Centre for Risk Research Inc.

Exhibit 1A

PGRx Information System
General Methodology

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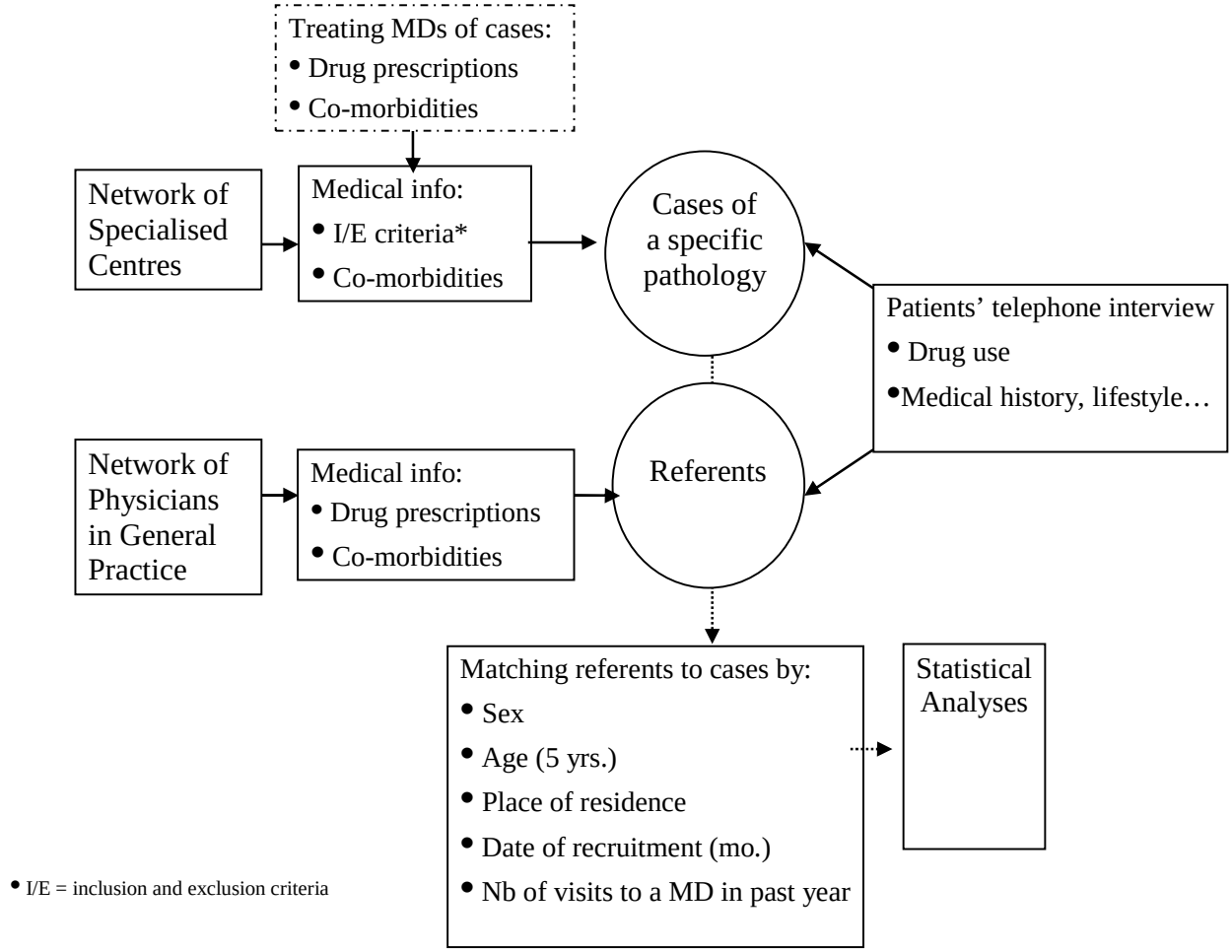
1. The PGRx Information System

PGRx is an information system that intends to bridge the resource gap to assess the effect of a drug on the risk of adverse events that are infrequent and/or with a long delay of onset. It uses some characteristics of the *ad hoc* case-control or case-referent design, transposed on a prospective, on-going, population-based recruitment plan. This particular design is called here systematic case-referent design in contrast to the *ad hoc* case-control or case-referent methodology. The PGRx information system is based on the routine and targeted recruitment of cases of a series of pathologies, compared to population-based referents for the study of exposure to a wide variety of drugs. The characteristics of the system are described in the following sections:

- Validity of case definition is insured by the systematic application of a strict clinical definition followed with confirmation by standard methods.
- Efficiency of recruitment of cases of adverse events in specialized centres where they tend to cluster with no compromise on exhaustivity by also recruiting alternative modes of presentation or access to medical care in a specified area.
- The routine recruitment of referents with medical general practitioners, covering a broad and representative population in a given area where the cases are identified, minimizes biases related to traditional *ad hoc* case control studies.
- By design, the effect of several drugs or drug classes and of drug interactions can be investigated for each adverse event. Drug exposure assessment is performed using up-to-date pharmaco-epidemiological methods.
- Information on potential confounding and interaction factors and on competing medical risks is systematically obtained from all cases and referents.
- High statistical power, especially for rare diseases.

The PGRx information system is schematically presented in the following figure.

General organisation of the PGRx Information System



• I/E = inclusion and exclusion criteria

2. PGRx Scientific Board and scientific committees

2.1 PGRx International Scientific Board (ISB)

The scientific development and guidance of the PGRx information system is under the auspices of an International Scientific Board composed of scientists and clinicians in the various pathologies that have been concerned with serious drug-related adverse events (appendix 1). The inclusion of a specific adverse event in the PGRx system is carefully evaluated for its pertinence and priority.

2.2 PGRx Pathology Specific Scientific Committee (PSC)

When a health problem is identified for inclusion in the PGRx system (see below "case typology"), a *pathology specific scientific committee* is formed with experts in the field to define all the specifics of the pathology under consideration (Exhibit 1B). This committee is fully independent from any sponsor.

The summary of existing protocols is available to prospective subscribers. The detailed protocols can be consulted by subscribers at the PGRx facilities.

2.3 Study Specific Scientific Committee (SSC)

If an in-depth analysis is planned, for the study of a given product, a class of products or other risk factor, a detailed scientific protocol is developed with an *ad hoc* committee of experts in the field. These experts may or may not be part of the pathology specific scientific committee.

Several formulas are possible as to the relations between sponsors and the study specific scientific committee.

3. Case typology in the PGRx system

3.1 Definition of adverse events

Cases are defined as adverse events and not adverse reactions. This means that no hypothesis is made *a priori* on the causality of the event (as opposed to spontaneous reports of adverse reaction commonly recruited by pharmacovigilance systems).

3.2 Typology of cases in the PGRx system.

Four types of cases are defined in the PGRx information system:

- Routine recruitment of "standard" cases of predefined conditions.
- Addition of standard cases.
- "Targeted" cases of new pathologies.
- Special cases.

3.2.1 Routine recruitment of “standard cases”

A minimal number of cases of adverse events will be routinely recruited in the system every year. The purpose of this recruitment is to establish a network of participating Centres to be mobilised whenever a more important sample is necessary for a given pathology. Adverse events that are recruited routinely (standard cases) correspond to pathologies selected according to the following criteria:

- They are serious or can potentially evolve into serious conditions.
- They correspond to some adverse events that led to the market withdrawal of products over the past decade (a list that is updated overtime).
- They are suggested to PGRx by regulatory authorities as significant issues.

Table

Causes of drug withdrawals worldwide 1963-2005

Cardiology	Myocardial Infarction, Arrhythmias, Stroke	**19%**
Haematology	Agranulocytosis, Aplastic anemia	**17%**
Hepatology	Acute Hepatitis	**17%**
Oncology	Carcinogenicity Leukemias	**9%**
Allergology	Stevens Johnson Syndrome, Photosensitivity	**7%**
Nephrology	Acute Renal insufficiency	**5%**
Neurology	Neurological disorders Demyelinating diseases	**5%**
Obstetric/Paediatrics	Malformations, Teratogenicity	**5%**
Others	Rhabdomyolysis, Anaphylaxis, Intestine intussusception, Etc.	**17%**

3.2.2. Addition of standard cases

The number of standard cases recruited for a given pathology in a given time span may be increased rapidly on demand, in case of alerts or pharmaco-vigilance notifications. The number of cases routinely recruited has been set up in such a way that it is not a problem for most Centres that usually treat a larger number of patients with the pathology at hand, to add more standard cases into the system. For very rare diseases, increasing the number of cases may require the participation of additional Centres. In some instances, the number of cases in the system is limited by the number of *existing* cases.

3.2.3 “Targeted” cases of new pathologies

On demand, the system enables the addition of cases of pathologies that are not routinely collected. This task is rendered much simpler than setting up a study *de novo* since it taps on the entire infrastructure edified for the collection and interview

of routine cases on the one hand, and uses the routine set of referents on the other hand. In many instances, *targeted* cases may be recruited in Centres that already contribute to the collection of routine cases since a large set of medical specialties are represented in the routine set up.

3.2.4 Special cases

The study of risks in certain situations may require the building of a new case and referent collection module. This is the case for instance of paediatric cases or those with mental deficits. Specialized recruitment modules must be created with the appropriate tools and appropriate referents must be defined and recruited. The treatment of the information is then similar to the other types of cases in the PGRx system.

4. Case definition, identification and recruitment

4.1 Case definition

In all types of cases in the PGRx information system, cases are ascertained using a strict case definition that can be used uniformly by all Centres and clinicians participating to the recruitment of cases. Case identification proceeds with inclusion and exclusion criteria based on detailed clinical signs and symptoms, laboratory and imaging findings and other clinical features where appropriate (severity grading, clinical evolution, etc.). Whenever possible, case definition mimics standard definition provided in clinical guidelines or consensus reports. The definition is adapted if variations exist in clinical practice over a territory, so it can fit all situations and at the same time only include cases that strictly correspond to the definition.

4.2 General inclusion and exclusion criteria

The PGRx has a set of general inclusion and exclusion criteria:
Inclusion:

- Male or female, 18 or older
- Patients can be interviewed by telephone.
- Patients can speak French or English
- Place of usual residence in the area of recruitment

Exclusion:

- Refuse to participate
- Cannot be reached by telephone

4.3 Identification of incident cases

For all types of adverse events considered, the PGRx system only includes incident cases. Incident cases are those who have been newly diagnosed (usually within 3 months of onset). Some case-control studies published in the literature also include cases who have been diagnosed a while back as an attempt to recruit patients more rapidly. However, the validity of this approach is not guarantied as long-term cases

often have longer survivals. Consequently, it is not possible to determine whether a drug is associated with the occurrence of the adverse event or with its prognosis. Increasing the retroactive time span for the collection of cases may be considered only if such a survival bias is excluded and the validity of information collected not jeopardized, in exceptional circumstances (such as the need for urgent addition of cases).

4.4 Recruitment of cases

For each condition, patients 18 year-old or older are identified in specialty units of participating hospital. In some cases, recruitment may be ascertained in private specialty practices. Both are referred below as "Centres". Within each Centre a number of board-certified medical specialists for pathologies at hand are recruited as investigators for the study. Each investigator is individually contacted to introduce the system and seek their participation in the recruitment of cases. Participating Centres are sent a description of the adverse events of interest and the specific case definitions with inclusion and exclusion diagnostic criteria.

Investigators are instructed to identify and refer to the system all cases who are discharged alive during a specified time span. The time span varies according to the pathology at hand. Very rare diseases (such as agranulocytosis for instance) require a full time-span of recruitment each year, while more frequent diseases (such as incident myocardial infarction) require limited time spans of recruitment. The time span is set up for each disease in order to maximise the chances of fulfilling recruitment objectives, while limiting the risks of selection biases. Time spans will be detailed in each disease's protocol.

Physicians obtain written consent of eligible patients and transfer the coordinates of the patients to the PGRx staff for the telephone interview, through a secured Internet connection. For each case that fits the case definition, investigators are requested to fill an electronic case report form that includes details concerning the clinical diagnosis, inclusion and exclusion criteria, as well as laboratory and imaging data specific to the case definition.
A non-nominal registry is kept of all non recruited eligible patients and reasons for not recruiting them is recorded.

