Supplementary Appendix 2. Study Protocol

The efficacy, safety, and cost-effectiveness of screening and eradication of *Helicobacter pylori* infection for gastric cancer prevention- a prospective cohort study

Abstract

Background: There is insufficient evidence to support the efficacy, safety, and cost-effectiveness regarding the mass screening and eradication of *H. pylori* is indicated in countries with various prevalence of *H. pylori* infection and incidence of gastric cancer.

Objectives: Therefore, we designed this prospective cohort study to survey the updated prevalence of *H. pylori* infection and antibiotic resistance of *H. pylori*, to assess whether the risk of gastric cancer can be reduced and whether the risk of extra-gastric diseases will be altered after the screening and eradication of *H. pylori* in the screened group as compared to the general population, to monitor the antibiotic resistance rate of other bacteria 1 year after the program (in a subgroup of subjects), to survey the changes in gut microbiota after *H. pylori* eradication (in subgroup of subjects), and to identify risk factors associated with gastric cancer development after the screening and eradication program.

Methods: Healthy adult subjects (N=14,400 in Taiwan) will be screened for *H. pylori* infection by serology test and *H. pylori* stool antigen test (HpSA). Those with only one positive test will be confirmed by ^{13}C-urea breath test (^{13}C-UBT). Endoscopy, histology, and culture for *H. pylori* will be done in subgroup of subjects. Antibiotic resistance of *H. pylori* will be

determined by agar dilution test and PCR followed by direct sequencing. Eradication therapy with clarithromycin-containing regimens or bismuth quadruple therapy will be offered for all infected subjects in other clinical trials or according to the local prevalence of antibiotic resistance. ^{13}C-UBT (or validated HpSA may be an alternative) will be used to confirm the status of *H. pylori* after eradication therapy. Rescue therapies will be offered and retested by ^{13}C-UBT. Fecal samples will be collected at baseline and 1 year after eradication therapy for analysis of microbiota and antibiotic resistance of gut flora in subgroups of subjects. Pepsinogen I and II levels will be measured. Buffy coat DNA will be used for genome wide single nucleotide polymorphisms. We will link to the database of vital statistics in Taiwan to assess the long-term outcomes.

Outcomes:

1. Cross-sectional: The age-standardized prevalence, the antibiotic resistance rate of *H. pylori*, and the aged-standardized and specific incidence and mortality of gastric cancer.
2. Changes of gut microbiota and antibiotic resistance before and year 1
3. Long-term: The standardized incidence rate (SIR) and standardized mortality rate (SMR) of gastric cancer and extra-gastric diseases in the screened group vs. general population. The cost-effectiveness of the program in countries with different prevalence of *H. pylori* and incidence of gastric cancer.

Introduction

Gastric cancer remains one of the leading causes of cancer related mortality worldwide.[1] More than 750,000 people died of gastric cancer each year. [1] The majority (>85%) of non-cardia gastric cancer can be attributable to *Helicobacter pylori (H. pylori)*.[2] Animal studies and randomized trials in human have shown that eradication of *H. pylori* can reduce the risk of gastric cancer.[3,4] Eradication of *H. pylori* before the development of precancerous gastric lesions offers higher protective effect against gastric cancer.[5] However, eradication of *H. pylori* may also reduce the risk of metachroneous gastric cancer in patients receiving curative endoscopic resection for early gastric cancer.[6,7] Therefore, eradication therapy is recommended for subjects with *H. pylori* infection, unless there are competing considerations.[8-10] However, whether mass screening and eradication of *H. pylori* in the general population for gastric cancer prevention is indicated remains controversial.[11] There are several unresolved issues and additional evidences are required to support such policy. First, there is a need to survey the current disease burden of gastric cancer and *H. pylori* infection. Second, surveillance of the updated prevalence of antibiotic resistance is necessary to decide the optimal regimen to be used for gastric cancer prevention. Third, whether the mass screening and eradication program may reduce the risk of gastric cancer and the cost-effectiveness in countries with different prevalence of *H. pylori* and different incidence of gastric cancer should be assessed. Fourth, the long-term changes of antibiotic resistance of other bacteria after mass screening and eradication program should be

monitored. Fifth, the long-term impacts of such program on the gut microbiota and extra-gastric disorders remain unknown. Finally, how to identify subjects who retain the risk of gastric cancer and require endoscopic surveillance remains unknown. Therefore, we conducted this trial to provide evidence on these issues.

