ABSTRACT

Title: PHARE study: An observational multicenter study on antibiotic resistance of *Helicobacter pylori* in France

Keywords: PYLERA®, antibiotic, resistance, observational, bismuth subcitrate potassium, metronidazole, tetracycline hydrochloride, *Helicobacter pylori*

Rationale and background: In countries where it is available, the quadruple bismuth therapy of a proton pump inhibitor (PPI) (omeprazole) + PYLERA® (bismuth subcitrate potassium, metronidazole, and tetracycline hydrochloride) has been proposed as first-line treatment for the eradication of *H. pylori* infections. PYLERA® has been available in France since 10 April 2013. Antibiotic (ATB) resistance is the main factor for treatment failure to eradicate *H. pylori* [i]. This observational, national, multicenter 5-year study on *H. pylori* resistance to ATBs was requested by the "Agence Nationale de Sécurité du Médicament et des Produits de Santé"– French Health Authorities) as part of the European Risk Management Plan for PYLERA®. It is intended to address the French regulatory agency requirements for the surveillance over 5 years of *H. pylori* resistance to ATBs following the marketing authorization of PYLERA® in France.

Research question and objectives: This study is intended to address a regulatory requirement for the surveillance of *H. pylori* susceptibility to ATBs following the marketing authorization of PYLERA® in France.

The primary objective is to assess the resistance rate in clinical isolates of *H. pylori* to ATBs (amoxicillin, clarithromycin, levofloxacin, metronidazole, rifamycin, and tetracycline). The secondary objectives are to monitor the evolution of strain resistance to ATBs over the 5-year period following the commercial launch of PYLERA® and to evaluate the impact of evolution of strain resistance to ATBs on the efficacy of PYLERA® treatment.

Study design: This is an observational, multicenter study in France. The participation of each subject in the study lasts 1 visit, which takes place during a scheduled endoscopy with biopsies by a gastroenterologist as part of a regular medical investigation. Biopsies collected by all gastroenterologists are sent to a central bacteriology laboratory where the detection of *H. pylori* and the resistance to ATBs are determined.

Setting: This study is conducted exclusively in metropolitan France using a representative sample of 100 gastroenterologists distributed in 5 regions of France: North East, North West, South East, South West, and Paris area. These gastroenterologists were randomly selected to participate in the study. Participating gastroenterologists include subjects, based on inclusion criteria, on 1 specific day per month.

Subjects and study size, including dropouts: The study population is defined as being comprised of subjects 18 years of age and older who have a scheduled or planned

endoscopy at one of the centers involved in the study, who exhibit a pathology potentially associated with an infection by *H. pylori* or who have had failure of a previous treatment for eradication, and who have signed the information leaflet.
The study size is up to 1000 subjects recruited in each of 3 collection periods: collection year 1 (1st trimester 2014 to 1st trimester 2015), collection year 2 (1st trimester 2016 to 1st trimester 2017), and collection year 3 (1st trimester 2018 to 1st trimester 2019), for a total of an estimated 3000 participating subjects over 5 years.

Variables and data sources: Gastroenterologists collect gastric biopsies in accordance with their center's usual practices. The required number of biopsies are collected for the usual anatomopathology evaluation by the center and 2 additional biopsies (1 antral and 1 fundic) are collected for resistance testing in the context of the study.

Safety variables: Not applicable.

Data to be collected:

- Endoscopy date and sample collection time
- Sociodemographic data: age, gender, country of birth, current place of residence, and education level
- Reason for consultation: epigastric pain, other signs of dyspepsia, anemia, and others
- Use of ATBs in the 30 days before the endoscopy including name, duration and end date
- Use of a PPI in the 7 days before the endoscopy including name, duration, and end date
- History of *H. pylori* infection: previous eradication attempts including number of attempts, and nature and duration of eradication attempts, such as the following:
    - Bismuth quadruple therapy: PPI (omeprazole or other name and dosage) + PYLERA® (10 days)
    - Triple therapy: PPI + amoxicillin + clarithromycin (7, 10, or 14 days)
    - Triple therapy: PPI + amoxicillin + metronidazole (7, 10, or 14 days)
    - Sequential treatment: PPI + amoxicillin (5 days) followed by PPI + clarithromycin + metronidazole (5 days)
    - PPI + amoxicillin + levofloxacin (10 days)
    - PPI + amoxicillin + rifabutin (10 days)
    - Others
- Biopsies for bacteriology: localization (antral, fundic) and number
- Results of endoscopy: rashes, superficial erosions, ulcers, esophageal diseases, others
- Resistance results from central laboratory