4.5 Recruitment of special cases

Special cases can be recruited as required by the nature of the adverse events of interest. These include:

- Paediatric patients
- Pregnant women
- Elders
- Patients with communication deficits
- Patients with mental deficits

4.6 Recruitment of Centres

For each pathology in the PGRx system, a network of specialized Centres and physicians in the domain of concern is identified for recruitment of cases. The identification of this network depends on the considered pathology. It is detailed on each specific pathology protocol of standard cases collection. The number of units enrolled depends on the sample size required for the cases. Together the recruitment units represent a catchment area and will include patients with various modes of presentation in a given region.

Each unit is contacted by the PGRx staff. Those who agree to participate to the recruitment of cases of a given pathology, are individually met or called by telephone for the installation procedure which includes information about the system, precise instructions for recruitment, setting recruitment goals (number of cases and times pan), setting a registry (if there is none) for the time of recruitment, training on the Internet data entry system and providing the paper tools for recruitment (information to patients, consent forms, drug list and drug display for patients). Participating Centres and physicians are asked to recruit cases on a rotating basis so that recruitment is not uninterrupted in a given region over the year.

Investigators are regularly contacted by the PGRx staff about their recruitment progress and queries on their recruited cases. A toll-free number is made available to participating physicians who need to inquire about specific cases or diagnostic criteria.

4.7 Validation of cases

Whenever required (i.e. uncertainty in the application of inclusion criteria, need for in-depth analysis, etc.), cases may be reviewed by an expert panel that verifies the diagnostic criteria listed on the case report form. To ensure that their assessment is not influenced by the drug(s) exposure of the patient, which could lead to selection bias, experts are blinded with respect to exposure.

5. Referent definition, identification and recruitment

5.1 Definition of the referent group

One characteristic of the PGRx information system is to recruit referents on a routine and ongoing basis from the population where cases emerge. This is the main feature that distinguishes the here called “case-referent” approach from the traditional *ad hoc* case-control methodology. Referents reside in the same regions where cases are identified and are patients seen by physicians in general practice with no restriction as to the motive for consultation. These motives are collected and used for adjustment in the analyses as needed.

In some particular instances (study of secondary or recurrent diseases), cases in the system can be used as referents.

5.2 Identification of referents

Consistent with a secondary base principle, the pool of eligible referents includes residents of the geographical region covered by the participating Centres for the recruitment of cases, and who have some opportunity for exposure. Physician-based referents have been shown to be valid and useful source of base sampling in pharmacoepidemiology.

5.3 Recruitment of referents

Each family physician (GPs) in the PGRx network recruits 10 patients consecutively. Physicians are asked to identify and propose participation to a total of 10 consecutive patients; 4 men and 4 women in each of the following age categories: 18-34, 35-49, 50-64, 65-79, and 1 man and 1 woman more in one of these age categories dependent on the most needed age group regarding the surveyed pathologies. Physicians obtain the consent of eligible patients and transfer the coordinates of the patients to the PGRx staff for the telephone interview, through a secured Internet connection.
A network of twenty physicians enrolled every month over 10 months in a given year (excluding the month of August and end of year holidays) provides a pool of 2,000 referents per year. Spreading out recruitment evenly over the year insures adequate matching with occurrence of cases over a year.

It is possible to increase on demand the number of referents collected in a given stratum (age, gender or both). It is also possible to recruit referents with special inclusion criteria such as in the paediatric population for instance. This is then considered in the Study Order.

As for cases, physicians who recruit referents are requested to fill an electronic medical data form that includes some medical and biological data (section 7 below).

5.4 Recruitment of physicians in general practice

Physicians in general practice are enrolled for the recruitment of referents in all regions where cases are recruited and in sufficient number so to insure matching capacity (at least two referents per case on average). Physicians are randomly selected from a general list of practicing physicians in a given region. A mailing is sent and physicians are contacted by phone for invitation to participate. In order to be enrolled, they must have access to Internet and use computerized prescriptions. Those who agree are provided with a secured access to the PGRx system on Internet and are instructed on recruitment of consenting patients, on filling the medical data form and the electronic transfer of their computerized drug prescriptions over the previous two years.

Participating physicians are asked to recruit patients once a year on a rotating basis so that recruitment is not interrupted in a given region over the year.
Investigators are regularly contacted by the PGRx staff about their recruitment progress and queries. A toll-free number is made available to participating physicians who need to inquire about specific cases or diagnostic criteria.

6. Drug exposure ascertainment

6.1 General methodology

Drug exposure ascertainment is obtained from two different sources in the PGRx system:

A) A structured patient interview
B) The medical data form with the computerized medical prescriptions

6.2 Index date

The index date is defined as the date of the first occurrence of signs, symptoms or diagnosis, whichever comes first, suggestive of the manifestation of the adverse event under consideration. In the case-referent approach, exposure to drugs is only pertinent for the period preceding the onset of the health event of interest. The index date can be different from the recruitment date by a period of time called recruitment delay which should not exceed three months in the PGRx data collection system. All exposure to drugs in the patients' interview and definition of exposure variables, refer to the period preceding the index date.

6.3 The structured patient interview

All cases and referents are submitted to a telephone-administered questionnaire by a trained interviewer within a few days of their recruitment (a maximum of 45 days after recruitment). In special circumstances where the interview of a patient cannot be done due to medical conditions, a proxy-responder may be interviewed instead. This is recorded to be taken into account in the analysis.

The content of the PGRx interview and tools is confidential. They can be consulted at PGRx facilities whenever a subscription is signed. Parts of the questionnaires are provided to the Subscribers.

In order to stimulate patients' memory of the drugs they have used, three approaches are used in sequence (Abenhaim et al, 1996, Strom et al, 2004):

- Spontaneous recall
- Guided recall with a drug list
- Guided recall with a detailed questionnaire

6.3.1 The interview guide

A printed interview guide is provided to all patients so it can be available to them at the time of the telephone interview. They are asked to look at the guide before hand and check all health conditions and drugs from the lists provided (see below). The goal of the interview guide is to limit the recall bias by doing a systematic review of health problems and therapeutic classes of drugs, and to facilitate spontaneous recall.

At the beginning of the interview, the interviewer asks the patient to have the guide in front of them along with their copies of prescriptions and packages of their current medication.

6.3.2 Interview time windows and time marks

Four time windows are used in sequence with patients for the memory recall of their use of medications, one week, two months, one year and two years previous to the index date.

For the longer time windows of one and two years, the patients are invited, at the beginning of the interview, to identify life events that can mark the boundaries of each of those two time windows. The life events do not have to be related to health and can be anything that helps the patient visualize the period of time for memory recall.

6.3.3 Review of health problems

The interview starts with the systematic review of a list of health problems. That list is provided to the patient in the interview guide so they had the time to look at it and check the health problems that they recognized as having had in the previous two years (appendix 2). Health problems in the list are grouped under large classes that roughly correspond to systems. The interviewer reads the list so that no health problem is omitted in the memory recall of patients who are asked if they have taken any drug for any of the health problems in the list.

6.3.4 Drug list and drug visual display

The interview guide contains a drug list for each of the 19 categories of health problems reviewed (above). The drug list (appendix 3) contains roughly 220 brand names, with an average of 20 drug names in each category that are selected with the following criteria:

- Drugs containing new active principles that have been on the market for three years or less.
- + Drugs under study including drugs targeted in risk management or surveillance plans.
- + To fill out each list to 20, drugs that have the highest sales and are used by at least 0.4% of the general population in one year.(125,000 users in Canada and 250,000 in France, for example)
  The sales figures considered here are those of the brand name products plus the generics.
  The rules for combining sales figures whenever different brand names or generics are available for the same active principles are detailed hereunder.
- + When only one drug meets one of the above-mentioned criteria in a health problem section, other drugs with the same indication are added, even if they do not meet a required criterion.

Up to 10 photographic visual displays of drug packages are provided in the interview guide, when available for the following drugs among the 20 appearing in the list:

- New active principles.
- +Drugs under study including drugs targeted in risk management or surveillance plans.
- To fill out each photographic series to 10, drugs that have the highest sales.
  The rules for combining sales figures whenever different brand names or generics are available for the same active principles are detailed hereunder.
- + When only one drug meets one of the above-mentioned criteria in a health problem section, other drugs with the same indication are added, even if they do not meet a required criterion

The drug lists and drug visual displays are systematically reviewed with the patient.

The rules for combining sales figures whenever different brand names or generics are available for the same active principles are:

1- Current criteria:

If a brand name drug or a generic is sold to 250,000 users:

- The brand name drug and the generic will be included in the PGRx drug list. (The generics are mentioned as follows: "rINN generics" where rINN is the recommended International Non-proprietary Name which is the name of the concerned active principle)
- Only the photography of the brand name drug will be displayed.

2- Criteria to be added, in the next updating of the interview guide

For a given active principle, when the sum of the brand names drug and the generics sales figures reaches the 0.4% of the population:

- The brand name drug and the generic will be included in the PGRx drug list. (The generics are mentioned as follows: "rINN generics" where rINN is the recommended International Non-proprietary Name which is the name of the concerned active principle)
- Only the photography of the brand name drug will be displayed. The question of the generics displays has to be discussed in the next scientific committee.

The drug list is specific for each country where PGRx is to be implemented.

6.3.5. Spontaneously reported drugs

Patients are instructed to report all drugs taken in the two years previous to the index date, whether they were obtained by prescription, over-the-counter or from the family (friends) pharmacy, even if they do not appear in the drug list of the interview guide.

The interview thus also contains:

- Questions and space in the interview guide, for any type of drugs reported as taken by the patient that are not on the drug list or are not recognized by the patient as belonging to any of the health problem reviewed.

- Patients are invited to remember OTC, homeopathic, phytotherapeutic, traditional medicines, pharmacists' preparations and other types of medications that they may have been taking.

6.3.6. Hospital drugs

The interview focuses on drugs taken on an ambulatory basis, whether they are prescribed initially in-hospital or in ambulatory settings. However hospital medications spontaneously reported by the patient are recorded.

6.3.7. Vaccines

A list of 50 vaccines is provided in a special section of the interview guide and used during the telephone interview.

For younger patients, the interview on the vaccines records information on the batch number.

6.3.8. Excluded drugs

The interview does not collect:

- Drugs only available in-hospital
- Drugs taken on a research protocol
- Drugs used for anti-cancer chemotherapies
- Anti-retrovirus drugs
- Throat lozenges for symptomatic relief
- Antiseptics and disinfectants for external use
- Emollients and hydrations for external use

6.3.9 Updating of the drug list

The drug list is revised three times a year using the criteria mentioned above.

6.3.10 Information collected on drugs reported

For each drug reported by the patient, the following information is collected:
- The name of the drug, its dosage and form.
- The amount taken in 24 hours, the last time the drug was taken.
- The dates of first and last takes.
- The mode of use (continuous, regular or sporadic).
- Substitutions with generic specialties of the same drug.

6.4 The medical data form

6.4.1 Referents

Primary care physicians are enrolled to participate to the PGRx recruitment of referents, only if they use an electronic patients' record system. When a patient accepts to be recruited, the physician transmits an anonymous extract of the electronic patients' record including the drug prescriptions over the previous two years.

6.4.2 Cases of adverse events

The usual primary care physician of cases recruited is identified by the specialist who recruits the patient. The information is transmitted to the PGRx staff who attempts to contact this physician, with the consent of the patient, to obtain information on prescriptions and chronic health conditions of the patients over the previous two years, as for the referents. As this is done for verification purposes (see below), the intensity of this search with the primary care physician depends on the relative rarity of the adverse event under consideration. For more frequent pathologies such as myocardial infarction, attempts to contact the primary care physician will be limited to a sample of 10% to 20% of the cases. For more rare diseases such as multiple sclerosis or acute hepatitis, the attempts can cover all cases recruited.