Earlier epidemiological studies have shown that more than half of the people in the world were infected with *H. pylori*.[12] However, many of these studies were conducted decades ago and the updated prevalence of *H. pylori* infection is lacking in many countries.[12] With the improvement in socioeconomic status and hygiene, the prevalence of *H. pylori* is expected to be declined in many countries.[13] Therefore, there is an unmet need to survey the updated prevalence of *H. pylori* infection. Besides, there were several limitations of previous studies. First, the tests used to survey the prevalence of *H. pylori* were heterogeneous.[12] Serology test, urea breath test, and *H. pylori* stool antigen (HpSA) tests were the most commonly used tests in these studies. However, the accuracies of serology test and HpSA used in these studies varied greatly. Second, the methods used to recruit study subjects and the inclusion and exclusion criteria varied greatly in different studies.[12] Studies that recruit patients with dyspepsia from outpatient clinic might overestimate the prevalence of *H. pylori*. Studies that recruit subjects from community setting are more representative of the actual prevalence of *H. pylori* infection in the general population. Third, the age-standardized prevalence of *H. pylori* infection was not reported in the majority of previous studies. Since the prevalence of *H. pylori* is usually higher in older people and lower

in the younger generations, studies that recruit higher proportion of older people may overestimate the actual prevalence of *H. pylori* infection. Therefore, it is necessary to report the age-standardized prevalence of *H. pylori* infection which is needed to estimate the burden of *H. pylori* infection in the general population. Therefore, we aimed to survey the updated prevalence of *H. pylori* infection in Asia-Pacific countries.

The prevalence of antibiotics varies greatly in different countries and may change over time.[14] Several studies have shown an increase of clarithromycin and levofloxacin resistance of *H. pylori*, leading to the reduced eradication rates of regimens containing these antibiotics.[14,15] International consensus reports recommended that the optimal first-line treatment in each country should be decided according to the local prevalence of antibiotic resistance.[19,10,16] Although the prevalence of antibiotic resistance of *H. pylori* has been reported in several studies, there are some limitations of these studies. First, the updated prevalence of antibiotic resistance is lacking in many countries. Second, the methods and the break points of minimum inhibitory concentrations (MICs) used to determine the antibiotic resistance varied in different studies. Third, the study periods of the published articles varied greatly. Therefore, surveillance of the updated prevalence of antibiotic resistance of *H. pylori* is warranted.

Few randomized controlled trials addressed on whether mass screening and eradication of *H. pylori* is effective and cost-effectiveness in the prevention of gastric cancer.[11,17] Most of the published studies and ongoing trials randomized *H. pylori* infected

subjects into eradication therapy or no eradication therapy.[4] Few of the previous trials randomized subjects into screening versus no screening.[4] Since the prevalence of *H. pylori* and incidence of gastric cancer varied greatly in different countries, the sample size required for the latter issue is expected to be large, especially in countries with low prevalence of *H. pylori* infection and low incidence of gastric cancer. Therefore, an alternative study design is to assess whether the risk of gastric cancer is the screened population is reduced as compared to the general population. Standardized incidence rate and standardized mortality rate can be used to assess whether such preventive strategy is effective for gastric cancer prevention. Cost-effectiveness may also be analyzed according to the prevalence of *H. pylori* and incidence of gastric cancer.

The widespread use of antibiotics in the mass screening and eradication of *H. pylori* for gastric cancer prevention may lead to the emergence of antibiotic resistant strains of other bacteria in the community.[18-21] However, there is little evidence to suggest whether the short-term increase in antibiotic resistance of other bacteria may persist or may be restored to basal state in the long run. Recently, we showed that the short-term increase in the antibiotic resistance of *Escherichia* coli and *Klebsiella pneumoniae* may be restored to basal state 2 months after triple therapy and concomitant therapy.[21] There were no significant increase of antibiotic resistance of *Escherichia coli* and *Klebsiella pneumoniae* after bismuth quadruple therapy.[22] However, whether the antibiotic resistance of other bacteria and the resistome of gut microbiota are increased after *H. pylori* eradication should

be assessed in further studies. Therefore, we will monitor the prevalence of antibiotic resistance of other bacteria after mass screening and eradication of *H. pylori* infection in a subgroup of subjects.

The use of antibiotics to eradicate *H. pylori* also leads to short-term perturbation of the composition and diversity of gut microbiota.[23-24] Some studies showed that the short-term use of triple therapy may lead to long-term perturbation of oral and gut microbiota.[23-24] However, our recent study showed that the short-term changes of alpha and beta-diversities were largely recovered months to years after *H. pylori* eradication.[22] Yet, the findings should be validated in other population. Besides, whether the changes in strain level are restored to basal state should be assessed. There are controversial associations of *H. pylori* and extra-gastric disorders, such as inverse associations with allergic diseases, inflammatory bowel disease, and obesity, and positive associations with cardiovascular diseases and colorectal cancer etc.[25,26] The associations might be attributed to gut microbiota. Whether mass screening and eradication of *H. pylori* eradication may increase or reduce the risks of these extra-gastric disorders deserves investigation. Another important issue is that gastric cancer may still develop in some patients after *H. pylori* eradication. It is important to identify factors predisposing to gastric cancer after eradication therapy.

