in ODC activity in the tumor tissue compared to the normal gastric mucosa (82). In a clinical trial examining the influence of *H. pylori* infection on polyamine levels, Linsalata *et al.* evaluated the polyamine levels via high performance liquid chromatography in the gastric antral and body biopsies from 20 *H. pylori*-positive and 6 *H. pylori*-negative patients undergoing gastroscopy (83). The *H. pylori*-positive patients exhibited higher polyamine levels in the antrum as opposed to the body, but both *H. pylori*-

positive biopsies contained higher polyamine levels compared to *H. pylori*-negative patients (83).

These data sustain the concept that enzymes involved in putrescine and spermidine synthesis –i.e. ODC and SMOX– are upregulated in *H. pylori*-infected patients and in GC.

3. The role of polyamines in gastric immune cell function

Upon infection, *H. pylori* primarily localizes to the gastric mucous layer and the gastric epithelial surface, but has also been shown to penetrate into the lamina propria (84). Colonization is followed by myeloid and lymphocyte infiltration into the gastric mucosa. These immune cells are activated by bacterial virulence factors, resulting in the release of inflammatory mediators that contribute to cellular damage and progression to cancer. The evasion of *H. pylori* from the innate and adaptive immune response leads to chronic persistence of infection and this may contribute to the progression to gastric adenocarcinoma (84). Several studies have implicated polyamines in the regulation of macrophage function, thus regulating *H. pylori* persistence and the potential risk for GC. The effect of polyamines on macrophage function is shown in Fig. 2.

Monocyte-derived phagocytic macrophages are vital to gastric mucosal homeostasis. Resident macrophages and infiltrating monocytes/macrophages present in the mucosa monitor and respond to invading pathogens. Stimulation by pathogen-associated molecular markers, damage-associated molecular markers, and the T helper (Th) 1 cytokine IFN-γ, polarize macrophages to the pro-inflammatory M1 phenotype (85,86). Th2 cytokines including IL-4 and IL-10, conversely, can stimulate alternatively activated M2 macrophages (87). Classically activated M1 macrophages produce high levels of antimicrobial effectors, such as nitric oxide (NO) or reactive oxygen intermediates, and pro-inflammatory cytokines TNF-α, IL-1β, IL-8, and IL-12 to recruit other immune cells to the site of infection; these cells thus exhibit antitumoral, antimicrobial and pro-inflammatory properties (85,86). In contrast, alternatively activated M2 macrophages have increased ARGI activity and express cytokines that dampen the immune response; these cells are associated with tissue repair and carcinogenesis (85). To assess the effect of polyamine regulation on macrophage activation, Hardbower *et al.* infected *Odc* [mye] macrophages *in vitro* with *H. pylori* SS1 and found increased M1 macrophage activation with *Odc* deletion and no effect on M2 markers (66). Subsequent addition of putrescine to *H. pylori*-infected *Odc* [mye] bone marrow-derived macrophages reversed this enhanced M1 macrophage activation (66), suggesting a role for ODC and putrescine as macrophage function regulators. *Odc* [mye] mice exhibited alterations in chromatin structure, specifically an increase in histone 3, lysine 4 monomethylation and histone 3, lysine 9 (H3K9) acetylation, along with decreased H3K9 di/trimethylation in *in vivo* and *ex vivo* primary macrophages, resulting in an upregulation of gene transcription, especially M1 gene expression (66). Together, these findings suggest that ODC reduces macrophage M1 response to bacterial infections through chromatin structure alterations, resulting in bacterial persistence and pathogenesis.