6.5 Concordance between drug exposure information from patients' interviews and medical data forms

For all referents, and all or a sample of cases recruited, two sources of information is available on drug exposure, the interview and the medical data form. The interview is considered as the primary source of information and is used in the analyses as the measure of exposure. Where the two sources disagree on drug exposure, it is the interview that is used, and the disagreement is recorded for use in sensitivity analyses where one source is substituted for the other (in-depth analyses).

7. Co-morbidities and risk factors

Characteristics that are associated with drug exposure and are also risk factors for the adverse event should be considered as potential confounders. Three types of information are used for the control of confounding as well as for performing interaction analyses.

- Participating physicians provide a co-morbidities chart (appendix 4).
- Participating physicians provide some basic biological data.
- Patients are asked about their general risk factors and occupation.

All these information are collected on a routine basis in the PGRx system.

7.1 Co-morbidities and biological data

For each patient recruited, cases and referents, physicians fill a co-morbidity chart constructed in part with National health reports from countries where PGRx operates (appendix 4). Co-morbidities are then systematically organised in systems and allow

statistical treatment that is consistent with the International Classification of Diseases 10^{th} revision and other existing co-morbidity scales and indices. The information is entered in the system by the physician through a secure Internet data entry and transmission protocol.

In addition to the co-morbidities, recruiting referents physicians are also requested to enter basic biologic data:

- Blood pressure.
- Glycaemic profile; fasting blood glucose and $HbA1_c$.
- Lipid profile; cholesterol and triglycerides.

The values entered are those that are the closest preceding the index date.

7.2 Other variables

The patients' interview includes information for description of cases and referents, matching, control for potential confounding and interaction analyses. It contains the health insurance coverage for adjustment in the analyses and, in certain situations, for validation of the drug exposure. Sections of the interview include:

- Socio-demographic data
  - Age
  - Sex
- Complete past medical history in the previous two years
  - Review of systems
  - Visits to a physician
  - Hospitalisations
  - Health insurance coverage
- General risk factors
  - Body mass index
  - Smoking
  - Alcohol use
  - Physical activity
- Usual occupation
- Quality of life

Other variables can be added for control of potential confounding depending on the type of pathology that is considered as an adverse event.

7.3 Additional collection of information

In addition to the information collected routinely (above), specific information can be collected based on the nature of an adverse event and the cases recruited. Specific statistical methods are then used to control for potential confounding using the information on a sample of the study population.

8. Centre for Risk Research Specialised Interview Team

Given the specialized nature of the interview administered to the patients, PGRx has its own call centre and recruits and trains its own interviewers. The Centre is located

in Montreal, Canada and supervised by an administrative director and a Scientific Director.

Each interviewer recruited receives a basic training on how to approach a patient on the telephone, on the patient interview with special emphasis on drug exposure ascertainment, and on data security. An instructors' manual has been developed to that effect. Trained interviewers participate in the training of newcomers in order to insure homogeneity of method across all interviews.

Interviewers are required to sign a confidentiality agreement before they start.

The call centre is equipped with an automated call monitoring system that coordinates the contacts with physicians and patients in the delays prescribed in the PGRx protocol.

9. Control of biases

Control of biases is based on routine procedures that are implemented at three levels.

- Participating Centres
- Pool of referents
- Patients' standardized interview

These procedures are updated regularly in order to ensure the best quality achievable in all aspects of the PGRx system, according to the highest current scientific and technological standards.

The PGRx system follows the guidelines for Good Pharmaco-epidemiology Practice (GPP).

9.1 Participating Centres

Participating Centres are responsible to recruit cases by strictly applying inclusion and exclusion criteria that correspond to the case definition at hand, and to make sure that participation is being offered to each eligible patient without selection effect. Three procedures are in place to insure quality of cases of various pathologies in the PGRx system.

9.1.1 Comprehensiveness of cases

Centres and physicians participating to the recruitment of cases are instructed to recruit consecutively all patients that correspond to the case definition of a specified pathology. However, not all potential cases eventually enter the system, either because of failure to recruit them due to time constraints, or because of patient refusal to participate. Apart from increasing the accrual time of rare events, this problem is not of particular concern if the reason for non-inclusion is independent from drug exposure. To ensure that there is no selection bias, in-depth case-referent studies may include the use of a validation module whereby the patient registry in some Centres is used to compare patients who have been included in the system to those who did not,

distinguishing between different types of non-inclusion such as severity of disease and death, or non-participation.

9.1.2. Case validation

Cases can be validated by an independent expert panel that is blinded to drug exposure. This validation applies to all cases for certain diseases and to a sample of cases for other diseases.

Other methods of validation are possible on an *ad hoc* basis and include internal consistency assessments and comparison with external health insurance databases where available.

The validation procedure is described in the methodological section for each disease studied (Exhibit 1B).

9.1.3 Case participation

Some cases are not included in the study either because they refused, are lost after patient discharge, or were not in a health condition to participate to the system and be interviewed. The socio-demographic characteristics of these patients are compared to those of patients included in the system.

9.2 Pool of referents

The pool of referents is monitored closely to insure representativity of the population where they come from. Two methods are used.

9.2.1 Monitoring of the recruitment base

Every year the recruitment base of participating physicians in general practice on a given territory is reviewed to insure representativity of general practitioners on that territory, using national data on medical manpower. A percentage of participating physicians is replaced (voluntary or attrition) every year.

9.2.2 Referent ascertainment

Physicians participating to the PGRx system are instructed to recruit patients in the system in a sequential manner and in a pre-specified time period. This minimises the risk that they select patients based on their level of comorbidity or frequency of visits.

9.3 Patients' standardized interview

Patients' interviews are performed by especially trained interviewers using a method that has been developed for PGRx taking advantage of the latest advances in pharmaco-epidemiology. The details are presented in section 6.3. The interview technique contributes to minimize biases under three features, content, support and conduct.

9.3.1 The content of the interview is standardized and adapted to each country participating to PGRx. Formulation of questions is consistent with regulatory, ethical and customary considerations. The interview plan is structured to facilitate administration by the interviewer, understanding by the interviewee and to reduce sources of tensions and fatigue during the interview.

9.3.2 Support tools are made to help recall and reduce risks of errors: an interview guide described in section 6.3 to help the recall of drugs and vaccines taken, an electronic calendar to help the interviewer locate the dates of interest with patients, a data entry template that signals omissions and discrepancies.

9.3.3 The conduct of interviews is planned at dates and times that correspond to patients' preferences and availabilities obtained by their recruiting physician. Interviewers are trained on etiquette when addressing patients or vulnerable persons, rigour, empathy and security issues. Special training is provided for interview of minors. The training guide is updated with new PGRx features as they are incorporated. A continuing education program is planned at regular intervals.

10. Quality control

10.1 Monitoring of interviews

All interviews are taped and stored in a secured database. Each interview is monitored for its duration and given a "difficulty score" by the interviewer according to the difficulty to obtain information from a patient. Statistics on duration and difficulty by interviewer and day and time of interview, are reviewed on a weekly basis.

10.2 Standard operating procedures

The PGRx operations for data collection, utilisation, transfer and storage, are coded in a Standard Operating Procedures (SOPs) manual. Each procedure is controlled by a specific monitoring system and subjected to regular internal audit. An audit trail is maintained through all SOPs in the PGRx process. Internal audit are performed on a routine basis.

10.3 Data security

Data security is embedded in all SOPs where pertinent on confidentiality, integrity and accessibility issues. Confidentiality is partly insured by the physical separation of databases containing the identity of participating physicians and their patients, and the health information used for statistical analyses. The identity of participants is used for scheduling interviews, sending reminders and organise internal audits. Linkage of the two databases is possible only with the written consent of patients. Integrity is partly checked by computerized routines that are programmed to detect errors and inconsistencies in the database. All personnel working with the PGRx system is

specially trained on data security and has a pre-determined access to the information. A strict code of conduct is maintained in all operations.

The particular security issues relevant to each pathology in the PGRx system are described in the exhibit 1B.

11. Statistical Analysis

The analytic plan proceeds according to the case-referent design. Two types of analyses are programmed; one without *a priori* hypothesis called the *crude* analysis and the other with specific *a priori* hypotheses called the *in-depth* analysis. In both cases, the analysis is preceded by a matching procedure whereby case-referent sets are formed.

11.1 Matching procedure

Each case of a pathology selected for analysis is matched to a pre-determined number of referents on five criteria:

- Sex
- Age (within 5 years)
- Time of recruitment (closest not exceeding 3 months)
- Place of residence (same recruitment region)
- Number of visits to a physician in the previous year.

These criteria have been set to balance the probability of exposure to drugs between cases and referents.

11.2 Crude Analysis

The goal of the crude analysis is a general surveillance of adverse events and exposure to drugs or therapeutic classes. These analyses are performed periodically on a routine basis as a crude comparison between sets of cases and sets of referents for their exposure to therapeutic products. The association between an exposure and the occurrence of an adverse event is quantified through a crude Odds ratio and its 90% confidence interval. The crude Odds ratios are not adjusted for the various confounding variables and not subjected to particular risk curve modeling, and should be regarded as indicative only.

Reports of crude analyses results of each study are proprietary to PGRx and are provided to subscribers periodically. These reports are also accessible by subscribers on the PGRx Website in a limited and secured access.

11.3 In-Depth Analysis

In-depth analyses are conducted on demand. In that case a specific hypothesis must be specified and is tested regarding an adverse event and exposure to a specific drug or therapeutic class. In the PGRx system, this implies the creation of a complete "*ad*

hoc" protocol (Exhibit 3C) under the auspices of a *Product Study Scientific Committee*.

The analysis is performed using multivariate techniques considering all risk factors for a specific pathology (potential confounding variables) as well as co-medications. The association between a drug and the occurrence of an adverse event is quantified through adjusted odds ratios and their 95% confidence interval. Specific risk curve modeling is tested when available from the literature or clinical experience.

Special analyses can be performed depending on the problem at hand. For example, *propensity score analyses* are indicated where cases and referents are suspected to have different probabilities of exposure to a given drug or class of drug. Another example of special analyses is to account for the "*depletion of susceptible"* effect. In that case, past drug use can be included in the model. Depletion of susceptible can occur when patients who have been on the drug for a long time are at lower risk than new users.

Interaction analyses can be performed to test the effect of age, sex, time of year, past medical history, etc.

11.4 Sensitivity analysis

Sensitivity analyses can be performed to assess the robustness of results to changes in a number of parameters:

- A different set of referents is randomly selected and all the analyses repeated. Results are compared to those obtained with the original one. This is done whenever the size of the study allows for this operation.
- Case-cross over analysis is performed where the case is used as its own control for a different passed time-window. This is possible for transient exposures and with certain hazard functions only. In some instances, the case-cross over design may be defined a priori as the main design for the study.
- Exclusion of certain cases or referents from the analysis (to be defined for each study).
- When the diagnosis is coded as certain, probable or uncertain, the analysis can retain only the "certain" category first and then proceed with including the other categories in descending order of certainty.
- Other effects can be used in sensitivity analyses depending on the problem at hand, such as accounting for the recruiting region or Centres.

12. Ethical considerations

Participation of cases and referents is sought through informed consent form. The form and a summary of the information system is provided to the patient by their physicians.

The system complies with regulatory requests regarding patient privacy protection and ethical requirements in each country where PGRx has patients' recruitment activities (IRBs in Canada, CNIL in France, etc).