Therefore, we designed this prospective cohort study to survey the updated prevalence of *H. pylori* infection and antibiotic resistance of *H. pylori* in Asia-Pacific regions, to assess whether the risk of gastric cancer can be reduced and whether the risk of

extra-gastric diseases will be increased or reduced after the screening and eradication of *H. pylori* in the screened group as compared to the general population, to monitor the antibiotic resistance rate of other bacteria 1 year after the program (in a subgroup of subjects), to survey the changes in gut microbiota after *H. pylori* eradication (in subgroup of subjects), and to identify risk factors associated with gastric cancer development after the screening and eradication program.

Objectives: We aimed to assess the efficacy, safety, and cost-effectiveness of mass screening and eradication of *H. pylori* for gastric cancer in countries with different prevalence of *H. pylori* infection and incidence of gastric cancer.

Specific aims

1. To survey the age-standardized prevalence of *H. pylori* infection in the world.
2. To survey the updated prevalence of antibiotic resistance of *H. pylori* in the world.
3. To compare the composition and diversity of fecal microbiota in *H. pylori* infected vs. non-infected subjects and the changes after *H. pylori* eradication.
4. To analyze the association of genome wide single nucleotide polymorphism among *H. pylori* infected vs. non-infected subjects; and among those with and without precancerous lesions among *H. pylori* infected subjects.
5. To analyze the changes of antibiotic resistance of E. coli, K. pneumoniae, Enterococcus at

baseline and 1 year after eradication of *H. pylori.*

6. Long-term outcomes: To assess the impact of screening and eradication of *H. pylori* infection on the standardized incidence rate (SIR) and standardized mortality rate (SMR) of gastric cancer, other GI cancers, major cardiovascular events, inflammatory bowel disease, autoimmune disease (SLE, RA), Parkinson's disease, etc. in the screened group as compared to the general population. The role of gut microbiota on the development of these diseases will also be assessed.
7. To identify factors associated with gastric cancer after screening and eradication of *H. pylori*.
8. To perform a cost-effectiveness analysis of the strategy of screening and eradication of *H. pylori* infection in countries with different prevalence of *H. pylori* infection and incidence of gastric cancer.

Materials and Methods

Study population

Adult subjects aged 20 years or greater with unknown history of *H. pylori* infection are eligible for this study. Subjects with the following conditions will be excluded: 1. History of *H. pylori* eradication therapy; 2. History of gastrectomy; 3. Severe underlying illness, such as end-stage renal disease, decompensated liver cirrhosis, decompensated heart failure, hemophilia; 4. Those taking antibiotics 4 weeks or proton pump inhibitors 2 weeks prior to

the study; 5. Failure to give written informed consent.