Biopsy analysis: The resistance of *H. pylori* to 6 different ATBs is determined by agar diffusion using 2 different tests: disk diffusion for tetracycline, rifampicin (for rifamycin resistance), and levofloxacin, and Epsilometer test (E-test) for clarithromycin, metronidazole and amoxicillin.
Real time polymerase chain reaction (PCR) is performed on all biopsy DNA extracts for detection of *H. pylori* and clarithromycin resistance.

Data analysis: The number and percentage of subjects who are considered as positive or negative for the presence of *H. pylori* are calculated. The number and percentage of *H. pylori*-positive subjects (and the 95% CI) with *H. pylori* resistant to each ATB are calculated in each of the 5 geographic regions (North East, North West, South East, South West, and the Paris area) and for all regions combined.

Milestones: The following milestones are projected for the study:

- First period of inclusion, collection year 1: 1st trimester 2014 to 1st trimester 2015
- Interim report for collection year 1: 2nd trimester 2015
- Second period of inclusion, collection year 2: 1st trimester 2016 to 1st trimester 2017
- Interim report for collection year 2: 2nd trimester 2017
- Third period of inclusion, collection year 3: 1st trimester 2018 to 1st trimester 2019
- Closure of the database: 2nd trimester 2019
- Final data analysis: 2nd trimester 2019
- Final clinical study report: 3rd trimester 2019

Results: This report represents the second interim analysis and covers the time period 24-FEB-2016 to 23-FEB-2017.

The following results were observed:

- In the 2nd collection year, 75 sites participated in this study, enrolling 970 subjects of whom 359 were culture positive for *H. pylori*. The most common reason for consultation was epigastric pain in 633 subjects (65.3%).
- Most subjects (767 [79.1 %]) had no previous eradication attempts and 189 subjects (19.5 %) had previous eradication attempts; of these 108 (57.1 %) had one eradication attempt.
- Among the 970 samples tested, 409 samples (42.2 %) showed positive PCR result for *H. pylori*, 359 samples (37.0 %) were culture positive for *H. pylori*, and 359 samples (37.0 %) were positive by both methods.

Resistance rates determined on the 359 positive isolates of *H. pylori* from all culture positive subjects are, as follows:

- amoxicillin 0.8 %, 3 subjects,
- clarithromycin 34.4 %, 123 subjects,
- levofloxacin 17.9 %, 64 subjects,
- metronidazole 62.3 %, 223 subjects,
- rifamycin 0.3 %, 1 subject,
- tetracycline, 0.0%, no subjects,
- at least 1 of the 6 ATBs, 74.0 %, 265 subjects (95% CI =66.9 ; 78.5).

For subjects (409) who were *H. pylori* positive by PCR test:

- 269 (65.8 %) were sensitive to clarithromycin,

- 140 (34.2%) were resistant:
    - 97 (23.7%) were resistant with mutation A2142G/A2143G,
    - 37 (9.0%) were resistant with a dual population of wild type and mutation A2142G/A2143G,
    - 3 (0.7 %) were resistant with mutation A2142C,
    - 1 (0.2 %) was resistant with a dual population of wild type and mutation A2142C,
    - 1 (0.2 %) was resistant with the double mutation A2142G/A2143G and A2142C (regardless of wild type presence),
    - 1 (0.2 %) was resistant with the double mutation A2142G/A2143G and A2142T (regardless of wild type presence).

When results were assessed for isolates from subjects with prior treatment for *H. pylori*, the following was observed for the 125 isolates:

- 1(0.8%) was resistant to amoxicillin
- 74 (59.7 %), were resistant to clarithromycin,
- 29 (23.4 %), were resistant to levofloxacin
- 100 (80.6%) were resistant to metronidazole
- 1 (0.8%), was resistant to rifamycin
- None were resistant to tetracycline
- 112 (90.3%) were resistant to at least 1 of the 6 ATBs

When results were assessed for 73 isolates from subjects who had received PYLERA® as prior treatment for *H. pylori*:

- No isolates of *H. pylori* were resistant to amoxicillin
- 39 (54.2 %) isolates of *H. pylori* were resistant to clarithromycin,
- 18 (25.0 %) isolates of *H. pylori* were resistant to levofloxacin,
- 60 (83.3%) isolates of *H. pylori* were resistant to metronidazole,
- 1 (1.4 %) isolates of *H. pylori* were resistant to rifamycin,
- No isolates of *H. pylori* were resistant to tetracycline,
- 65 (90.3%) were resistant to at least 1 of the 6 ATBs.

