SUPPLEMENTARY METHODS

Study Participants and Eligibility

Thirteen villages were selected at random from four townships in Linqu County, Shandong Province, and a census identified 4010 inhabitants aged 35–64 years. Of these, 3599 subjects underwent gastroscopy with biopsy in 1994 and were invited to participate in the randomized Shandong Intervention Trial. A total of 3411 subjects who were thought to be eligible and provided written informed consent were then randomly assigned to treatment (Supplementary Figure 1). Eligibility exclusions before randomization included lack of written informed consent, residence outside the study villages, allergy to penicillin, deceased, missing baseline *Helicobacter pylori* serology, age outside the range of 35–64 years, previous treatment for *H. pylori*, previous cancer diagnosis (except nonmelanoma skin cancer), bleeding disorder, heart failure, emphysema, renal or liver disease, or other life-threatening illness. After randomization, which took place on July 23, 1995, investigators who did not know the treatment assignments or gastric cancer or gastric histopathology status reviewed eligibility data in January 2005, in preparation for a publication on the results of the trial (1). That review determined that 46 subjects were initially ineligible (two had died, 38 had a diagnosis of gastric cancer, and six had another cancer other that skin cancer before randomization). Data from the remaining 3365 eligible randomly assigned subjects were presented in the earlier publication (1) and in this paper. The original randomized trial and the study extension were approved by the Institutional Review Boards of the Beijing Institute for Cancer Research, the National Cancer Institute, and Westat, and separate written informed consent was obtained for the extended follow-up. The Shandong Intervention Trial is registered at the National Cancer Institute PDQ database (trial number NCI-OH-95-C-N029; available at http://www.cancer.gov/clinicaltrials/).

Design, Randomization, and Masking

The trial was stratified into *H. pylori*–seropositive vs *H. pylori*–seronegative subjects, as determined from serum drawn in 1994 (2). *H. pylori*–seropositive subjects were randomly assigned to three interventions or their placebos in a 2×2×2 factorial design, whereas *H. pylori*–seronegative subjects were randomly assigned in a 2×2 factorial trial of vitamin and garlic supplements but also received placebo for antibiotics to preserve masking (Supplementary Figure 1). Random treatments assignments were constrained to be balanced within groups of patients defined by age and sex, and participants and investigators were masked to treatment

assignment (2). Random treatment assignments were generated at Westat in the United States after eligibility was determined, and coded pill bottles were then distributed in Linqu County.

Treatments

Amoxicillin (1 g) and omeprazole (20 mg) (n = 1130) or placebo (n = 1128) were given twice daily for 2 weeks from September 15 to November 29 of 1995. A repeat 2-week course of active treatment was offered to the 382 subjects for whom the initial course of active therapy did not eradicate *H. pylori*, as determined by ^{13}C-urea breath tests conducted in January to March of 1996. To preserve masking, 383 subjects assigned to placebo and matched on village, age, and sex were offered retreatment with placebo. We call this treatment "*H. pylori* treatment." Vitamin and garlic oral supplements began in November of 1995 and continued to March 31, 2003. The vitamin supplement (n = 1677) contained 250 mg of vitamin C, 100 IU of vitamin E, and 37.5 μg of selenium or placebo (n = 1688), given twice daily. We call this intervention "vitamin treatment." The garlic supplement (n = 1678) contained 400 mg of Kyolic aged garlic extract (Wakunaga Pharmaceutical Co., Osaka, Japan) and 2 mg of steam-distilled garlic oil or placebo (n = 1687), given twice daily. Bottles with garlic placebo capsules contained trace amounts of steam-distilled garlic oil to preserve masking. We call this intervention "garlic treatment." From December 1995 to May 1996, the vitamin supplement also contained beta-carotene (7.5 mg, twice daily). Garlic and vitamin supplements were not given in June and July of 1999, and garlic supplements were not given in September of 2002 (1).