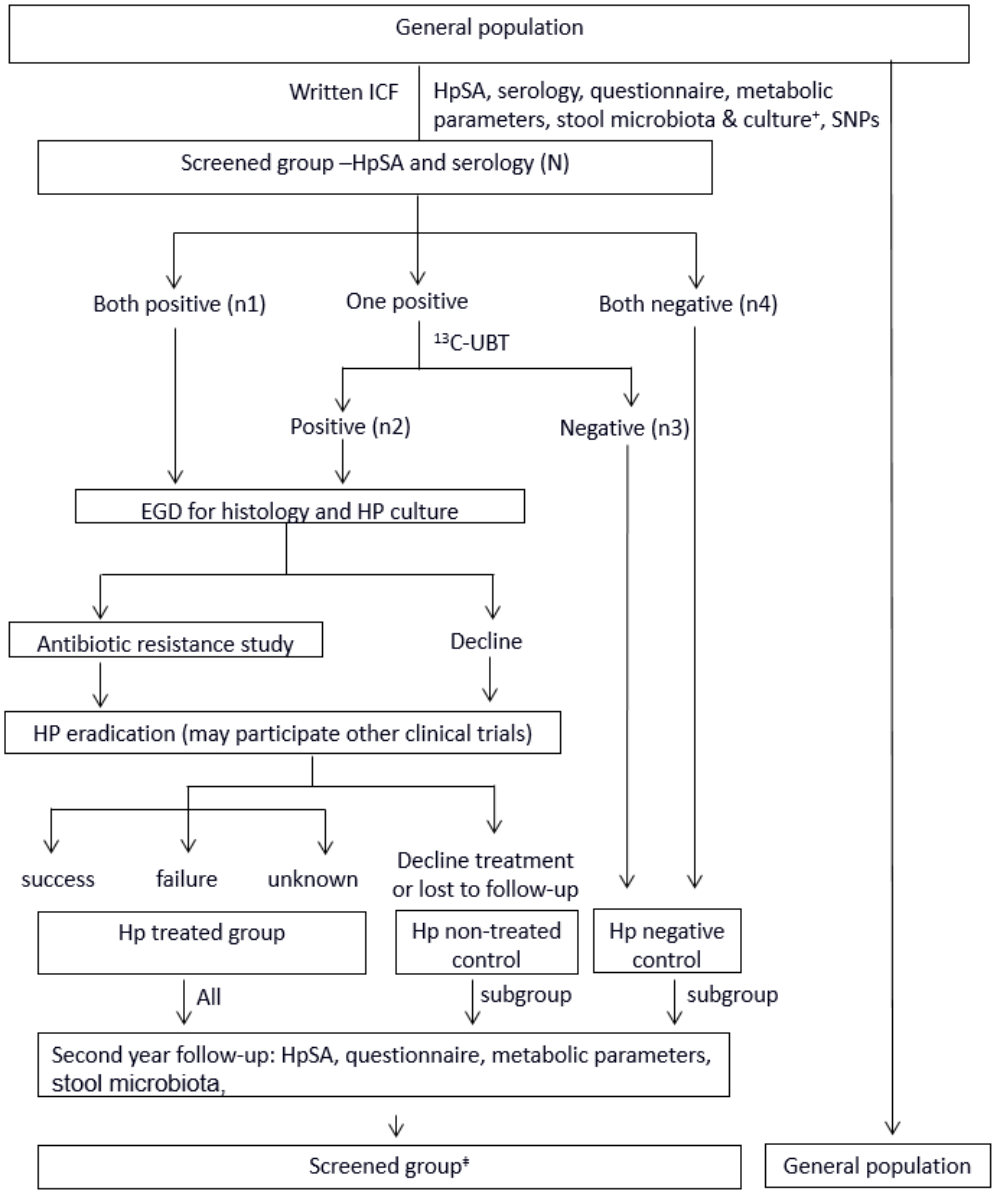
Study flow (Figure 1)

The message of the screening program will be posted in the website, in the participating hospitals, and will be transmitted by internet media. Participants will be screened for eligibility by the study staff. Eligible subjects will be screened for *H. pylori* by HpSA and serology after written informed consents are obtained (Figure 1). About 20ml of blood samples will be collected for *H. pylori* serology test, pepsinogen I/II levels, and biochemistries. The demographic characteristics, lifestyle, dietary habits, symptoms, personal history, family history, and medication history will be obtained by validated questionnaire. Subjects with both negative tests of HpSA and serology are considered *H. pylori* non-infected subjects. Those with only one positive test will be confirmed by ^{13}C-UBT and will be considered as *H. pylori* non-infected subjects if UBT is negative. Those with two positive tests or those with positive UBT will be invited for screening of early gastric cancer and susceptibility testing of *H. pylori* by esophagogastroduodenalscopy (EGD). Subjects without *H. pylori* infection will be categorized as *H. pylori* negative control.

Eradication therapy will be provided to all *H. pylori* infected subjects. They may participate clinical trials or may be treated empirically according to the local prevalence of antibiotic resistance. ^{13}C-UBT will be used to determine the eradication status (success, failure). Those with unknown status after eradication therapy will be categorized as

"unknown eradication status). Those who declined treatment or lost to follow-up will be categorized as *H. pylori* non-treated control.

Figure 1. Study flow of this cohort study



- ✓ Prevalence of *H. pylori* defined as (n1+n2/N) x 100%
- ✓ Outcomes: standardized incidence rate (SIR) and standardized mortality rate (SMR) of gastric cancer, other GI cancers, major cardiovascular events, inflammatory bowel disease, autoimmune disease (SLE, RA), Parkinson's disease, etc. in the screened group as compared to the general population

- ✓ [+]Stool culture for E. coli, K. pneumoniae, Enterococcus, in subgroup of subjects (Hp treated, Hp non-treated, and Hp negative) at baseline and year 1
- ✓ Analyze factors associated with gastric cancer after eradication therapy

Table 1. Case number to be recruited in each age category (Taiwan)