Appendix 1 PGRx International Scientific Board

[redacted]	[redacted]	Pharmacoepidemiology	[redacted]
[redacted]	[redacted]	Neurology	[redacted]
[redacted]	[redacted]	Pulmonary medicine	[redacted]
[redacted]	[redacted]	Rheumatology	[redacted]
[redacted]	[redacted]	Pharmacology	[redacted]
[redacted]	[redacted]	Biostatistics	[redacted]
[redacted]	[redacted]	Pharmacoepidemiology	[redacted]
[redacted]	[redacted]	Nephrology	[redacted]
[redacted]	[redacted]	Infectious diseases	[redacted]
[redacted]	[redacted]	Cardiology	[redacted]
[redacted]	[redacted]	Geriatrics	[redacted]
[redacted]	[redacted]	Neurology	[redacted]
[redacted]	[redacted]	Endocrinology	[redacted]
[redacted]	[redacted]	Haematology	[redacted]
[redacted]	[redacted]	Pharmacology	[redacted]
[redacted]	[redacted]	Pharmacoepidemiology	[redacted], Canada
[redacted]	[redacted]	Hepatology	[redacted]
[redacted]	[redacted]	Epidemiology	[redacted]
[redacted]	[redacted]	Psychiatry	[redacted]

Observers

[redacted]	Emea	[redacted]
[redacted]	Afssaps	[redacted]
[redacted]	DGS	[redacted]

Appendix 2 Classification of principal drug indications

Complete list of 79 specific items can be consulted upon request

Chronic Pathologies

- Cardiovascular and Cerebro-vascular risk factors and pathologies, Hypertension
- Obesity, Diabetes, Thyroid disorders and other Endocrinology and Metabolic Pathologies
- Pain
- Osteoarthritis, Low-Back pain, Musculo-Tendinous Pain, Osteoporosis, Gout, Rheumatisms and other Musculoskeletal Disorders
- Respiratory and Pulmonary Problems
- Flue, Throat Angina, Bronchitis, Sinusitis, and other Respiratory Infections
- Gastric Problems
- Intestinal Problems
- Liver Disorders
- Allergies
- Acne, Psoriasis, Eczema and other Dermatologic Problems
- Anxiety, Depression, Sleep Disorders and Psychiatric Disorders
- Neurological Disorders
- Contraception, Infertility, Menopause
- Urinary, Kidney or Genital Problems
- Sexually Transmitted Infections; HIV, HBV and HPV infections, AIDS
- Cancer and Malignant Tumours
- Blood Disorders
- Eyes and ears pathologies; Glaucoma, infectious diseases and others

Appendix 3 The PGRx Drug List

List* of drugs and vaccines systematically documented in the PGRx System (*Lasr update December 1st 2007)

- FRANCE -

Aérius®
Aclasta®
Acomplia®
Act-Hib®
Actonel®
Adartrel®
Advil®
Agréal®
Allopurinol génériques
Almogran®
Alprazolam génériques
Amarel®
Amlor®
Amodex® Gé
Amoxicilline Acide clavulanique génériques
Amoxicilline génériques
Apranax®
Aprovel®
Aranesp®
Arava®
Aricept®
Art 50®
Aspégic®
Atacand®
Atarax®
Augmentin®
Avaxim®
Avonex®
Bactrim®
Baraclude®
Béfizal®
Bétaféron®
Bi-Profénid®
Bi-Profénid®
Birodogyl®
Bonviva®
Brexin®
Bronchodual®
Cardensiel®
Cartrex®
Célébrex®
Célestène®
Cervarix®
Champix®
Chibroproscar®
Chondrosulf®
Cialis®
Ciprofibrate génériques
Clamoxyl®
Clarityne®
Coaprovel®
Codoliprane®
Copaxone®
Cortancyl®
Cotareg®
Coversyl®
Cozaar®
Crestor®
Curacné®
Dafalgan®
Déroxat®
Dextropropoxyphène
Paracétamol génériques
Dialgirex® Gé
Diamicron®
Di-Antalvic®
Doliprane®
Donormyl®
DT Polio®
Dukoral®
Duphaston®
Efferalgan Codéine®
Efferalgan®
Effexor®
Elisor®
Enbrel®
Equanil®
Eupantol®
Ezétrol®
Fégénor®
Fénofibrate génériques
Flanid® Gé
Flécaine®
Fludex®
Fosamax®
Fractal®
Fucidine®
Furosémide génériques
Gardasil®
Gaviscon®
Glucophage®
Gripguard®
HBVaxPro®
Hepséra®
Hexaquine®
Humira®
Imigrane®
Imiject®
Immugrip®
Imovane®
Imovax Polio®
Inégy®
Inéxium®
Infergen®
Influvac®
Inipomp®
Inspra®
Ixprim®
Januvia®
Josacine®
Kardégic®
Kestin®
Ketec®
Kétoprofène génériques
Kineret®
Lamaline®
Lamisil®
Lantus®
Lanzor®
Laroxyl®
Lasilix®
Lercan®
Lescol®
Lévothyrox®
Léxomil®
Lipanor®
Lipanthyl micronisé®
Lipanthyl®
Lipirex®
Lipur®
Lodalès®
Lodoz®
Loxen®
Lumirélax®
Lutényl®
Lyrica®
Lysanxia®

Maalox®
MabThéra®
Maxepa®
Médiator®
Meningitec®
Meninvact®
Menjugate®
Metformine génériques
Méthotrexate génériques
Mopral®
Mutagrip®
Naramig®
Neisvac®
Nétromicine®
Nexen®
Niaspan®
Nifluril®
Noctamide®
Nureflex®
Nurofen®
Ogast®
Omacor®
Oméprazole génériques
Orbénine®
Orélox®
Oroken®
Paracétamol génériques
Pariet®
Paroxétine génériques
Pégasys®
Pentavac®
Permixon®
Piasclédine®
Plavix®
Pneumo 23®
Pravadual®
Pravastatine génériques
Prévenar®
Prévgrip®
Préviscan®
Primalan®
Procuta® Gé
Propanolol génériques
Propofan®
Pyostacine®
Questran®
Rabipur®
Rébétol®
Rébif®
Relenza®
Relpax®
Repevax®
Revaxis ®
Rivotril®
Roaccutane®
Rocéphine®
Roféron A®
ROR Vax®
Rudivax®
Sébivo®
Sécalip®
Séresta®
Sérétide®
Simvastatine génériques
Singulair®
Skenan®
Solu-Médrol®
Solupred®
Spiriva®
Spirolept®
Stablon®
Stamaril®
Stilnox®
Structum®
Subutex®
Surgam®
Symbicort®
Tahor®
Tamiflu®
Tareg®
Témesta®
Tercian®
Tétagrip®
Tétravac®-Acellulaire
Ticovac®
Toco®
Topalgic®
Tranxène®
Triatec®
Triflucan®
Tyavax®
Typhim Vi
Vaccin BCG SSI®
Vaccin Boostrixtetra®
Vaccin Engerix B®
Vaccin Fluarix®
Vaccin GenHevac B® Pasteur
Vaccin Havrix®
Vaccin Infanrixhexa®
Vaccin Infanrixquinta®
Vaccin Infanrixtetra®
Vaccin Méningococcique A+C polyosidique
Vaccin Priorix®
Vaccin Rabique Pasteur
Vaccin Tétanique Pasteur
Vaccin Twinrix®
Vaccin Typherix®
Vaccin Varilix®
Varivax®
Vastarel®
Vasten®
Vaxigrip®
Ventoline®
Viagra®
Viraféron Peg®
Virlix®
Vogalène®
Voltarène®
Xanax®
Xatral®
Xolaam®
Xolair®
Xyzall®
Zaldiar®
Zanidip®
Zéclar®
Zélitrex®
Zinnat®
Zithromax®
Zocor®
Zoloft®
Zolpidem génériques
Zomig®
Zomigoro®
Zonégran®
Zovirax®
Zyban®

Appendix 4 List of chronic conditions (co-morbidities)

- ❑ Cardiovascular Pathologies
  - ❑ Coronaropathy
  - ❑ Cardiac Insufficiency
  - ❑ Cardiac Rhythm and Electric Conduction Disorders
  - ❑ Valvulopathy
  - ❑ Congenital Heart Disorders
  - ❑ Arterial Hypertension
  - ❑ Chronic Arterial Diseases
  - ❑ Stroke (sequel)
  - ❑ Others: _____
- ❑ Pulmonary Pathologies
  - ❑ Asthma
  - ❑ Chronic Respiratory Insufficiency
  - ❑ Others: _____
- ❑ Endocrinology and Metabolic Disorders
  - ❑ Diabetes
  - ❑ Hyper-thyroidal / Hypo-thyroidal Disorders
  - ❑ Others: _____
- ❑ Hepatic Pathologies
  - ❑ Viral Hepatitis
  - ❑ Auto-immune Hepatitis
  - ❑ Cirrhosis
  - ❑ Others: _____
- ❑ Nephrologic Pathologies
  - ❑ Chronic Nephropathy

- ❑ Others: _____

- ❑ Neurological Pathologies
  - ❑ Epilepsy
  - ❑ Parkinson Disease
  - ❑ Multiple Sclerosis
  - ❑ Alzheimer Disease and other Dementia
  - ❑ Others: _____

- ❑ Psychiatric Diseases
  - ❑ Schizophrenia
  - ❑ Others: _____

- ❑ Haematological Disorders
  - ❑ Haemoglobinopathy
  - ❑ Haemolysis
  - ❑ Haemophilia and Haemostasis Disorders
  - ❑ Bone Marrow Insufficiency and Chronic Cytopenia
  - ❑ Others: _____

- ❑ Systemic Inflammatory Pathologies
  - ❑ Lupus Erythematosus
  - ❑ Rheumatoid Arthritis
  - ❑ Ankylosing Spondylarthritis
  - ❑ Crohn Disease
  - ❑ Ulcerative Colitis
  - ❑ Sclerodermia
  - ❑ Others: _____

- ❑ Infectious / Parasitic Diseases
  - ❑ Active Tuberculosis
  - ❑ HIV Infection

- ❑ Leprosis
- ❑ Bilharziozis
- ❑ Others: _____

❑ Neoplasia and Malignant Tumors:

_____

❑ Others:

_____

PGRX

STUDY OF CERVARIX® & AUTOIMMUNE THYROIDITIS AND GRAVES DISEASE

USING THE PGRx INFORMATION SYSTEM

February 26, 2009

PGRx
Centre for Risk Research Inc.
LA-SER sarl

Table of content

NOTE

This protocol is provided with the *Exhibit 1A: The general methodology of PGRx (Appendix 1),* which applies to all studies conducted with the PGRx Information System.

The Exhibit 1A is up-dated on a yearly basis by the International Scientific Board of PGRx, taking into account evolution of the System resulting form the actual conduct of data collection and studies. For the purpose of the study of Cervarix®, in the case of any difference or apparent discrepancies between the Exhibit 1A and the present Protocol, it is this Protocol that prevails at any time.

1. Introduction

1.1. Overview of the study

1.1.1. Study Objective

The objective of the study is to assess whether the use of Cervarix® is associated with a modified risk of autoimmune thyroiditis and Graves disease ("the disease").

1.1.2 .General inclusion & exclusion criteria for the cases and referents in the study

Study subjects are cases and referents from the PGRx system satisfying with the following criteria:

Inclusion criteria

- Female gender
- Age 14 to 26 years-old
- Patient residing in France (continental)
- Patient accepting to participate in the study

Exclusion criteria

- Prior reported history of the disease;
- Patient or Patient's parent cannot read the interview guide or answer a telephone interview questionnaire in French.

1.1.3. Study design

1.1.3.1. Case-control (or case-referent) methodology

This study is a systematic case-referent study. It consists in using the PGRx information system to:

a) Monitor a large number of neurology centres for the occurrence of the disease,
b) Match general practice-based controls to these cases, selected from the pool of PGRx potential referents
c) Document the previous vaccination by Cervarix® in both cases and controls,
d) Estimate the relative risk of the disease in Cervarix® vaccinated females by the odds ratio (adjusted for a series of confounders and interaction factors, including other drug use).

1.1.3.2. Rationale for the choice of the case-control design using PGRx

The case-control (or case-referent) methodology is the design of choice for the study of rare events, such as autoimmune disorders in epidemiology. Its power is not affected by the small incidence of diseases and has proved efficient in pharmacoepidemiology (Abenhaim, 1996). When based on field collection of data, this design allows for the documentation of individual risk factors.

Ad hoc case-control studies in pharmacoepidemiology are however cumbersome and require a large amount of work and procedure to control for the various sources of biases (Wacholder, 1992).

The PGRx Information System (PGRx) has been developed to minimise these difficulties and biases.

PGRx is a systematisation of the case-control referent (or case-referent, Miettinen, 1976) methodology. It is available in France and Canada. It addresses most of the concerns usually raised with ad hoc case-control studies. Autoimmune disorders have been listed as conditions of interests for PGRx since the inception of the system.