Regarding the secondary objectives of the study:

- Comparison of resistance rates of the first and second collection years was performed. The only statistically significant change was found for the resistance rate of *H. pylori* to clarithromycin in the *total population who received prior treatment* for *H. pylori*. Indeed, in Year 1 clarithromycin resistance rate was 73.9 % (95% CI = 64.9 ; 81.7) versus 59.7% (95% CI = 50.5 ; 68.4) in Year 2 (p-value = 0.02).
- It should be noted that no statistically significant changes occurred in resistance rates to tetracycline and metronidazole in patients who received prior treatment with PYLERA®.
- Subgroup analyses of resistance rates of the first and second collection years did not reveal any statistically significant differences.

Discussion: This 5-year, national, observational study is being performed in metropolitan France using a representative sample of approximately 100 gastroenterologists distributed in 5 regions of France. Each participating gastroenterologist is to include up to 20 subjects each year over a period of 5 years. Overall enrollment is not to exceed 1000 subjects per year.

The primary objective of the study is to assess the resistance rate in clinical isolates of *H. pylori* to ATBs (amoxicillin, clarithromycin, levofloxacin, metronidazole, rifamycin, and tetracycline).

The secondary objectives of the study are to monitor the evolution of strain resistance to ATBs over the 5 years period following the commercial launch of PYLERA®, and to evaluate the impact of evolution of strain resistance to ATBs on the efficacy of PYLERA® treatment.

When susceptibility results were assessed for isolates from subjects who were *H. pylori* positive (overall), who never received prior treatment for *H. pylori*, who had any *H. pylori* eradication attempt, and who had *H. pylori* eradication attempt with PYLERA®, the following was observed:

	Resistance* Rate, % (Collection Year 2)			
	Overall *H. pylori* Positive Population (N=359)	Treatment-naive Population (N=231)	Any *H. pylori* Eradication Therapy Attempted (N=125)	PYLERA® Eradication Therapy Attempted (N=73)
Amoxicillin	0.8	0.9	0.8	0
Clarithromycin	34.4	20.3	59.7	54.2
Levofloxacin	17.9	14.7	23.4	25.0
Metronidazole	62.3	52.4	80.6	83.3
Rifamycin	0.6	0.4	0.8	1.4
Tetracycline	0	0	0	0
At least 1 of the 6 antibiotics	74.0	64.9	90.3	90.3

*Resistance or Intermediate resistance

PYLERA® combines bismuth subcitrate potassium, metronidazole and tetracycline hydrochloride. Bismuth subcitrate potassium resistance is not expected[34]. Therefore, resistance to metronidazole and tetracycline is of main interest.

For Collection Year 1, 30.5 % (57/187) of patients who received prior treatment were treated with PYLERA® against 53.2% (100/189) for Collection Year 2.

As seen during Collection Year 1, the percentage of *H. pylori* isolates collected from culture positive subjects that were resistant to metronidazole was higher for patients who received any treatment for *H. pylori* and for patients who reported treatment with

PYLERA®. We should be cautious about this result since no statistical test confirming this difference was performed.

As seen during Collection Year 1, even in the group of patients who received prior treatment with PYLERA®, no *H. pylori* isolates were resistant to tetracycline.

Regarding the evolution of strain resistance, the only statistically significant change was for the resistance rate of *H. pylori* to clarithromycin in the total population who received prior treatment for *H. pylori*. Indeed, there was a significant reduction in the resistance rate of *H.pylori* to clarithromycin in that population. Moreover, no statistically significant changes occurred in resistance rates to tetracycline and metronidazole in patients who received prior treatment with PYLERA®.

The wide distribution of patients studied and the absence of stringent admission criteria for the study suggest that the results of this study are probably generalizable. Nevertheless, sample size may be a limitation to generalizability as only 37.0% of 970 samples were culture positive for *H. pylori*.