Age	HP prevalence	HP resistance	Resistance of gut flora	Gut microbiota	Gastric cancer risk	
					Male	Female
0 ~ <10	100		100	100		
10 ~ <20	100		100	100		
20 ~ <30	100	20	100	100		
30 ~ <40	100	20	100	100		
40 ~ <50	100	20	100	1500	1000	500
50 ~ <60	200	30	200	5000	2500	2500
60 ~ <70	200	30	200	5000	2500	2500
≥70	100	20	100	2500	1000	1500
Total	1000	150	1000	14400	7000	7000

Stool sampling for *H. pylori* stool antigen test and ^{13}C-urea breath test

All of stool samples will be collected in a stool container that is free of media, preservatives, animal serum, or detergents and the samples will be collected within three days before returning to the study site. Stool samples will be placed in the freezer at a -18°C or colder environment, while test samples will be placed in the refrigerator at 2-8°C. All stool samples will be tested with Vstrip® HpSA at designated laboratories. Subjects with only one positive test among HpSA and serology will be confirmed by ^{13}C-UBT in the study sites and samples will be sent for designated laboratories. ^{13}C-UBT will be performed by the site technician. Positive computer-generated ^{13}C-UBT results will be defined as a delta value of ≥4 units and negative results as < 4 units.

Endoscopic features and severity of gastritis (in subgroup of patients)

The endoscopic examination will be recorded by video. The extent of gastric atrophy and intestinal metaplasia will be recorded. Random biopsy from antrum (2 pieces) will be taken for *H. pylori* culture and determination of genotypic resistance. Target biopsy from suspected gastric atrophy or intestinal metaplasia at antrum (x1), angle (x1), and body (x1) will be done for histology. The severity of gastritis will be graded by the updated Sydney Classification (0=none, 1=slight, 2=moderate, and 3=marked) among those who underwent EGD and biopsy for histological examination. In subjects with atrophic gastritis or intestinal metaplasia, additional biopsy (x2) will be taken for determination of epigenetic alteration and mutations of somatic genes.

Eradication of *H. pylori* infection

Subjects with *H. pylori* infection will be treated with the clarithromycin containing regimen or bismuth quadruple therapy, depending on the local prevalence of antibiotic resistance. They may participate clinical trials or be treated empirically according to the local prevalence of antibiotic resistance. In regions with clarithromycin resistance of 15% or lower, clarithromycin containing regimens will be given, but bismuth quadruple therapy may be an alternative option. In regions with clarithromycin resistance greater than 15%, bismuth quadruple therapy is the treatment of choice, but non-bismuth quadruple therapy may be an alternative option if bismuth quadruple therapy is not available in that institute. ^{13}C-UBT will be done to determine the eradication status at least 6 weeks after completion of eradication therapy. The adverse events and compliance to the treatment will be recorded.

Second-line or third-line rescue therapies will be given for those fail after first-line treatment. ^{13}C-UBT will be used to confirm the eradication status at least 6 weeks after completion of eradication therapy.

Determination of phenotypic and genotypic resistance, and genome wide single nucleotide polymorphism

The minimum inhibitory concentrations (MICs) will be determined by agar dilution test using the Brucella chocolate agar with 7% sheep blood and incubated for 7 days under microaerobic conditions. The resistance breakpoints for amoxicillin, clarithromycin, metronidazole, tetracycline and levofloxacin, and are defined as greater than ≥0·5, ≥1, ≥8, >0·5 and >1, respectively. Polymerase chain reaction (PCR) followed by direct sequencing using the automatic sequencer will be used to genotype the *gyr*A and 23S rRNA mutations (ABI PRISM 3100 Genetic Analyzer, Applied Biosystems). The genome wide single nucleotide polymorphisms will be determined by TWB2.0 SNP array in the National Genotyping Center. Fasting serum collected at the time of study entry will be centrifuged and stored at −80°C until used.

Sequencing of 16S rRNA and shotgun metagenomics

Stool specimens for fecal microbiota analysis and culture and susceptibility testing will be collected at baseline (before treatment) and 1 year. The stool samples will be collected into a DNA stabilizer kit (Stratec Biomedical, Birkenfeld, Germany) for microbiota analysis and another swab culture tube for culture and susceptibility testing. The liquid DNA stabilization