Endpoints: Follow-up for Gastric Cancer Incidence and for Cause-Specific Mortality

The primary endpoints were gastric cancer incidence and mortality; secondary endpoints included death from gastric or esophageal cancer, death from any cancer, and mortality from any cause. Gastric cancer incidence was ascertained either from scheduled gastroscopies, with biopsies at seven standard sites, for all trial participants in 1999 and 2003; from gastroscopies conducted from May 2, 2003 to August1, 2010 for those diagnosed with moderate or severe dyplasia at any biopsy site or with mild dysplasia at two or more sites in 2003 (285 participants had one or more endoscopic examinations with biopsies during the extended follow-up); or from cancer registry or autopsy reports, which were supported by medical records. Gastroscopy and biopsy procedures and histopathologic criteria are described elsewhere (3, 4). Study participants were visited monthly for distribution of supplements through March of 2003, and initial trial

follow-up ended on May 1, 2003. During the extended follow-up from May 2, 2003, to August 1, 2010, a village doctor supervised the follow-up in each village. Staff from the Beijing Institute of Cancer Research visited each village every 3 months to gather information on gastric cancer incidence and cause-specific mortality. Patients diagnosed with gastric cancer were interviewed, and their hospital records were reviewed to determine the method of diagnosis and the location of the cancer in the stomach. Copies of pathology and other medical reports were acquired. Causes of death were obtained from death certificates and hospital records. Deaths were categorized as death with a gastric cancer diagnosis before or at the time of death. In secondary analyses, we assessed death with gastric or esophageal cancer diagnosed before or at the time of death; death with any cancer (except nonmelanoma skin cancer) diagnosed before or at the time of death; and death from any cause.

Statistical Analysis

Intention-to-treat analyses were performed. Odds ratios (ORs) of gastric cancer incidence and corresponding 95% confidence intervals (CIs) were estimated from conditional logistic regression. The regression models included indicators for treatment and the following covariates: age (<40, 40–44, 45–54, ≥55 years), sex (male, female), ever smoker (yes, no), and ever drinker of alcohol (yes, no). The age categories were chosen so that approximately one-quarter of the participants fell into each category. These analyses were stratified on baseline histopathology in 1994 into 1) moderate chronic atrophic gastritis or less severe; 2) severe chronic atrophic gastritis or superficial intestinal metaplasia; 3) deep intestinal metaplasia; and 4) mild, moderate, or severe dyplasia. For analysis of *H. pylori* treatment, only participants who were *H. pylori*–seropositive in 1994 were included. Analogous stratification and covariate adjustment were used to estimate mortality hazard ratios (HRs) and 95% confidence intervals for gastric cancer and other causes of death using Cox proportional hazards analyses on the scale of time since randomization (July 23, 1995). Schoenfeld residuals did not identify violations of the proportional hazards assumption for treatment main effects, nor did tests for interaction of treatment with time on study. All *P* values were two-sided, and *P* less than or equal to .05 was considered statistically significant. Analyses were performed using SAS software (version 9.2, SAS Institute, Cary, NC).

SUPPLEMENTARY RESULTS

Alternative Covariate Adjustment for Gastric Cancer Incidence Data

An alternative logistic analysis of the effects of *H. pylori* treatment on gastric cancer incidence in the *H. pylori*–seropositive stratum included indicator variables for each intervention, sex, age, and histopathology categories. The adjusted odds ratio for *H. pylori* treatment was 0.60 (95% CI = 0.38 to 0.94) with *P* value of .026. These results are very similar to those presented in Table 2.

Cause-specific Numbers of Deaths by Treatment in the Trial and in the Entire Study

The cause-specific numbers of deaths are shown in Supplementary Table 1 below. Marginal counts are shown for each treatment and for the corresponding placebo group. These counts are listed separately for the Shandong Intervention Trial period ending on May 1, 2003, for the extended follow-up from May 2, 2003, to August 1, 2010, and for the entire trial plus extended follow-up period ending on August 1, 2010. From these data, one can determine whether treatment effects on a particular cause of mortality occurred during the trial, during the period of extended follow-up, or during both periods. For example, nine gastric cancer deaths occurred during the trial among those assigned to vitamins, compared with 12 deaths in the corresponding placebo group. During the extended follow-up, eight deaths occurred on vitamins, compared with 18 deaths on placebo.