1.2. Overview of the PGRx Information System (PGRx)

1.2.1. General Description and Methods of PGRx[1]

The PGRx general methodology is described in PGRx Database & Information System Exhibit 1 A – *General Methodology*.

In brief, PGRx has been developed in response to the paucity of databases or information systems available for the study of rare diseases and/or delayed adverse events associated to medicines, with sufficient power and specificity on disease diagnosis and individual risk factors. It operates since 2007.

The system prospectively and routinely collects information on:

1) Cases[2] of a dozen diseases[3] collected in more than two hundred specialized referral centres and validated through a series of procedures. The collection ensures for a control of selection bias;
2) A large pool of general practice-based potential referents from which controls or referents can be selected and matched to cases of diseases under study. Matching can be made on calendar time, age, gender, region and any other relevant parameter available and can be individual matching or frequency-matching. The selection of referents is performed in such a way to ensure a fair representation of the population-time experience with the drugs studied in the relevant source populations,
3) 300 drugs (including vaccines) documented through: (i) guided telephone interviews and (ii) medical prescription records (in a sample of either treating physicians'

[1] See Exhibit 1A attached

[2] In the PGRx DIS, cases are defined as adverse *events* and not necessarily adverse *reactions*. No hypothesis is made *a priori* on the causality of the event (as opposed to spontaneous reports of adverse reactions frequently reported in pharmacovigilance systems).

[3] The diseases routinely surveyed in the PGRx Information System are presently: myocardial infarction, multiple sclerosis (first central demyelination), Guillain-Barré syndrome, lupus erythematosus, cutaneous lupus, myositis and dermatomyositis, inflammatory arthritis, unspecified connectivitis, type I diabetes, thyroiditis, thrombocytopenia, suicide attempts, torsade de pointes and acute liver injuries. First results have been presented in various conferences (ICPE, 2008; ISOP, 2008).

computerized prescriptions or treating physician's reports). All new molecules, products targeted in risk management plans and up to 24 products used by more than 250 000 persons in the country are listed, including most vaccines. Cervarix® is one of the vaccines routinely studied. The lists of drug or vaccines specifically studied at the different dates are provided with the Exhibit 1A.

4) Individual behavioural, medical and family risk factors: smoking, alcohol use, physical activity, occupation, chronic co-morbidities, familial history of certain diseases, others.

For each AID a PGRx Scientific Committee, called PGRx Pathology Specific Scientific Committee (see Exhibit 1A), has been organised and the general methodology for the study of each AID in PGRx has been developed under the auspices of those committees. The collection of data in PGRx follows the criteria developed by these committees. Out of these collected data, the scientific committee for each individual study (e.g. the one for Cervarix® and autoimmune disorders assembled by the manufacturer) may select those that it considers appropriate for its study.

1.2.2. PGRx Network for Autoimmune disease

A network of centres treating patients for these diseases has been assembled to participate in the PGRx Database and Information System.

Table A2.1 and Figure A2.1 in the Appendix 2 reports the number of centres participating in the collection of cases of autoimmune thyroiditis and Graves disease, the date of start of the surveillance of this disease in the system, the number of cases recruited so far by age group (l4-26 years old, all age groups) and the objectives of recruitment per year in the System.

1.3. Overview of the literature

1.3.1 Epidemiology of thyroïditis

Epidemiological studies of dysthyroidism are more frequent than studies on thyroiditis. When incidence rates of the different causes of dysthyroidism are assessed, it is possible to consider all spontaneous dysthyroidism cases as auto-immune disorders.

Carlé (2006) conducted a prospective population-based study to assess incidences of subtypes of hypothyroidism in a Danish population cohort. Between 1997 and 2000, incidence rate of hypothyroidism was 32.8 per 100 000 person-years. Nosological types of hypothyroidism were: spontaneous (presumably auto-immune) 84.4%, post-partum 4.7%, amiodarone-associated 4.0%, subacute thyroiditis 1.8%, previous radiation or surgery 1.8%, congenital 1.6% and lithium-associated 1.6%. Hypothyroidism was more common among females with a female/male incidence rate ratio of 3.5; the ratio was 3.7 for spontaneous hypothyroidism. According to those results, thyroiditis incident rate on the whole study population was 27.7/100,000 person-year and was 21.8/100,000 women-year among women.

A large study from Denmark has shown the incidence of thyrotoxicosis to be 65.4/100,000 person-year (mild iodine deficiency region), and 92.9/100,000 person-year (moderate iodine deficiency) (Bulow Pedersen I, 2002).
In the study of Laurberg (1991) the incidence of GD was:

- 19.7/100,000 person-year in Iceland (a high iodine intake region);
- 14.8/100,000 person-year in Denmark (region of low average iodine intake).

Epidemiological surveys from iodine sufficient regions have shown incidences of GD in caucasian populations approximately of 20–25/100,000 person-year (Brownlie, 1990; Berglung 1990 ; Heraldsson, 1985 ; Furszyfer, 1970 ; Mogensen , 1980).

The Whickham Survey assessed the incidence of thyroid disorders in a randomly selected sample of adults of Great Britain with a twenty-year follow-up (Vanderpump, 1995). The mean incidence of spontaneous hypothyroidism in women was 350/100,000 person-year ($IC_{95\%}$: 280-450) rising to 410/100,000 person-year ($IC_{95\%}$: 330-500) for all causes of hypothyroidism and in men was 60/100,000 person-year ($IC_{95\%}$: 30-120). The mean incidence of hyperthyroidism in women was 80/100,000 person-year ($IC_{95\%}$: 50-140) and was negligible in men.

A study from Sweden (Berglund, 1996) showed no significant change in the incidence of GD over a period of 20 years (the incidence was 17.7/100,000/yr in 1970–1974 and 22.2/100,000/yr in 1988–1990).

1.3.2. Risk factors associated with thyroïditis

Many arguments suggest that thyroiditis is associated to an interaction between susceptibility genes and environmental triggers. Genetic susceptibility, in combination with external factors (e.g., dietary iodine), is believed to initiate the auto-immune response to thyroid antigens. Epidemiological data from family and twin studies, point to a genetic implication on the development of thyroiditis.

Genetic susceptibility:
The familial occurrence of thyroiditis has been reported in the literature by several studies (Tomer, 2003). Such studies reported a family history of thyroid disease in up to 60% of patients with GD. It was shown that 33% of siblings of patients with GD or HT developed thyroiditis themselves. Others studies have reported the presence of thyroid autoantibodies in up to 50% of the siblings of patients with GD. 36% of those GD patients with ophthalmopathy reported a family history of thyroiditis, and 23% of them had a first-degree relative with thyroiditis.
Twin studies are based on comparison of the concordance (simultaneous occurrence) of a given disease among monozygotic twins (MZ) with the concordance among dizygotic twins (DZ). Several twin studies have reported a higher concordance of thyroiditis in MZ twins than in DZ twins. For GD, the concordance was 35% in MZ twins and 3% in DZ twins. Twin studies in HT have shown concordance rates of 55% in MZ and 0% in DZ twins. The concordance rates for thyroid autoantibodies (TAb) were also reported to be higher in MZ twins compared with DZ twins. Twin data may confirm the hypothesis of an inherited susceptibility to thyroiditis.

Environmental risk factors:

- Iodine intakes have been associated with the increase of incidence of thyroiditis (Bulow Pedersen I, 2002 ; Caturegli, 2007 ; Teng, 2006) ;

- Tobacco consumption increase risk of hypothyroidism (Vestergaard 2002; Bindra,2006) ;
- Selenium deficiency.

1.4. Drugs allegedly associated with thyroïditis

1.4.1. All drugs

Drug exposures have been described with the occurrence of thyroiditis: lithium (Miller, 2001), amiodarone (Martino, 2001), interferon-alfa (Carella, 2004), interleukin-2 (Schuppert, 1997).
Yu (2007) conducted a case-control study, within the Vaccine Safety Datalink project in the USA. Cases of GD and HT, among persons aged 18-69 years, following hepatitis B vaccine have been reported to the Vaccine Adverse Events Reporting System. Hepatitis B vaccination was not associated with risk of GD (OR=0.90; $CI_{95\%}$: 0.62-1.32) or HT (OR=1.23; $CI_{95\%}$: 0.87-1.73). No association was found between the time interval since vaccination and either outcome.

1.4.2. Time windows at risk used in studies

In the above mentioned studies, time-windows varying from less than 1 year to several years have been used for the study of the relation between thyroïditis and vaccines.

Table 1 summarizes the main features stemming from the literature review.

Table 1: Epidemiology of thyroïditis and data stemming the literature review

Socio-demographics (age, gender)	50-64 years old Female/male incidence rate ratio 3.5
Incidence	Denmark:(hypothyroidism) $32.8/10^5$person-years GB: (women only) $350/10^5$person-year ($IC_{95\%}$: 280-450)
Prevalence	France: from 0.5 to 5%
Time to event tested	≤1 year, 1-5 years, ≥5 years

2. Cases

2.1. Populations for case recruitment

2.1.1. Source population

The source population for the study is made of patients who are:

- Hospitalised for the occurrence of the disease in one of the centres participating in the PGRx Network for AID;
- Or addressed to a centre participating in the PGRx Network for the diagnosis or the management of the disease.

2.1.2. Study population for cases

The study population is made of patients from the source population above who are:

- Incident cases patients presenting with the set of symptoms and signs retained for the diagnosis of the disease defined further below;
- Reported in PGRx by the specialist participating in PGRx;
- Recruited within 12 months after the date of the occurrence of the first clinical sign identified by a physician;
- Meeting all inclusion and exclusion criteria for the study.

2.2. Identification of cases

2.2.1 PGRx Centres for the recruitment of cases

Centres eligible to participate to the PGRx Network for the recruitment of contemplated events are [redacted] and [redacted] that have a specialized unit or a health care network for the management of this disease. These units are selected on the volume of incident cases of the disease that they treat per year.

2.2.2 Recruitment of cases

Participation must be proposed to all consecutive patients who respond to inclusion and exclusion criteria for the event in the PGRx participating centres.

2.2.3. Web entry

Each specialist recruiting a case fills out a medical data form directly on a secured Internet data entry system on which they have been individually provided with a login and a password.

2.3. Information collected

2.3.1. Medical form[4]

General information

When the case is included the following data are collected by the recruiting specialist:

- Date of the consultation;
- First and last name, date of birth and gender of the patient;
- Inclusion and exclusion criteria;
- Name and address or phone number of the usual treating general practitioner of the case recruited.

Medical information

The following sections of the medical form are used for case ascertainment:

[4] The web-based Clinical Research Forms are available for consultation to interested parties upon request.

- Date of the first symptoms evocative of the disease
- Description of the symptoms and signs of the first evocative episode
- Description of biological and imaging findings (if appropriate and/or available)
- Current and previous chronic diseases
- Personal history of autoimmune disorders
- Elements of differential diagnosis

2.4. Case definition

Cases for the study are *incident cases* (i.e. newly diagnosed patients) reported as having occurred in the previous twelve months before the recruitment consultation.

2.4.1 Case ascertainment

Cases will be validated by an independent expert review panel blind to the medications and vaccinations status. The panel will review the medical forms of all the cases recruited. At the end of their review of each case, the expert review panel will qualify the cases as:

a) Definite
b) Possible
c) Rejected

Definite cases only will be used in the main analysis. Possible cases may be used for potential "unplanned analysis" (see further below). Rejected cases are used for the identification of biases (see special section "Identification of biases" further below). The diagnostic criteria to classify the patients are described below; they have been adapted from internationally accepted definitions to allow for the recruitment of cases at the early stages of the disease at hand and to better take into account the age groups concerned by the vaccination.

Every year, PGRx centres are contacted to assess the potential evolution of the diagnosis of the cases reported previously. Any change in the diagnosis of the case is recorded and the case is reclassified as definite, possible or rejected. .

2.4.2 General definition of cases for the study

Cases for the study are *incident cases* of disorders evocative of auto-immune thyroiditis or of Graves's disease.
A personal history of auto-immune thyroiditis excludes the patient. Patients presenting a recurrence or a relapse of Graves'disease are excluded.

2.4.3. Definition of definite possible and rejected cases

Cases for the study are ascertained by the following algorithm, simplified in table 2A and 2B.