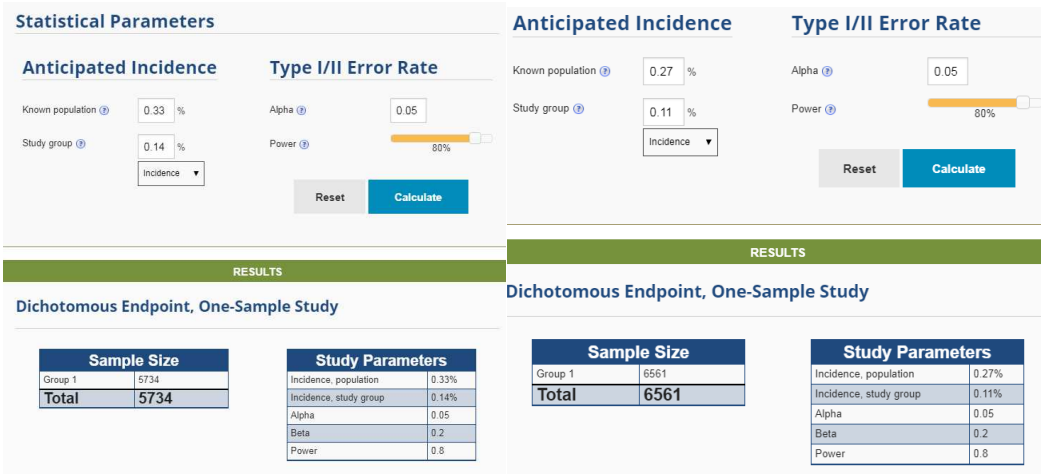
buffers may preserve the microorganism titre and prelyses bacteria through the inactivation of DNases and prevents degradation of DNA. Participants will be requested to return the fecal specimen to the research assistant in the hospital on the day of specimen collection. The stabilized samples will then be stored at -80℃ refrigerator immediately. The QIAamp Fast DNA Stool Mini Kit (Qiagene,MD,USA) will be used to extract the genomic DNA of fecal microbiota. High throughput sequencing of 16S rRNA will be performed using the Illumina miseq. The methods for metagenomic shotgun sequencing are as follows: a). Quantify input DNA: Use fluorometric-based methods for quantification, such as Qubit to provide accurate quantification for dsDNA; b).Fragment DNA: required concentration 20ng/μl for a 350 bp insert size, volume 55μl, splicing of DNA into small segments (about 350bp) using Covairs; c).Repair ends and select library size; d).Ligate adapters; e).Check libraries by real-time PCR (KAPA Library Quantification Kit)(550bp); f).Normalize and pool library; g).Library quantification: real-time PCR (KAPA Library Quantification); h).Sequencing by NextSeq.

Sample size estimation for long-term outcomes (gastric cancer)

In male, the average annual incidence of gastric cancer among male subjects aged between 40~75 years are 33.4 per 100000 in Taiwan. The **10-year** cumulative incidence is expected to be 334 per 100000=0.334%. We assumed that the 60% of gastric cancer can be prevented and the expected 10-year cumulative incidence is 0.334%x0.4=0.14%. It is estimated that at least 5734 subjects are required to give a power of 80% at significance level of 0.05. In females, the average annual incidence of gastric cancer among female

subjects aged between 45~75 years are 27 per 100000 in Taiwan. The 10-year cumulative incidence is expected to be 270 per 100000=0.27%. We assumed that the 60% of gastric cancer can be prevented and the expected 10-year cumulative incidence is 0.334%x0.4=0.14%. It is estimated that at least 6561 subjects are required to give a power of 80% at significance level of 0.05.

Figure 2. Sample size estimation in Taiwan



https://clincalc.com/stats/samplesize.aspx

Table 2. Incidence of gastric cancer and colorectal cancer according to age in males and females in Taiwan

	Gastric cancer		Colorectal cancer	
Age	Male	Female	Male	Female
40~44	5.09	3.33	30.22	26.97
45~49	11.21	7.29	48.15	39.84
50~54	14.82	11.09	84.67	61.57
55~59	27.29	12.28	116.02	79.39
60~64	44.91	22.96	180.43	104.31
65~69	54.15	24.81	227.66	134.64
70~74	76.32	36.51	296.50	190.70

75~79	109.60	56.89	358.83	234.38
80~84	153.25	75.90	457.96	349.97
85+	206.36	100.69	452.91	351.63

Long-term outcomes

We will link to the database of vital statistics in Taiwan to assess the long-term outcomes, including the cancer registry, mortality, and National Health Insurance database.

