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* [1]. 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)
    - o 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.

Results: This report represents the 1st interim analysis and covers the time period 24 Feb 2014 to 24 Feb 2015.

The following results were observed:

- In the 1st collection year, 75 sites participated in this study, enrolling 984 subjects of whom 386 were culture positive for H. pylori. The most common reason for consultation was epigastric pain in 667 subjects (67.8 %)
- Most subjects (783 [79.6 %]) had no previous eradication attempts and 187 subjects (19.0 %) had previous eradication attempts; of these 117 (62.6 %) had 1 eradication attempt
- Of a total 984 samples tested, 416 samples (42.3 %) showed positive PCR result for H. pylori, 386 samples (39.2 %) were culture positive for H. pylori, and 386 samples (39.2 %) were positive by both methods
- Resistance rates determined on the 386 positive isolates of H. pylori from all culture positive subjects are, as follows:
    - amoxicillin 0.5 %, 2 subjects
    - clarithromycin 37.6 %, 145 subjects
    - levofloxacin 15.0 %, 58 subjects
    - metronidazole 56.0 %, 216 subjects
    - rifamycin 0.8 %, 3 subjects
    - tetracycline, 0.0%, no subjects
    - at least 1 of the 6 ATBs, 71.0 %, 274 subjects (95% CI = 66.2; 75.5)
- For subjects (416) who were H. pylori positive by PCR test, 259 (62.3 %) were sensitive to clarithromycin; 151 (36.3%) were resistant with mutation A2142G/A2143G, and 6 (1.4%) were resistant with mutation A2142C. A total of 157 (37.7%) were resistant with mutation A2142G/A2143G or mutation A2142C
- When results were assessed for isolates from subjects with prior treatment for H. pylori, the following was observed for the 115 isolates:
    - None were resistant to amoxicillin
    - 85 (73.9%), were resistant to clarithromycin,
    - 17 (14.8%), were resistant to levofloxacin
    - 90 (78.3%) were resistant to metronidazole
    - 1 (0.9%), was resistant to rifamycin
    - None were resistant to tetracycline
    - 108 (93.9%) were resistant to at least 1 of the 6 ATBs
- When results were assessed for isolates from subjects who had received PYLERA® as prior treatment for H. pylori:
    - No isolates of *H. pylori* were resistant to amoxicillin
    - 21 (61.8 %) isolates of *H. pylori* were resistant to clarithromycin, which was a greater percentage of resistance than in isolates from subjects who had not received prior treatment and a smaller percentage of resistance than in isolates from subjects who had received other prior treatment
    - 3 (8.8%) isolates of *H. pylori* were resistant to levofloxacin
    - 31 (91.2%) isolates of *H. pylori* were resistant to metronidazole, which was a greater percentage of resistance than in isolates from subjects both who had and who had not received other prior treatment
    - 1 (2.9 %) isolates of *H. pylori* were resistant to rifamycin
    - No isolates of *H. pylori* were resistant to tetracycline
    - 33 (97.1%) were resistant to at least 1 of the 6 ATBs

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 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. Given the nature of secondary endpoints (comparative rates over time), for obvious reasons, these endpoints are not treated in this first interim report.

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 1)			
	Overall *H. pylori* Positive Population (N=386)	**Treatment-naive Population (N=266)**	**Any *H. pylori* Eradication Therapy Attempted (N=115)**	**PYLERA® Eradication Therapy Attempted (N=34)**
Amoxicillin	0.5	0.8	0	0
Clarithromycin	37.6	22.2	73.9	61.8
Levofloxacin	15.0	15.4	14.8	8.8
Metronidazole	56.0	45.9	78.3	91,2
Rifamycin	0.8	0.8	0.9	2.9
Tetracycline	0	0	0	0
At least 1 of the 6 antibiotics	71.0	60.9	93.9	97.1

The percentage of H. pylori isolates collected from culture positive subjects that were resistant to clarithromycin and metronidazole increased after any treatment for H. pylori and after reported treatment with PYLERA®. The number of H. pylori isolates that were resistant to amoxicillin, levofloxacin, rifamycin, and tetracycline did not increase after reported treatment with PYLERA®. Resistance rates to clarithromycin increased from 22.2% in treatment-naive subset of subjects to 73.9% in the subset having any eradication treatment reported and to 61.8% in the subset having previous PYLERA® eradication treatment reported. Resistance rates to metronidazole increased from 56.0% in treatment-naive subset of subjects to 78.3% in the subset having any eradication treatment reported and to 91.2% in the subset having previous PYLERA® eradication treatment reported. It is worth noting that the small numbers (particularly in PYLERA® pretreated subset, N = 34) may have greatly influenced the resistance rates.

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.

The analysis of the results of susceptibility tests of isolates of H. pylori collected between 24 Feb 2014 to 24 Feb 2015 to ATBs demonstrates higher rates of resistance to metronidazole and clarithromycin in patients previously treated for H. pylori compared to those not treated.