Table 2A: Definition of cases for the study of incident auto-immune thyroiditis evocative disorders

	Clinical presentation	Biologic examinations
Definite cases	Hypothyroidism consistent with incident auto-immune thyroiditis	**AND** anti-peroxydase (anti-TPO) **AND** increased TSH > 7 mU/L
Possible cases		**AND** anti-thyroglobuline (anti-TG) **AND** 4 mU/L< TSH < 7 mU/L

Table 2B: Definition of cases for the study of incident Graves's disease evocative disorders

	Thyrotoxicosis	Thyroid gland	Auto-antibodies	TSH
Definite cases	Presence of exophthalmia or palsy or tachycardia or weight loss or weight gain	-	**AND** anti-TSH-receptor	**AND** decreased TSH
Possible cases *Subclinical thyroiditis*	Discrete symptoms or absence of symptoms	**AND** thyroid gland with normal or borderline size	**AND** anti-peroxydase (anti-TPO) and/or Anti-thyroglobulin (anti-TG)	**AND** decreased TSH

3. Referents and matching rules

3.1. Definition of referents

Referents to the cases are patients selected from the pool of potential referents reported by physicians in general practice, who meet the same general inclusion and exclusion criteria as the cases.

Patients with no reported previous history of the disease considered for the cases, as reported by themselves or their physician will be selected from the pool of potential referents in the PGRx system to serve as referents to cases.

3.2. Recruitment of referents

3.2.1. PGRx Pool of Potential Referents

A network of *ca.* two hundred and fifty (250) general practitioners (GPs) enrols a pool of *ca.* 2,000 referents each year in the PGRx database and Information system. Each GP in the network is asked to recruit 1 male and 1 female in the following age categories: 18-34, 35-49, 50-64, 65-79 (age strata may be more detailed or doubled if needed).

For the purpose of the study of autoimmune disorders in younger age groups, voluntary GPs have been asked to also recruit patients 14 to 17 y.o (2 males and 2 females per year of age and by physician).

Physicians who recruit potential referents are requested to fill an electronic medical data form that includes medical information on the patient (current prescriptions with their motives and diagnoses, chronic diseases, medical risk factors and some biological data).

Physicians obtain consent of eligible patients to participate and transfer the coordinates of the patients to the PGRx staff for the telephone interview, through a secured Internet connection.

PGRx GPs are enrolled for the recruitment of referents in all telephone regions of the country. Physicians are randomly selected from a general list of practicing physicians in a given region. In order to be enrolled, they must have access to Internet and use computerized prescriptions. Those who agree are provided with a secured access to the PGRx system on Internet and are instructed on recruitment of consenting patients, on filling the medical data form and the electronic transfer of their computerized drug prescriptions over the previous two years.

Participating physicians are asked to recruit a set of potential referents patients one to three times a year on a rotating basis so that recruitment is not interrupted in a given region over the year. This recruitment spread out overtime facilitates matching of selected referents to cases on calendar time.

3.2.2. Referents selected for the study of autoimmune disorders

The selection of referents from the PGRx pool of potential referents proceeds in order to apply the same inclusion and exclusion criteria as in cases.

3.3. Matching

To each case is matched at least one referent. As many referents as possible meeting the criteria for the study and allowing proper matching to case are retained. It is estimated than an average of 4 referents will be available per case with the following priority rules:

1) Date of recruitment of the cases and referents: Cases and referents are organised by trimester of recruitment in a given year (Q1 to Q4): for each matching criteria below, a referent is looked for in the same quarter of recruitment as the case or, if none is found, in the next adjacent quarter of recruitment, and then the next one again. If no matched referent is found, the case is not retained.

2) Age: matching will be done with the following order of priority: ± 1 month, then ± 3 months; then ±6 months, then ±1 year (for age ≤ 17), then ±2 years (for age ≥ 18); if no matching referent is found to a case, the case is not retained.

3) Number of visits to a physician in the previous year (0-5, >5). If no matching referent is found to a case, this matching criterion is dropped.

4) Place of residence (region or telephone zone): cases will be match to referents of the same region, if necessary matching will be performed with referents from contiguous regions; if necessary, referents from all France are considered.

4. Drug exposure ascertainment

The ascertainment of exposure follows 3 steps:

1 – Identifying and ascertaining drugs and vaccines used in the last 2 years

2 – Defining the index date for exposure

3 – Defining the relevant time window at risk for the exposure before that index date.

A subject is considered as 'exposed' whenever a vaccine use is ascertained during the time window at risk.

4.1. Identifying drug and vaccine use

4.1.1. Sources of information

Information on drug exposure is obtained from:

A) A structured telephone interview of the patient (cases and referents) or of one of the patient's parent (see below)using:
   - an interview guide,
   - a list of 19 General Health Conditions,
   - a list of up to 20selected drugs for each General Health Condition (see below)
   - and visual photographic displays of up to 10 drug packages per General Health Conditions
   - a list of all vaccines (with up to 10 visual displays of packages)

B) Medical records obtained from the Treating Physician[5] of the cases and the PGRx GPs reporting referents:
   - Either copies of computerized medical prescriptions
   - And/or medical prescription forms filled by the treating physician

For cases, the name of the treating physician and consent to contact him/her is obtained from the patient. They are contacted by the PGRx research team

Exposure is defined by a combination of the information from these two sources (see further below).

The interview is conducted by trained telephone interviewers belonging to the PGRx Call Centre specialised in pharmacoepidemiology. Patients are conducted through a list of questions. The duration of the interview is recorded. Interviews may be taped for quality control (with the information of the patient).

Consent is confirmed from the patient (case or referent), or from the patient' parent at the beginning of the interview. If the patient is minor (under 18 y.o in France), both the parent and

[5] To obtain reimbursement of certain health services, including drug prescribed, from the national health insurance, French patients must identify a so-called 'Treating Physician'.

the minor are asked to be present during the interview. The person actually interviewed is decided by the parent.

4.1.2. Drug list and drug visual display for the guided interview

The drug list used in the interview contains roughly 325 brand drug names (including *ca.* 50 vaccines, see below), with up to 20 drug names in each of the 19 General Health Conditions categories (see Exhibit 1A) ; they are selected with the following criteria (in order of selection):

- Drugs containing new active principles that have been on the market for 3 years or less.
- Drugs targeted in risk management or surveillance plans under study.
- Drugs that are used by at least 250,000 patients per year (selected in order of sales' figures)

Up to 10 photographic visual displays of drug packages are provided in the interview guide for each General Health Condition and for the vaccines (same order of selection as above).
The drug lists and drug visual displays are systematically reviewed with the patient.

The drug list and drug visual displays are renewed three times a year using the criteria mentioned above.

4.1.3. Ascertainment of vaccine use

4.1.3.1. Vaccines in the guided interview

A list of ca. 50 vaccines is provided in a special section of the interview guide and used during the telephone interview. Cervarix® is one of these vaccines.

For each Cervarix® use reported by the patient, the following information is sought for:

- The number of shots received with their date
- The availability at the patient's of evidences of the vaccination: medical prescription, health record, the vaccine package or other, and the possibility to obtain the copy of the evidence if needed
- The batch number of the reported vaccine (if the package is available to the patient or if this number is available in the health record)
- The settings of the vaccination (general practice, specialised physician settings, vaccination centres or other).

4.1.3.2. Confirmation of Cervarix® use

Reported use of Cervarix® will be considered as 'confirmed' when: reported by the patient as used with at least one of the following source of confirmation obtained:

- Vaccine batch number reported by the patient (from the drug package or his/her health record)
- Copy of the doctor's vaccine prescription or of the health record or of other evidence sent by the patient
- Record of the vaccine prescription sent by the treating physician or the GP of the referent

Only confirmed vaccines reported by the patient are considered for 'definite exposure' (see further below) in the main analysis of the study. Thus 100% of definite exposure to vaccines used in the main analysis will be confirmed by at least one objective source.

4.1.4. Spontaneously reported drugs

Patients are instructed to report all drugs taken in the two years previous to the index date, whether they were obtained by prescription, over-the-counter or from the family pharmacy, even if they do not appear in the drug list of the interview guide.

- Patients are invited to remember OTC, homeopathic, phytotherapeutic, traditional medicines, pharmacists' preparations and other types of medications that they may have been taking.
- Hospital medications spontaneously reported by the patient are recorded.

4.1.5. Records of medical prescriptions

AID Cases: The treating physician of cases recruited is tentatively identified by the specialist who recruits the patient into PGRx. Or during the interview of the case Attempts are made (with the consent of the patient) to contact this physician and to obtain information on prescriptions and chronic health conditions of the patients over the previous two years. This is usually successful for 50% of the cases in PGRx.

Referents: The PGRx GPs are asked to transmit extracts of the patients' electronic records for the drug prescriptions over the previous two years. Approximately 90% of them usually do so in an exploitable way.

4.2 Index date

4.2.1. Definition of index date

The index date is the date before which drug use may be considered as exposure and after which drug use is considered as non exposure.

Within a given case-referent set, the index date is the reported date of the first clinical sign evocative of the disease in the case; it is applied to all matched referents of the set.

4.2.2. Ascertainment of the index date

The index date is ascertained by:

- The date of the first symptoms reported by the recruiting physician in the medical form of the case;

- The date of the first symptoms which led to a contact with a physician (GP, specialist or hospital), reported by the case patient during the telephone interview. During this interview, it is tempted to trace back the history of the event with the patient.

The earliest of these dates will be used as the principal index date for the study if they are not more than 1 month apart. If the difference is longer the expert review panel will decide of the retained index date of the case, blind on exposure.

4.3. Time windows at risk

4.3.1. Cervarix® vaccination

- The full vaccination with Cervarix® requires 3 shots over a period of 6 months (T0 and ideally T1 and T6, with 1 month minimum between any two shots).
- Each shot is considered as a 'vaccine use'.
- Exposure is defined as the presence of a vaccine use during the time-window considered at risk for developing the event (see below).

4.3.2. Risk associated with each shot

The following assumptions have been retained for the main analysis:

a) A user may be a person receiving any one shot or the entirety of the Cervarix® vaccination during the at risk time window :
b) The risk does not vary according to the number of shots received.
c) The risk does not vary according to the rank of the shot
d) After a given shot, and during the time considered at risk, the instantaneous risk or 'hazard' is constant

4.3.3. Mortal & immortal times

Table 3 presents the time-windows considered at risk or not at risk for the study. It is based on the following definitions or mortal and immortal times (Miettinen *et al.*, 1989):

1) *The initial 'immortal' time window*: the time following a contemplated shot during which an event, if it occurred, could not be considered as resulting from this contemplated use and should consequently be considered as "unexposed" if no relevant previous shot (as described just below) had occurred.

2) *The time at risk after vaccination or "mortal time"*: the time after the initial immortal time window, during which an event, if it occurred, could theoretically be attributable to a contemplated shot of the vaccination and should consequently be considered as "exposed". This period of time applies to each vaccine use (shot)

Mortal times of 24 months, 6 months and 2 months are considered for the study of autoimmune diseases and Cervarix® using the PGRx system. Table 3 identifies which have been retained as the primary, secondary and exploratory time-windows in this study according to the Scientific Committee. These different time-windows have been selected by consensus in the absence of definitive biological or epidemiological data on this respect.

3) *The final 'immortal' time window after last drug use*: After the last of the mortal time windows defined above, the time will be considered as at no risk or "immortal".

Table 3: Time considered potentially at risk after each individual shot of the vaccine for the study of thyroiditis

	1st 24 Hours	2 months*	6 months*	24 months*	>24 months*
Risk	Immortal	Exploratory Mortal	Secondary Mortal	Primary Mortal	Immortal

* After the first 24 hours

4.4. Definite and uncertain exposure

Exposure to Cervarix® will be considered as 'Definite' only if:

- The reported use is confirmed by an objective source
- The index date for the event (in case and referents) occurred during one of the time-windows at risk (or "mortal" time windows) following of the reported shots

Other reported use of Cervarix®, including reported uses not confirmed by an objective source, confirmed reported uses occurring in one of the immortal time windows and vaccine prescription records not reported by patients, whatever the time window, will be considered as "uncertain exposures to Cervarix®" and controlled for in the analysis (no odds ratios to be published).