Statistical analysis

- ✧ Prevalence of *H. pylori* infection: Those with positive HpSA and positive serology tests (n1) or those with a positive test of HpSA/ or serology and positive UBT (n2). Thus, the crude prevalence of *H. pylori* infection is (n1+n2/N) x 100%, where N is the total number of screened subjects. The population distribution of that population will be used to calculate the age-standardized prevalence of *H. pylori* infection.
- ✧ Prevalence of antibiotic resistance of *H. pylori*: The prevalence of genotypic resistance of clarithromycin (23S rRNA mutation) and levofloxacin (gyrase A mutation) will be reported. The prevalence of phenotypic resistance of clarithromycin, levofloxacin, amoxicillin, metronidazole, tetracycline, and rifabutin will also be reported.
- ✧ The composition and diversity of fecal microbiota in *H. pylori* infected vs. non-infected subjects will be analyzed.
- ✧ The genome wide association (GWA) will be analyzed among *H. pylori* infected vs.

non-infected subjects. The GWA will also be analyzed among those with and without precancerous lesions among *H. pylori* infected subjects.

- ✧ The antibiotic resistance of E. coli, K. pneumoniae, Enterococcus at baseline and year 1 will be analyzed (Hp treated vs. Hp non-treated, and Hp negative). The changes of antibiotic resistance at baseline and year 1 will be analyzed in different subgroup.
- ✧ Long-term outcomes: The standardized incidence rate (SIR) and standardized mortality rate (SMR) of gastric cancer, other GI cancers, major cardiovascular events, inflammatory bowel disease, autoimmune disease (SLE, RA), Parkinson's disease, etc. in the screened group as compared to the general population will be analyzed. SIR = (Observed Cases/Expected Cases) x 100% (as shown in Table). 95% confidence interval for the SIR will be calculated by the Epi_Tools.XLS spreadsheet.
- ✧ Factors associated with gastric cancer (demographic characteristics, lifestyle, dietary habits, SNPs, severity of gastritis, atrophy, intestinal metaplasia on endoscopy (video recorded, AI/machine learning) and histology (OLGIM), and ethnicity. (***Note:** The case number of gastric cancer is expected to be only about 20-30 in the screened group in Taiwan. Therefore, collaboration with other countries is required for the analysis.)

Table 3. Analysis of standardized incidence rate (SIR) and standardized mortality rate (SMR) of gastric cancer

Age	**Male**				**Female**			
Age at recruitment (baseline)	10 year cumulative age-specific rate* (A)	Study sample (B)	Expected Cases (A x B)	Observed # of Cases	Age-specific rate (A)	Study sample (B)	Expected Cases (A x B)	Observed # of Cases
40~44	81.5	500	0.4					
45~49	130.1	500	0.7		91.9	500	0.5	
50~54	210.6	1250	2.6		116.9	1250	1.5	
55~59	361	1250	4.5		176.2	1250	2.2	
60~64	495.3	1250	6.2		238.9	1250	3.0	
65~69	652.4	1250	8.2		306.6	1250	3.8	
70~74	929.7	500	4.6		467	750	3.5	
75~79	1314.3	500	6.6		664	750	5.0	
80~84	-	-	-	-		-		
85+	-	-	-	-		-		
Total			33.8				19.4	

10-year cumulative age-specific rate* (per 100000). We will obtain the cumulative case number of gastric cancer in each age cohort during the 10 years and may calculate the 10-year cumulative age –specific rate of gastric cancer.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68:394–424.

2. Plummer M, Franceschi S, Vignat J, Forman D, de Martel C. Global burden of gastric cancer attributable to Helicobacter pylori. *Int J Cancer* 2015;136:487–90.

3. Lee CW, Rickman B, Rogers AB, Ge Z, Wang TC, Fox JG. Helicobacter pylori eradication prevents progression of gastric cancer in hypergastrinemic INS-GAS mice. *Cancer Res* 2008;68:3540–8.

4. Lee YC, Chiang TH, Chou CK, et al. Association Between Helicobacter pylori Eradication and Gastric Cancer Incidence: A Systematic Review and Meta-analysis. *Gastroenterology* 2016;150:1113–24.e5.

5. Wong BC, Lam SK, Wong WM, et al. Helicobacter pylori eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. *JAMA* 2004;291:187–94.

6. Fukase K, Kato M, Kikuchi S, et al. Effect of eradication of Helicobacter pylori on incidence of metachronous gastric carcinoma after endoscopic resection of early gastric cancer: an open-label, randomised controlled trial. *Lancet* 2008;372:392–7.

7. Choi IJ, Kook MC, Kim YI, et al. Helicobacter pylori Therapy for the Prevention of

Metachronous Gastric Cancer. *N Engl J Med* 2018;378:1085–95.

8. Sugano K, Tack J, Kuipers EJ, et al. Kyoto global consensus report on Helicobacter pylori gastritis. *Gut* 2015;64:1353–67.