The polyamine spermidine is also required to support translation through hypusination. Recently, we demonstrated that *H. pylori* induces DHPS expression in murine macrophages (Fig. 2), resulting in enhanced hypusination of EIF5A (88). Humans and mice infected with

this bacterium also exhibited increased levels of EIF5A^{Hyp} in CD68$^+$ cells. To understand the role of hypusination in macrophage function, mice were generated with specific deletion of *Dhps* in myeloid cells (*Dhps* ^{mye}). Innate immune proteins induced by *H. pylori* and regulated by hypusination were identified from *Dhps* ^{mye} bone marrow-derived macrophages and GC7-treated macrophages. The proteins included major antimicrobial effectors, such as NOS2, IRG1, and proteins involved in autophagy (IRGM1 and SQSTM1). Accordingly, *H. pylori* survival was increased in macrophages with *Dhps* deletion or DHPS inhibition *in vitro* (88). These data indicate that translational regulation by hypusination is a hallmark of eukaryotic host defense against pathogenic bacteria. Thus, although blockade of DHPS results in inhibition of cancerous cell growth (61), it could be questioned whether this strategy might be efficient for GC since it could support the persistence of *H. pylori* in the stomach. In this context, further studies about the role of DHPS in animal models of *H. pylori*-mediated carcinogenesis are needed and are underway in our laboratory.

Besides the general effect of hypusination on translation, polyamines have been shown to specifically regulate the translation of inducible NO synthase (NOS2). It was reported that addition of spermine, and to a lower extent spermidine, to *H. pylori*-stimulated macrophages does not affect *Nos2* mRNA expression, but reduces NOS2 translation and consequently NO synthesis, thus inhibiting NO-dependent *H. pylori* killing; this was similarly observed with *Odc* knockdown using siRNA (89). Similarly, *Smox* knockdown using shRNA in RAW 264.7 murine macrophages, which was associated with increased spermine concentration, resulted in a marked decrease in NOS2 protein induction, NO production, and *H. pylori* killing (80). Further, Chaturvedi *et al.* demonstrated that addition of spermine to *H. pylori*-stimulated RAW 264.7 macrophages *in vitro* blocked L-arginine uptake by the inducible L-arginine transporter solute carrier family 7 member 2 (SLC7A2, previously known as CAT2), whereas knockdown of *Odc* had the opposite effect (65). These findings demonstrate that the polyamine spermine can also affect the post-translational regulation of immune effectors during *H. pylori* infection.

It should be also noted that spermidine and spermine have been shown to stimulate apoptosis in *H. pylori*-stimulated macrophages (77,90) and that DFMO inhibited this cell death (72). Consistent with these *in vitro* data, Lewis *et al.* found that mice infected with *H. pylori* for 48 hours exhibited gastric macrophage apoptosis, which was eliminated in *Arg2*$^{-/-}$ mice (91). As a mechanism, Chaturvedi *et al.* proposed that H_2O_2 release during spermine catabolism by SMOX, downstream of ARGI2 and ODC, was responsible for mitochondrial membrane depolarization and subsequent apoptosis (77).

Collectively, these data indicate an important role for polyamines in macrophage function and subsequent persistence of *H. pylori* infection, implicating polyamines as potential players in progression of chronic gastritis to GC.

4. The role of polyamines in GECs

Since ODC is not markedly upregulated in GECs in response to *H. pylori*, the role of ODC in gastric epithelial cell function has not been deeply investigated. Nonetheless, transfection of GC cells SGC7901 and MGC803 with antisense RNA to *ODC* led to significantly suppressed tumor cell proliferation, G_1 cell cycle arrest, and reduced invasiveness (90).

Furthermore, Xu *et al.* 2012 demonstrated that knockdown of ODC protein production in the human GC cell line BGC-823 led to a decrease in cell proliferation, with or without *H. pylori* infection (71), indicating that ODC is essential for expansion of gastric cancerous cells, as observed with tumor cells derived from neuroblastomas (92), prostate cancer (93), or colorectal adenomas (94). Supporting this concept, 3% DFMO in the drinking water has been shown to inhibit the growth of human gastric tumor xenografts in athymic BALB/c mice (95); concomitantly, putrescine and spermidine levels were significantly decreased in the tumors, whereas spermine was not affected.