5. Co-morbidities and risk factors

Information is recorded for the control of confounding as well as for performing interaction analyses:

5.1. Comorbidities

The following comorbidities are recorded:

- Chronic co-morbidities: documented with the list described with Exhibit 1A (Appendix 1). Co-morbidities reported spontaneously are systematically organised. Both sources allow classification that is consistent with the International Classification of Diseases 9th revision. Further coding is performed by trained medical archivists at PGRx when necessary.
- Past medical history in the previous two years
  - Review of 19 categories of morbid conditions
  - Number of visits to a physician in the previous year
  - Hospitalisations

5.2. Risk factors

Table 4 lists the risk factors considered *a priori* for the study.

Table 4: Risk factors considered a priori for the study of thyroiditis

Risk factors considered a priori
- Family history of autoimmune disorder (1st degree) - Geographical origin - Recent pregnancy - Smoking - Number of vaccines received

6. Procedures for the minimization of biases in data collection and management

6.1. Practices and Procedures

PGRx complies with the Good Pharmacoepidemiological Practices (GPP) issued by the International Society for PharmacoEpidemiology (ISPE) revised in 2004 (http//www.pharmacoepi.org/resources/guidelines_08027.cfm). The PGRx Standard Operating Procedures are applied, both to data collection and data management.

6.2. Minimisation of selection bias

Several techniques are used to limit and/or assess the extent of this potential bias:

Recruiting centres are instructed to report all cases to PGRx, whatever their exposure, during their time of participation in the system. External sources of information on the recruitment of patients are sought for in each centre. The number of patients included is compared to the expected number in each centre and reasons for deviations are discussed with investigators. The sites recruiting autoimmune disorders are visited very frequently (on a bi-monthly basis on average) by trained clinical research assistants to elicit reporting and try and document non reported cases.

6.3. Minimisation of information bias

6.3.1. Classification of case/referent status

- The exclusion of the occurrence of a previous thyroiditis diagnosis in cases and referents is achieved through 2 sources (physician and patient). The data collected on the selected referents will further be checked for the presence of elements in favour of endocrine disorders (co-morbidities, personal histories, symptoms spontaneously reported, drug use). Any referent with a possible or definite antecedent or presence of thyroiditis will be excluded from the set of referents.

6.3.2. Classification of exposure status

- 100% of exposure considered in the study is uses confirmed with an objective source as described in section 4.4.2.

- Index date: two sources of information are used to define the index date (the medical form filled by the physician and the interview of the patient).

6.4. Information collected on potential confounders

Information on family history of AID is especially collected for this study, as patients with a family history of auto-immune disease may be at a lower probability of being vaccinated while having a higher probability of developing the disease and/or the vaccine may interact with a familial predisposition to develop the disease. It is however anticipated that the frequency of this risk factor in referents is expected to be very low.

7. Statistical issues

7.1. Sample size

7.1.1. Recruitment expected in PGRx

Table 5 identifies the number of female cases 14-26 years old with the disease expected per year and for 3 years in PGRx and the corresponding number of referents on average. This number was first derived from the declarations of the investigators of the first centres entered in the PGRx system and is consistent with the actual recruitment reported in Appendix A2.

Table 5 also reports the date of first case recruitment and the expected date of termination (3 years after).

Table 5: Expected number of cases and referents for thyroiditis in PGRx and dates of start and of expected end of the study

Females 14-26 y.o Cases/.y. N	Females 14-26 y.o Cases/. 3 y. N	Matched Referents 3 y. N	Date 1st effective surveillance	Expected Date end
15	45	180		

7.2. Exposure estimation

7.2.1. Expected rates of exposure

For the time-window of 24 months, the mean expected rate of exposure in the referents is estimated at xxxx%.

Table 6: Estimated exposure to the vaccine used for power calculation according to the time window considered

	24 months
Expected % of referents exposed in the time-window	

7.3. Odds ratios detectable

7.3.1. Direction of effect

The scientific committee has considered that some vaccines may as well decrease or increase the risk of auto-immune disease. Statistics are consequently presented as two-sided.

Tables 7 presents the odds ratio ascertainable as different from unity with 80% power and 95% confidence (2-sided)using the expected sample of cases and referents expected to be recruited over 3 years according to Table 5, and using the exposure rate displayed in Table 6 for the primary mortal time defined in Table 4 for this study.
Estimates have been made using StatCalc® in EpiInfo®, Version 6 and verified with the formula provided in Schlesselman[6]. Both estimates are close enough.

Table 7. Odds ratio (OR) detectable in the primary analysis for the risk of thyroiditis in vaccine users

14-26 y.o Expected Female Cases* N	14-26 y.o Expected Referents N	Expected exposure of referents†	OR detected # StatCalc®	OR detected # Schlesselman formula
45	180			

* 3 years recruitment
# With 95% 2-sided confidence and 80% power
†Primary time window at risk of 24 months after each shot (mortal time),

8. General Analytical Plan

Analysis will be performed with the SAS 9.1.3 Service Pack 4, Windows version 5.1.2600 (copyright © 2003 SAS Institute Inc. Cary, NC 2713, USA) or a more recent version if it becomes available.

8.1. Descriptive Analysis

Cases and referents will be described for the variables listed in the previous sections of this protocol, including socio-demographics (age, region, ethnicity, socio-economic status) clinical features (according to Table 2); presence of severe co-morbidities; individual risk factors (see below); exposure to Cervarix® vaccine (by time-windows), separately by age (<18; ≥ 18 y.o) and case/referent status.

[6] Case-control studies: Design, Conduct, Analysis. New-York: Oxford University Press, 1982. 354pp

8.2. Univariate comparisons

8.2.1. Risk factors to be considered a priori

The distribution of the risk factors listed in Table 4 plus other risk factors that may arise in the literature and are retained by the Scientific Committee before the analysis (if available in PGRx) will be described in cases and referents.

8.2.2. Risk factors to be listed *a posteriori*

Classes of drugs and categories of co-morbid conditions will be tested for their difference in distribution between cases and referents. Any of these variables associated with case/referent status with a $p<0.1$ will be retained for the main multivariate model analysis.

8.2.3. Assessment of potentially strong confounders or risk factors

Matched odds ratios for exposure will be compared between sets of subjects presenting with and without the confounders identified *a priori* and *a posteriori* The position of the observed odds ratios will be examined (within or outside the interval) and decision taken on the analysis. If the number of cases and referents with the potentially strong confounders do not allow for an adequate control of their influence through modelling, the sample of sets used in the modelling for the sensitivity analysis will be censored of those with at least one subject presenting with the confounder. – The same approach will be applied by the comparison of odds ratios for exposure to the vaccine in strata of 25^{th}, 50^{th}, 75^{th}, 100^{th} percentile of 'multivariate confounding scores'.

8.3. Modelling and Analysis using Multiple variables

8.3.1. Main model

All retained risk factors identified will be used in a multiple modelling of the risk of thyroiditis associated with exposure to Cervarix®. A priori suspected and risk factors identified a posteriori from the univariate analyses will be controlled for. The analysis will be also controlled for the use of another HPV vaccine reimbursed in France[7]. The risk associated with the number of shots received will be assessed.

Results will be presented as adjusted odds ratios with their 95% confidence intervals (two-sided, estimated with 80% power).

The model considered is the conditional logistic regression for the assessment of relative risks through odds ratios.

[7] Gardasil®

8.4. Analysis performed for the identification of biases

A series of descriptive analyses will be performed to identify potential biases. No results will be reported as arising from these analyses. Statistical tests will be applied when possible to help in the interpretation of potential differences or interactions.

8.4.1. Selection bias

- Participant patients will be compared to non-participants on age, time and centre.

- Centres will be described for their recruitment, percentage of rejected cases, and the mean exposure to Cervarix® in the patients reported. Face comparisons between centres will be made on the mean exposure prevalence. Cases rejected and interviewed will be compared to retained cases and to referents for their use of Cervarix®

Decision will be taken by the Scientific Committee to retain or reject centres with obvious outlying results in the above analyses.

8.4.2. Information bias

- Diagnostic bias:

Referents identified with any elements in favour of a disorder consistent with or evocative of the disease, including its *forme fruste*, will be excluded from the set of referents. Exposure to vaccine reported in the patients' interviews will be compared to prescriptions recorded by the physicians. A separate study of the validity of exposure ascertainment in PGRx is conducted. Its results will be presented to the Scientific Committee and potential consequences for the study protocol considered before the final analysis

8.5. Timing of the analysis

8.5.1. Planned analysis

The main analysis will be performed at 36 months after the first index case included in the PGRx system. This delay may be extended if necessary to achieve the recruitment of the sample size displayed in Table 5.

8.5.2. Unplanned analysis

An unplanned analysis may be performed before the end of the study:

- At the request of the Health Authorities and with the formal agreement of the Cervarix Scientific Committee.
- Or at the request of the Cervarix Scientific Committee, justified by a possible alert identified in the literature or through pharmacoviligance reports.

This unplanned analysis will use all the methods described in the analytical plan and will be applied to the sets of cases and referents satisfactorily documented and to the data considered as consolidated at that time.

Whatever the results of this unplanned analysis, the study will be pursued until the planned completion since, according to the assumption of this study; cases may arise as far as 24 months after exposure.

9. Discussion of the general study methodology

9.1. Limits of observational research

Biases associated with medical practice
This study presents limitations associated with observational research such as possible indication bias for the vaccine and preferential diagnosis in exposed. While the first one is more likely to bias the results towards a lesser risk associated with vaccination in the present context, the second may act in the reverse direction. These two biases are associated with medical practice rather than with the study methods itself and may also be present in so-called 'record-linkage' or medical database research as they pertain to the nature of medical activity. Note than they are also present in unblinded cohort studies. Only double blind randomised clinical trials may completely eliminate their effect, when the blind is not actually broken in practice. The feasibility of such trials to assess the incidence of a rare disease is very low (published trials did not actually have the power to do so). The ethical justification of larger trials in this respect is debatable in the absence of any alert.

The very high specificity of the diagnosis and the potential comparisons between the various degrees of certainty in the diagnosis, as well as the medical information recorded for both cases and referents will provide useful information on this respect. Documenting for a number of potential confounders such as family history of disease or behavioural confounders will help in minimizing the effect of indication bias.

9.2. Limits of field case-referent studies

As opposed to studies nested in medical or prescription databases, the field case-referent nature of recruitment raises the question of potential selection bias, *i.e.* the preferential recruitment into the study of cases associated with exposure. The selection bias of concern here is notoriety bias where cases exposed to Cervarix® would be more likely to be reported than other, non-Cervarix®, patients. This would bias the results away from the null. The PGRx methodology, by collecting cases systematically in the absence of any alert, and announcing the surveillance of *ca.* 300 drugs to clinicians, limits the potential extent of this bias as compared to ad hoc case-referent studies. Important efforts are devoted at minimising this bias (section 7.2) and assessing its potential magnitude (section 9.4.1).

Note that the case-referent methodology allows for a volume of recruitment which is possible only with very large databases, especially if only definite cases of the disease are considered.

9.3. Nature of referents

The use of physicians as the source of referents in PGRx is a compromise between population-based referents and hospital based referents. They have been successfully used in pharmacoepidemiology (Abenhaim, 1996). Sampling of population-based referents may provide more valid estimates of exposure and behavioural risk factors than sampling of patients visiting physicians, but they are less likely to provide valid information on co-morbidities, antecedents and medical risk factors than the data collected through physicians. Also, the objective source of information on vaccination through medical records may be of great help in this instance. Hospital-based referents are frequently used because of the convenience of sampling and on the assumption that they may help control for referential biases. They are however frequently associated with exposure and reporting biases, as well as with actual referential bias. The pool of potential referents recruited in PGRx is less subject to this later bias while offering a convenient source of sampling of referents to be matched to the cases.

The matching of referents to cases on the number of visits to physician limits the extent of a bias associated with increased opportunity to exposure which may be feared with physician-based referents as opposed to population-based referents (although this bias is less likely to play a role in the contemplated age groups here). Another, to a certain extent symmetrical, concern is the so-called ‘overmatching’. Overmatching is not a validity bias but may impair the efficiency of a study.