Pooled Analysis of Trials of *H. pylori* treatments for Gastric Cancer Incidence

A 2009 meta-analysis of seven randomized trials, including the Shandong Intervention Trial, concluded that *H. pylori* treatment reduced the incidence of gastric cancer (5). However, two of the reports (6,7) included in the meta-analysis concerned the same trial and reported inconsistent findings (8), and in another included trial (9), the primary endpoint was a new gastric cancer following a gastric cancer diagnosis. In an unreported pooled analysis that excluded those three trials, we calculated a relative risk of gastric cancer incidence of 0.69 (95% CI = 0.44 to 1.08), suggesting lingering uncertainty about the effectiveness of *H. pylori* treatment in preventing gastric cancer. When we combined our data from extended follow-up of the Shandong Intervention Trial with the counts of gastric cancers and population sizes from other published studies (5,8), but excluded the three trials (6,7,9), we estimated the relative risk of gastric cancer

incidence as 0.66 (% CI = 0.46 to 0.95), with a *P* value of .027 (Supplementary Table 2). When we also included data from the trial of second gastric cancers following a primary gastric cancer (9), the estimated relative risk was 0.58 (95% CI = 0.42 to 0.81), with a *P* value of .0014 (Supplementary Table 2). In these analyses, we pooled the gastric cancer counts and population sizes across studies and computed relative risks comparing the group on *H. pylori* treatment vs the group on placebo by analyzing the ratio of binomial proportions with the use of StatXact-3 statistical software (Cytel Software Corporation, Cambridge, MA, 1995). All statistical tests were two-sided.

Supplementary Table 1. Cause-specific numbers of deaths by treatment group during the trial through May 1, 2003, during the extended follow-up from May 2, 2003 through August 1, 2010, and during the entire study through August 1, 2010*

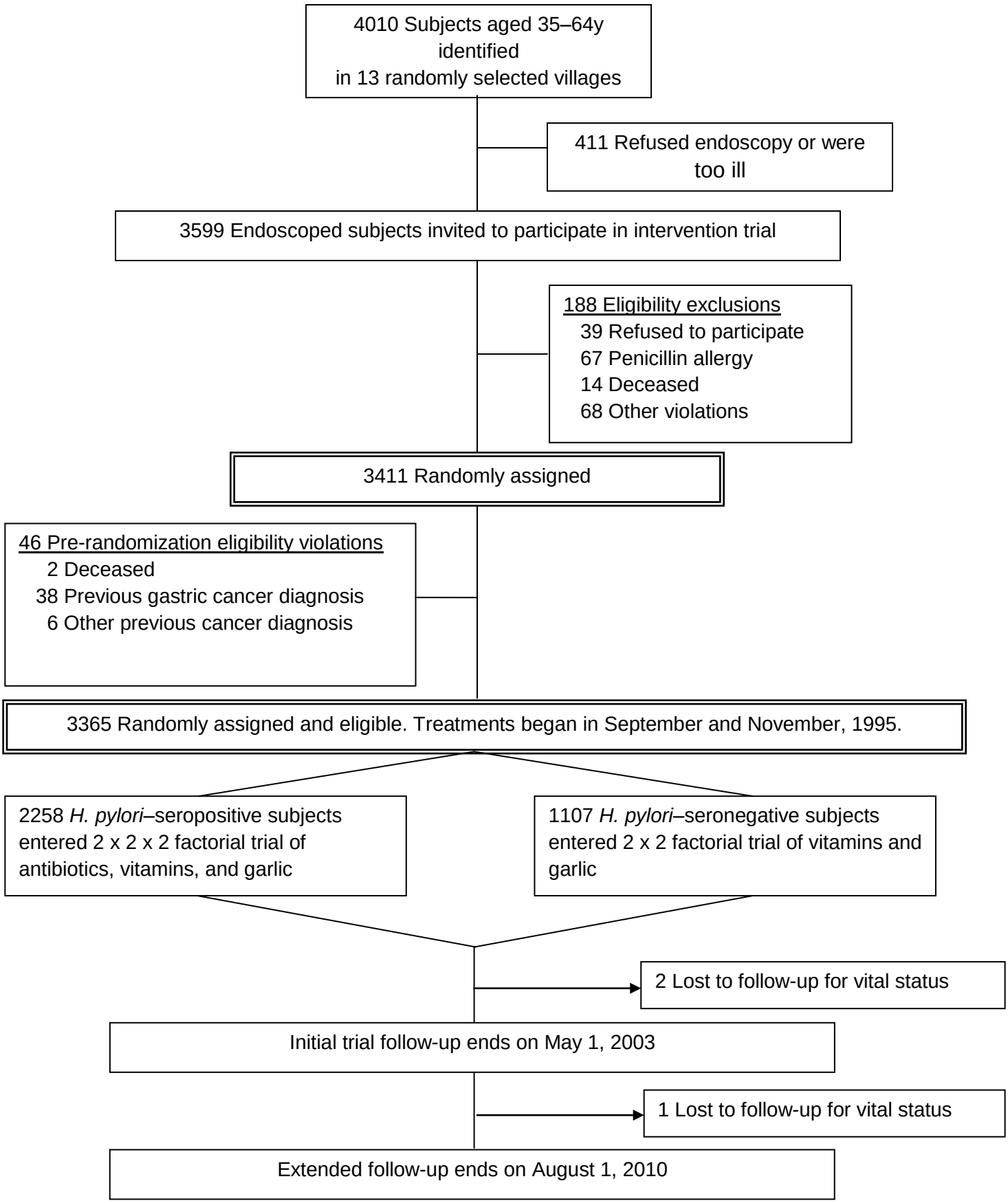
Treatment, study period	Cause of death							
	Gastric cancer	Esophageal cancer	Other cancer	Stroke	Other cardiovascular	Accident	Other	Total
H. pylori treatment (n = 1130)								
Trial only	8	7	17	12	6	8	9	67
Extension	9	5	24	19	14	7	12	90
Entire study	17	12	41	31	20	15	21	157
Placebo (n = 1128)								
Trial only	10	6	15	5	10	6	6	58
Extension	14	1	23	13	15	8	10	84
Entire study	24	7	38	18	25	14	16	142
Garlic (n = 1678)								
Trial only	12	6	25	13	14	13	12	95
Extension	9	3	34	27	16	8	22	119
Entire study	21	9	59	40	30	21	34	214
Placebo (n = 1687)								
Trial only	9	8	23	11	13	7	17	88
Extension	17	5	31	17	25	9	21	125
Entire study	26	13	54	28	38	16	38	213
Vitamins (n = 1677)								
Trial only	9	5	27	10	8	12	11	82
Extension	8	3	36	21	25	7	19	119
Entire study	17	8	63	31	33	19	30	201
Placebo (n = 1688)								
Trial only	12	9	21	14	19	8	18	101
Extension	18	5	29	23	16	10	24	125
Entire study	30	14	50	37	35	18	42	226