In contrast, the enzyme SMOX has been shown to be induced in GECs by *H. pylori* (96). SMOX inhibition with MDL72527 or transfection with siRNA reduces H_2O_2 synthesis and oxidative DNA damage, assessed by measurement of levels of 8-oxoguanosine, in gastric epithelial AGS cells. Moreover, *H. pylori*-induced DNA damage is attenuated by catalase, further evidencing the critical role of the SMOX/H_2O_2 pathway in DNA alteration. In parallel, inhibition of SMOX reduces mitochondrial membrane depolarization, cytochrome *c* release, and activation of caspase-3 (96). These results indicate that the induction of SMOX is involved in the development of GECs with DNA damage and increased apoptosis. These findings were recently recapitulated *in vitro* in primary GECs as Sierra *et al.* showed that gastric organoids from *Smox*-deficient mice exhibit reduced levels of phosphoserine 139 of H2FA histone family member X (pH2AFX), a reliable marker of DNA damage, compared to gastric organoid monolayers from wild-type mice (76). Importantly, β-catenin was less activated in *H. pylori*-infected gastric organoids from *Smox* deficient mice compared to WT gastroids, and this was recapitulated in human gastric organoids treated with the SMOX inhibitor SLH150–54 (76). The effect of *Smox* deletion or SMOX inhibition was reversed by adding back spermidine (76); however, the effects of SMOX silencing and spermidine supplementation were not observed in uninfected cells (76), demonstrating that spermidine facilitates β-catenin activation in *H. pylori*-infected cells, but does not initiate the process. Elucidation of the underlying molecular mechanisms deserves further investigation.

Of note, the knockdown of AOC1 via siRNA results in the inhibition of proliferation, cell invasion/migration, AKT signaling, and EMT transition with co-current promotion of apoptosis in the human GC cell lines AGS and MKN45 (79). This suggests an important role for AOC1 not only in polyamine homeostasis, but also in GC tumor progression.

Thus, although ODC is not induced in GECs during *H. pylori* infection, its activity plays a major role in GC progression, notably by supporting cell growth and proliferation; polyamine catabolism, notably by SMOX activity, in GECs is implicated in gastric carcinogenesis and GC progression (Fig. 2).

5. Polyamines as a target in GC

Polyamines are critical regulators of cell growth and differentiation in the GI tract and play essential roles in repairing gastric mucosal damage (97). However, uncontrolled production of polyamines can lead to aberrant differentiation and proliferation, which are hallmarks of carcinogenesis and cancer progression.

5.1. Effect of polyamines on gastritis and colonization—Studies focused on inhibiting ODC with a chemical inhibitor or through genetic knockout in mice were employed to elucidate its role in carcinogenesis progression and immune response. Administration of DFMO to C57BL/6 mice during a four-month infection with *H. pylori* SS1 revealed a two-log-order reduction in bacterial levels and a significant decrease in gastric inflammation; in addition, the two-fold increase in polyamine levels found in the gastric tissue of infected compared to control animals were reverted back to basal levels with DFMO treatment (65). Furthermore, isolated gastric macrophages from infected mice treated with DFMO exhibited an increase in L-arginine uptake, NOS2 protein, and NO (65). These data suggest that inhibition of ODC dampens gastric macrophage activation, and reduces *H. pylori* burden and development of gastric inflammation. Our data in Odc^{mye} mice with the same *H. pylori* strain and infection length again showed decreased *H. pylori* colonization, but increased gastritis (66). There was a significant increase in the number of CD68$^+$ODC$^+$ cells in the *H. pylori*-infected versus control patients, which demonstrates an association between upregulation of ODC and bacterial infection in human gastric macrophages. Luminex multiple array analysis of the Odc^{mye} mouse gastric tissues revealed that chemokines were significantly increased in Odc^{mye} gastric tissues. This enhanced chemokine production suggests that ODC deletion in macrophages correlates with enhanced immune cell infiltration into the stomach, contributing to the enhanced inflammation observed (66). Additionally, the cytokines interleukin (IL)-17, a hallmark of the Th17 response (98), specifically in *H. pylori* infection (99), and tumor necrosis factor (TNF-α), were detected in significantly higher levels in the Odc^{mye} gastric tissues (66). This emphasizes that ODC expression in myeloid cells reduces inflammation and thus the innate responses and antimicrobial activity against *H. pylori*. It should be also noted that DFMO exhibits a direct effect on *H. pylori* growth and virulence (100,101), which may explain the discrepancy in gastritis observed between the results observed with *Odc* knockdown and DFMO treatment; note that the effect of DFMO on *H. pylori* occurs independently of an effect on polyamines, but by causing direct oxidative damage (101). The infection of mice with specific deletion of *Odc* in GECs is warranted to understand the role of epithelial ODC on gastritis and bacterial persistence and is underway in our laboratory.