9. Malfertheiner P, Megraud F, O'Morain CA, et al. Management of *Helicobacter pylori* infection-the Maastricht V/Florence Consensus Report. *Gut* 2017;66:6-30.

10. Graham DY. *Helicobacter pylori* update: gastric cancer, reliable therapy, and possible benefits. *Gastroenterology* 2015;148:719-31 e3.

11. Liou JM, Lee YC, El-Omar EM, Wu MS. Efficacy and Long-Term Safety of *H. pylori* Eradication for Gastric Cancer Prevention. *Cancers (Basel)*. 2019;11:593.

12. Hooi JKY, Lai WY, Ng WK, et al. Global Prevalence of Helicobacter pylori Infection: Systematic Review and Meta-Analysis. *Gastroenterology* 2017;153:420–9.

13. Chen MJ, Fang YJ, Wu MS, et al. Application of Helicobacter pylori stool antigen test to survey the updated prevalence of Helicobacter pylori infection in Taiwan. *J Gastroenterol Hepatol* 2020;35:233–40.

14. Kuo YT, Liou JM, El-Omar EM, et al. Primary antibiotic resistance in *Helicobacter pylori* in the Asia-Pacific region: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2017;2:707-15.

15. Chen PY, Wu MS, Chen CY, et al. Systematic review with meta-analysis: the efficacy of levofloxacin triple therapy as the first- or second-line treatments of Helicobacter pylori

infection. *Aliment Pharmacol Ther* 2016;44:427–37.

16. Fallone CA, Chiba N, van Zanten SV, et al. The Toronto Consensus for the Treatment of *Helicobacter pylori* Infection in Adults. *Gastroenterology* 2016;151:51-69 e14.
17. Høgh MB, Kronborg C, Hansen JM, Schaffalitzky de Muckadell OB. The cost effectiveness of Helicobacter pylori population screening-economic evaluation alongside a randomised controlled trial with 13-year follow-up. *Aliment Pharmacol Ther*. 2019;49:1013–25.
18. O'Connor A, O'Morain CA, Ford AC. Population screening and treatment of *Helicobacter pylori* infection. *Nat Rev Gastroenterol Hepatol* 2017;**14**:230-40.
19. Sjölund M, Wreiber K, Andersson DI, Blaser MJ, Engstrand L. Long-term persistence of resistant *Enterococcus* species after antibiotics to eradicate *Helicobacter pylori*. *Ann Intern Med* 2003;**139**:483-7.
20. Jakobsson H, Wreiber K, Fall K, Fjelstad B, Nyrén O, Engstrand L. Macrolide resistance in the normal microbiota after *Helicobacter pylori* treatment. *Scand J Infect Dis* 2007;**39**:757-63.
21. Blaser MJ. Antibiotic use and its consequences for the normal microbiome. *Science* 2016;**352**:544-5.
22. Liou JM, Chen CC, Chang CM, et al. Long-term changes of gut microbiota, antibiotic resistance, and metabolic parameters after *H. pylori* eradication- a multicentre randomized trial. *Lancet Infectious Diseases* 2019;19:1109-20.

23. Yap TW, Gan HM, Lee YP, et al. *Helicobacter pylori* eradication causes perturbation of the human gut microbiome in young adults. *PLoS One* 2016;**11**:e0151893.

24. Jakobsson HE, Jernberg C, Andersson AF, Sjölund-Karlsson M, Jansson JK, Engstrand L. Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. *PLoS One* 2010;**5**:e9836.

25. Upala S, Jaruvongvanich V, Riangwiwat T, Jaruvongvanich S, Sanguankeo A. Association between *Helicobacter pylori* infection and metabolic syndrome: a systematic review and meta-analysis. *J Dig Dis* 2016;**17**:433-40.Upala S, Sanguankeo A, Saleem SA, Jaruvongvanich V. Effects of *Helicobacter pylori* eradication on insulin resistance and metabolic parameters: a systematic review and meta-analysis. *Eur J Gastroenterol Hepatol* 2017;**29**:153-9.

26. Upala S, Jaruvongvanich V, Riangwiwat T, Jaruvongvanich S, Sanguankeo A. Association between *Helicobacter pylori* infection and metabolic syndrome: a systematic review and meta-analysis. *J Dig Dis* 2016;**17**:433-40.

27. Upala S, Sanguankeo A, Saleem SA, Jaruvongvanich V. Effects of *Helicobacter pylori* eradication on insulin resistance and metabolic parameters: a systematic review and meta-analysis. *Eur J Gastroenterol Hepatol* 2017;**29**:153-9.