* The number of subjects assigned to *H. pylori* treatment or its corresponding placebo is smaller than the number assigned to garlic or vitamins or the corresponding placebos because only subjects who were *H. pylori*–seropositive at baseline were assigned to *H. pylori* treatment or the corresponding placebo. The total number of deaths in the entire study was 427, which is the sum of the number of deaths on the active treatment arm and on the placebo arm, both for vitamins and for garlic treatments.

Supplementary Table 2. Combined analysis of *H. pylori* treatment effects on gastric cancer (GC) incidence in randomized trials*

First author, year of publication (reference)	No. of incident GC/ No. of patients on *H. pylori* treatment	No. of incident GC/ No. of patients on placebo	Comments
Correa, 2000 (10)	3/491	2/485	
Wong, 2004 (11)	7/817	11/813	
Saito, 2005 (12)	2/379	3/313	
Ma, 2012 (this study)	34/1130	52/1128	
Subtotal	46/2817	68/2739	RR = 0.66, 95% CI = 0.46 to 0.95 (*P* = .027)
Fukase, 2008 (9)	9/272	24/272	Secondary GC incidence following treatment of primary GC
Total	55/3089	92/3011	RR = 0.58, 95% CI = 0.42 to 0.81 (*P* = .0014)
Leung, 2004 (6)	4/295	6/292	Excluded
Zhou, 2008 (7)	2/276	7/276	Excluded

* All references and data (except for this study) were taken from the meta-analysis by Fuccio et al. (5). The last two papers were not included in the combined analysis because of possible redundancy and inconsistency as discussed in a correspondence (8) regarding the original meta-analysis. RR = relative risk; CI = confidence interval.



Supplementary Figure 1. Trial design, accrual, and follow-up of trial participants.

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