Smox-deficient mice exhibit a significant reduction in gastric spermidine levels and inflammation, but increased colonization (76), indicating that spermidine in the stomach supports gastritis and the antimicrobial effect. Interestingly, we also observed that the specific deletion of DHPS, which uses spermidine as a substrate to generate EIF5A^{Hyp}, in myeloid cells resulted in a significant increase of stomach colonization by *H. pylori* and gastritis (88), demonstrating that spermidine metabolism by the SMOX/DHPS metabolic pathway is critical for the antimicrobial response *in vivo*. It is now important to determine why SMOX and DHPS favor and dampen inflammation of the stomach, respectively.

5.2. Polyamines regulate gastric carcinogenesis—Investigation into the role of a carbonate supplemented diet on Wistar rats induced with spontaneous GC through gastric resection by Ehrnström *et al.* revealed that animals fed the carbonate diet exhibit an increase in ODC protein expression, which is correlated with enhanced cell proliferation and augmented incidence of GC (102). In addition, in the models of GC induced by N-methyl-

N'-nitro-N-nitrosoguanidine (MNN) plus sodium chloride or by MNN plus tyrosine methyl ester, the ODC inhibitor 1,3-diaminopropane significantly reduces putrescine and spermidine production, and the number of cancers (103,104).

Further, the number of GECs with oxidative DNA damage and development of dysplasia and carcinoma were also significantly reduced by DFMO in Mongolian gerbils infected with the cancer-inducing clinical strain PZ5056G (74). In this study, the SMOX inhibitor MDL72527 has a similar effect as DFMO and that the combination of both compounds had a modest additive effect (74).

Thus, although the effect of polyamines on gastritis and colonization is complex and cell-specific, it seems that overall, polyamines are essential for gastric carcinogenesis and that inhibition of ODC and/or SMOX might represent a potent chemopreventive strategy in *H. pylori*-infected patients.

5.3. Human clinical trials—There is increased interest in targeting polyamine synthesis and metabolism for cancer prevention and therapy (Table 1). DFMO was one of the first drugs developed in the 1970s to target cancer cell growth via ODC activity inhibition (105). Initial *in vitro* studies with rat hepatoma and mouse leukemia cells revealed that DFMO administration decreased putrescine and spermidine concentrations, but barely affected spermine levels (106). However, DFMO was less effective *in vivo* in advanced small cell lung cancer and metastatic colon cancer clinical trials both alone and in combination with other agents (107). The ineffectiveness of DFMO was likely due to compensatory mechanisms that were activated upon polyamine depletion. Despite its mixed success, DFMO remains a possible anticancer agent due to its low toxicity, with only reversible ototoxicity reported (107). Other polyamine biosynthesis blockers, methylglyoxal bis (guanylhydrazone) and 4-amidinoindan-1-one-2'-amidinhydrazone (SAM486A), were investigated to target SAMDC. However, guanylhydrazone administration to hamsters resulted in undesired mitochondrial toxicity (108) and SAM486A displayed mixed success as a single agent in a phase II trial for relapsed or refractory non-Hodgkin's lymphoma (109) and in combination with other anti-proliferative agents, including 5-fluorouracil and leucovorin, in a phase I metastatic colorectal cancer trial (110). SAM486A was also tested in a phase II clinical trial for patients with metastatic melanoma, however many patients experienced severe toxicity with no confirmed partial response (111).

Recently in a phase II trial to evaluate DFMO as a potential maintenance therapy for children with high-risk neuroblastoma, Sholler *et al.* demonstrated that twice daily oral administration of DFMO for two years was well tolerated and was associated with high event free survival and overall survival (112). In a randomized placebo-control, double blind trial for sporadic colorectal adenoma prevention, Meyskens *et al.* reported a reduction in recurrent adenomatous polyps, with few side effects, after oral administration of DFMO and sulindac, a known nonsteroidal anti-inflammatory drug, once daily for 36 months (113). The clinical success of DFMO in these two cancers has spurred interest for clinical trials in other polyamine-implicated cancers. To this end, based on our data showing reduced GC in *H. pylori*-infected gerbils (74) and other work presented in this review, our group has been conducting a clinical trial of DFMO in patients with precancerous gastric lesions in Latin

America (ClinicalTrials.gov Identifier: NCT02794428); the study will be completed in late 2021.

Conclusions

Polyamine research has progressed considerably over the past few decades, elucidating the cellular and molecular functions of polyamines. Polyamines are essential for a variety of critical cellular functions including cell growth, proliferation, differentiation, and survival. Overall, in the context of *H. pylori*-induced GC development, polyamines are critical for carcinogenesis and for progression. Nonetheless, each polyamine can have differential effects in GECs and immune cells, and further deeper investigations are required to decipher their roles in the global oncogenic picture of the stomach. Regardless, recent advances have prompted clinical trials targeting key enzymes in the polyamine metabolic pathway including ODC.

Acknowledgements:

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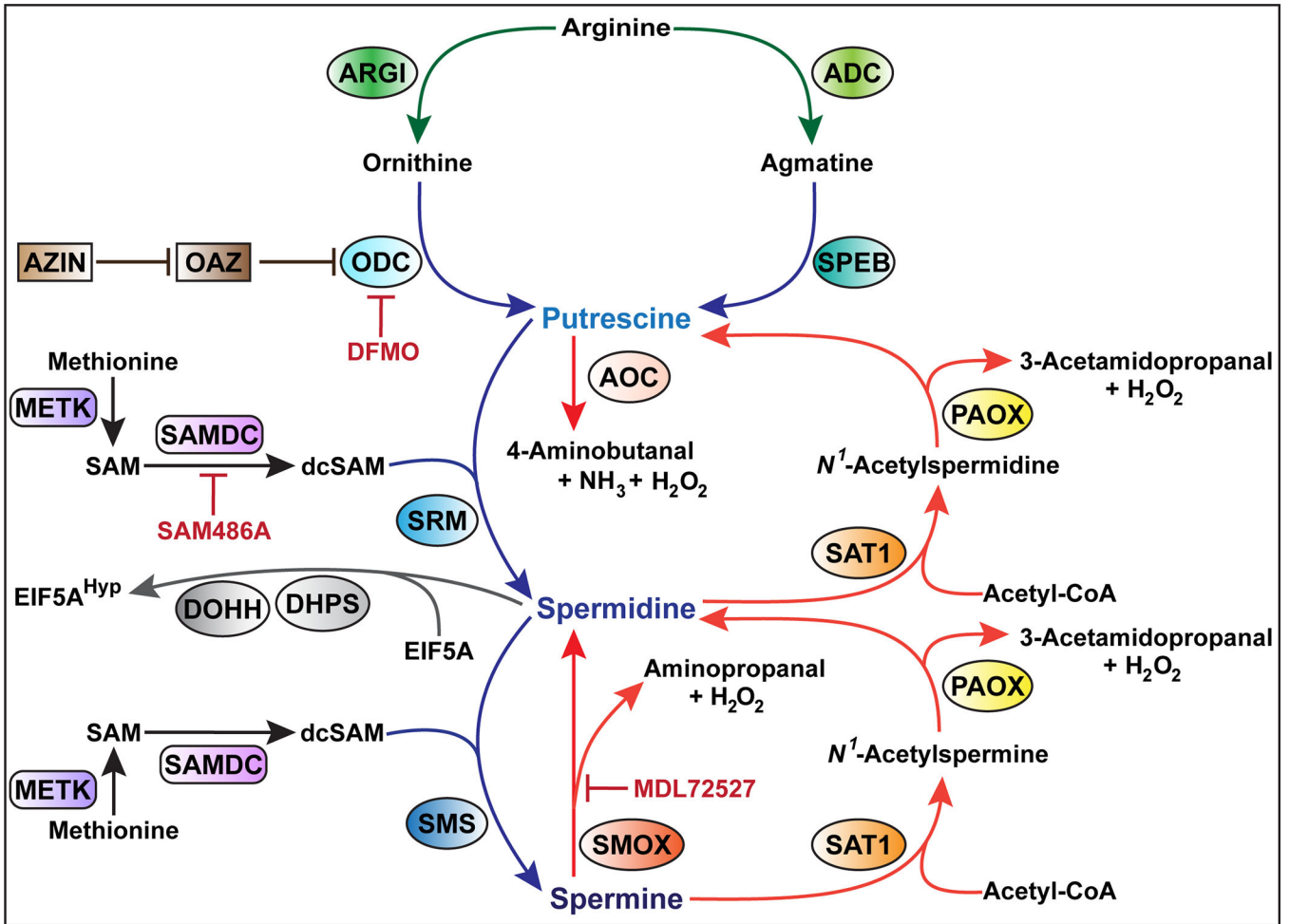
Fig. 1:

The polyamine metabolic pathway

Biosynthesis, interconversion, and degradation of putrescine, spermidine and spermine in mammals. Proteins abbreviations are in accordance with the guidelines from the Uniprot database (https://www.uniprot.org); note that the new abbreviation for ODC is DCOR.
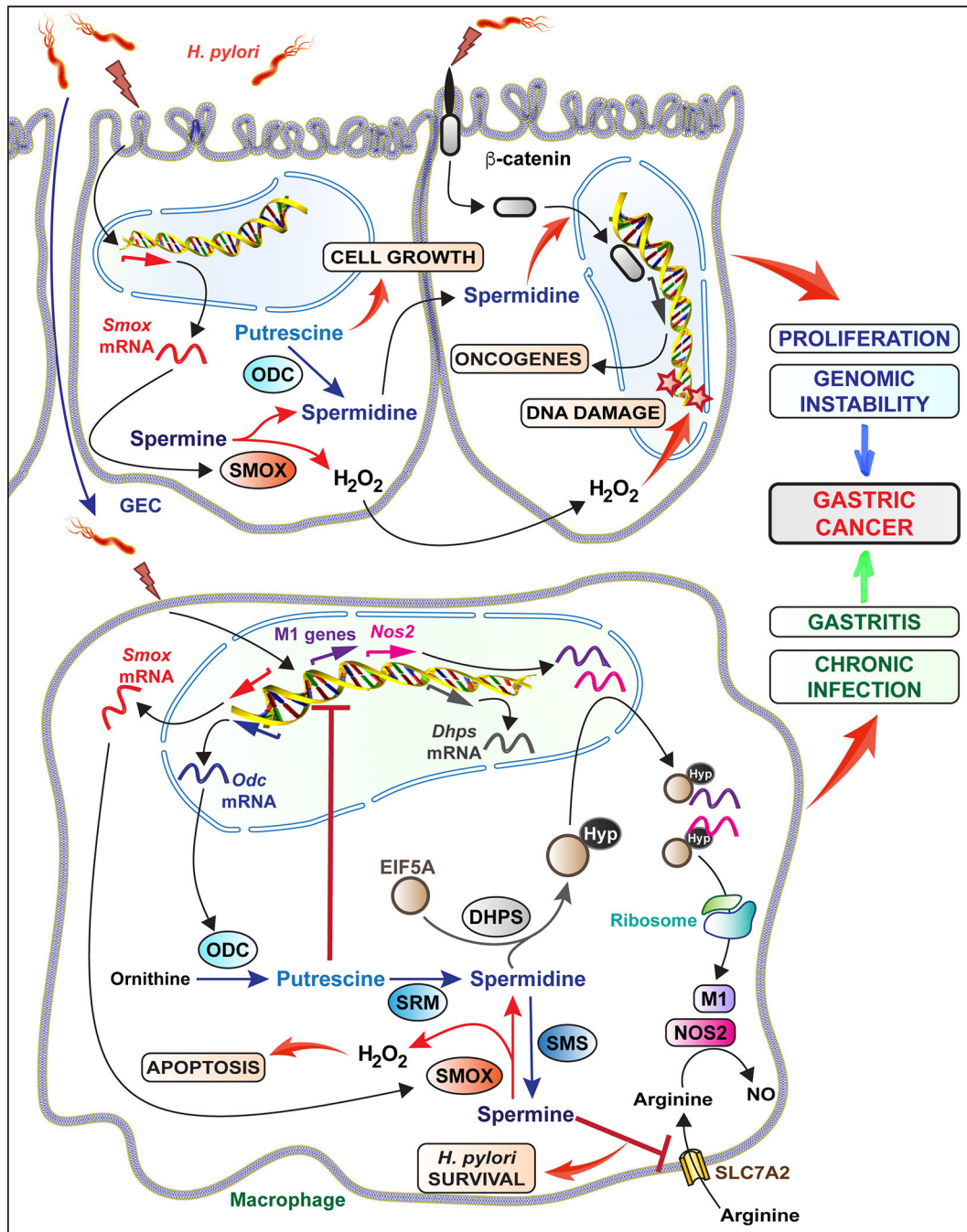
Fig. 2:

The role of polyamines on *H. pylori*-induced GC

The induction and the effect of polyamines in GECs and macrophages upon *H. pylori* infection are depicted.

Table 1.

Summary of clinical trial outcomes discussed in this review.

Cancer Type	Target	Drug	Combination Therapy	Clinical Trial	Patients Enrolled	Outcome	Toxicity	Ref.
Carcinoma (breast, prostate, stomach, female genital organ) or metastatic carcinoma of unknown origin	ODC	DFMO	Conventional chemotherapy	Phase I-II	38	No difference in disease progression in patients receiving DFMO + conventional chemotherapy vs. conventional chemotherapy only	Ototoxicity resulted in discontinuation of therapy for 6 patients	107
Relapsed or refractory non-Hodgkin's lymphoma	SAMDC	SAM486A	None	Phase II	41	Overall response rate (complete plus partial response): 18.9% for the 7 evaluable patients	Hematological: anemia, febrile neutropenia, thrombocytopenia Nonhematological: nausea, vomiting, diarrhea, asthenia, abdominal pain, flushing	109
Metastatic colorectal cancer	SAMDC	SAM486A	5-fluorouracil/leucovorin	Phase I	27	8/26 patients received a partial response & 9/26 had stable disease	Fatigue, dizziness, facial paresthesia, somnolence, skin toxicity	110
Metastatic melanoma	SAMDC	SAM486A	None	Phase II	15	No significant therapeutic potential & no reduction in tumor metabolism	Fatigue/lethargy, myalgia, neutropenia	111
Neuroblastoma	ODC	DFMO	None	Phase II	140	- Maintenance therapy to prevent relapse after standard therapy: 2-year survival: 84% ± 4%; overall survival: 87 ± 2%. - Maintenance therapy after salvage therapy for relapsed/refractory disease: 2-year survival: 54 ± 8%; overall survival: 84 ± 6%	67% of patients reported no treatment-related adverse events; the most common reported toxicity was Grade 2–3 liver injury	112
Sporadic colorectal adenoma	ODC	DFMO	Sulindac	Randomized placebo-control, double blind trial	375	Reduced recurrence of all adenomas (70%), advanced adenomas (92%), and recurrence of more than 1 adenoma (95%)	No differences in toxicity between groups	113