9.4. Information biases

For the case/referent status, the specificity achieved in PGRx for the diagnosis of cases and also for the exclusion of referents with history of the disease at hand is very high as compared to any systematic collection of data available, especially in comparison to so-called ‘record-linkage’ databases or usual medical databases.

The infamous ‘recall bias’ feared in studies using retrospective interviews is limited in this study as 100% of reported exposure will have to be based on objective information or documentation. The use of two sources of data on drug use (patients and physicians) helps in this process. A separate validation study of the validity of the ascertainment of exposure in PGRx is planned. Its results will be made available to the Scientific Committee before the final analysis is conducted.

A comparison of observed exposure of referents to expected exposures based on the data available at the end of the study on the reimbursement of vaccination will allow for the documentation of these biases if they exist. A crude case-population comparison of exposure will be done using these reimbursement data for the assessment of the exposure of the base population and the results compared with those obtained in this case-referent study.

9.5. Residual confounding

Few potentially strong risk factors are known for the diseases at hand (personal and familial history of auto-immune disorders, the existence of severe chronic co-morbidities, ethnicity, and some drugs). Whether they may interact with vaccination and/or represent potential confounders of an association is unknown. Personal or familial history of AID is thought to lower the probability of vaccination, but no data is available on this subject. All these variables are expected to have low or very low prevalence in the sample.
Despite the statistical procedures listed above, in addition to the matching of referents to cases, to minimize and control for the effect of potential confounders, it is always possible that some residual confounding may still exist at the end of the study. The potential magnitude of this residual confounding effect and its likelihood to explain any potential observation or association will be discussed based,

10. Timelines & Reports

Item	Date
Network of PGRx central demyelination Centres	Done On-going for paediatric centres
Recruitment of 1st case	
Recruitment of potential Referents	On-going
Finalisation of PGRx autoimmune thyroiditis and Graves disease -Cervarix® protocol	May 2009
1st Annual Descriptive report and blind analysis	
2nd Annual Descriptive report and blind analysis	
Final PGRx autoimmune thyroiditis and Graves disease -Cervarix® Study report	

Recruitment reports are issued every month. Descriptive reports provide data on all the variables listed in the document.

Persons in charge of the analysis and reports

The statistical analysis and reports will be conducted under the supervision of Profs. [redacted], [redacted], [redacted], [redacted] and Dr [redacted].

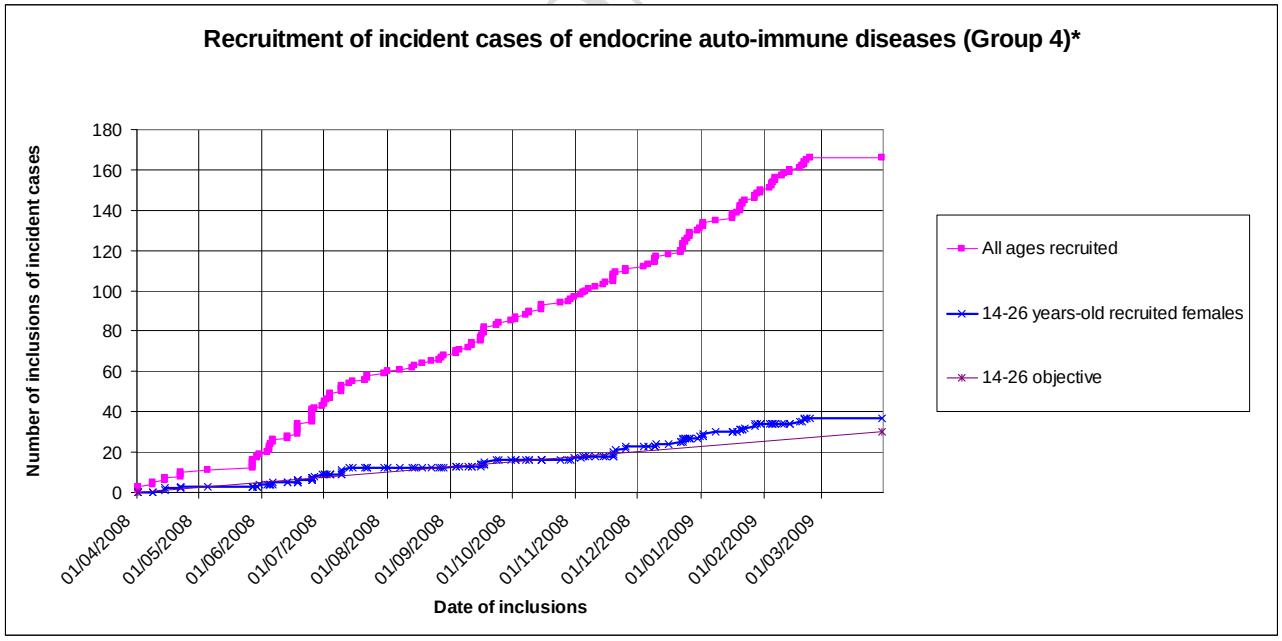
Appendix 1: Exhibit 1A: PGRx Information System General Methodology

Appendix 2: Recruitment of autoimmune thyroiditis and Graves disease in PGRx

Table A2.1 Recruitment of cases of endocrine disorders evocative of autoimmune thyroiditis and Graves disease in the PGRx System as of March 2, 2009

	Date of first inclusion	Participating centers N	Cases (all age) N	Recruited female cases 14-26 y.o. N	Target recruitment Females cases 14-26 y.-o.	
					per year N	3 years N
Group 4 (type 1 diabetes, autoimmune thyroiditis, Gaves'disease)			166	37	30	90
Cases of disorders evocative of Autoimmune thyroiditis	22/04/2008	15	36	4	-	-
Cases of disorders evocative of Graves'disease	22/04/2008	19	53	13	-	-

Figure A2.1 Recruitment of cases of endocrine disorders evocative of autoimmune thyroiditis and Graves disease in the PGRx System as of March 2, 2009



*Group 4 : Incident cases of type 1 diabetes, auto-immune thyroiditis and Graves'disease.

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PGRx

STUDY OF CERVARIX® & CENTRAL DEMYELINATION

USING THE PGRx INFORMATION SYSTEM

VERSION 2

February 26, 2009

PGRx
Centre for Risk Research Inc.
LA-SER sarl

Table of content

NOTE

This protocol is provided with the *Exhibit 1A: The general methodology of PGRx (Appendix 1),* which applies to all studies conducted with the PGRx Information System.

The Exhibit 1A is up-dated on a yearly basis by the International Scientific Board of PGRx, taking into account evolution of the System resulting form the actual conduct of data collection and studies. For the purpose of the study of Cervarix®, in the case of any difference or apparent discrepancies between the Exhibit 1A and the present Protocol, it is this Protocol that prevails at any time.

1. Introduction

1.1. Overview of the study

1.1.1. Study Objective

The objective of the study is to assess whether the use of Cervarix® is associated with a modified risk of central demyelination ("the disease").

1.1.2 .General inclusion & exclusion criteria for the cases and referents in the study

Study subjects are cases and referents from the PGRx system satisfying with the following criteria:

Inclusion criteria

- Female gender
- Age 14 to 26 years-old
- Patient residing in France (continental)
- Patient accepting to participate in the study

Exclusion criteria

- Prior reported history of the disease;
- Patient or Patient's parent cannot read the interview guide or answer a telephone interview questionnaire in French.

1.1.3. Study design

1.1.3.1. Case-control (or case-referent) methodology

This study is a systematic case-referent study. It consists in using the PGRx information system to:

a) Monitor a large number of neurology centres for the occurrence of the disease,
b) Match general practice-based controls to these cases, selected from the pool of PGRx potential referents
c) Document the previous vaccination by Cervarix® in both cases and controls,
d) Estimate the relative risk of the disease in Cervarix® vaccinated females by the odds ratio (adjusted for a series of confounders and interaction factors, including other drug use).

1.1.3.2. Rationale for the choice of the case-control design using PGRx

The case-control (or case-referent) methodology is the design of choice for the study of rare events, such as autoimmune disorders in epidemiology. Its power is not affected by the small incidence of diseases and has proved efficient in pharmacoepidemiology (Abenhaim, 1996). When based on field collection of data, this design allows for the documentation of individual risk factors.

Ad hoc case-control studies in pharmacoepidemiology are however cumbersome and require a large amount of work and procedure to control for the various sources of biases (Wacholder, 1992).

The PGRx Information System (PGRx) has been developed to minimise these difficulties and biases.

PGRx is a systematisation of the case-control referent (or case-referent, Miettinen, 1976) methodology. It is available in France and Canada. It addresses most of the concerns usually raised with ad hoc case-control studies. Autoimmune disorders have been listed as conditions of interests for PGRx since the inception of the system.

1.2. Overview of the PGRx Information System (PGRx)

1.2.1. General Description and Methods of PGRx[1]

The PGRx general methodology is described in PGRx Database & Information System Exhibit 1 A – *General Methodology*.

In brief, PGRx has been developed in response to the paucity of databases or information systems available for the study of rare diseases and/or delayed adverse events associated to medicines, with sufficient power and specificity on disease diagnosis and individual risk factors. It operates since 2007.

The system prospectively and routinely collects information on:

1) Cases[2] of a dozen diseases[3] collected in more than two hundred specialized referral centres and validated through a series of procedures. The collection ensures for a control of selection bias;
2) A large pool of general practice-based potential referents from which controls or referents can be selected and matched to cases of diseases under study. Matching can be made on calendar time, age, gender, region and any other relevant parameter available and can be individual matching or frequency-matching. The selection of referents is performed in such a way to ensure a fair representation of the population-time experience with the drugs studied in the relevant source populations,
3) 300 drugs (including vaccines) documented through: (i) guided telephone interviews and (ii) medical prescription records (in a sample of either treating physicians'

[1] See Exhibit 1A attached

[2] In the PGRx DIS, cases are defined as adverse *events* and not necessarily adverse *reactions*. No hypothesis is made *a priori* on the causality of the event (as opposed to spontaneous reports of adverse reactions frequently reported in pharmacovigilance systems).

[3] The diseases routinely surveyed in the PGRx Information System are presently: myocardial infarction, multiple sclerosis (first central demyelination), Guillain-Barré syndrome, lupus erythematosus, cutaneous lupus, myositis and dermatomyositis, inflammatory arthritis, unspecified connectivitis, type I diabetes, thyroiditis, thrombocytopenia, suicide attempts, torsade de pointes and acute liver injuries. First results have been presented in various conferences (ICPE, 2008; ISOP, 2008).

computerized prescriptions or treating physician's reports). All new molecules, products targeted in risk management plans and up to 24 products used by more than 250 000 persons in the country are listed, including most vaccines. Cervarix® is one of the vaccines routinely studied. The lists of drug or vaccines specifically studied at the different dates are provided with the Exhibit 1A.

4) Individual behavioural, medical and family risk factors: smoking, alcohol use, physical activity, occupation, chronic co-morbidities, familial history of certain diseases, others.

For each AID a PGRx Scientific Committee, called PGRx Pathology Specific Scientific Committee (see Exhibit 1A), has been organised and the general methodology for the study of each AID in PGRx has been developed under the auspices of those committees. The collection of data in PGRx follows the criteria developed by these committees. Out of these collected data, the scientific committee for each individual study (e.g. the one for Cervarix® and autoimmune disorders assembled by the manufacturer) may select those that it considers appropriate for its study.

1.2.2. PGRx Network for Autoimmune disease

A network of centres treating patients for these diseases has been assembled to participate in the PGRx Database and Information System.

Table A2.1 and Figure A2.1 in the Appendix 2 reports the number of centres participating in the collection of cases of Central Demyelination, the date of start of the surveillance of this disease in the system, the number of cases recruited so far by age group (l4-26 years old, all age groups) and the objectives of recruitment per year in the System.

1.3. Overview of the literature

1.3.1 Epidemiology of central demyelination

Worldwide, the distribution of multiple sclerosis (MS) is not uniform. A north -to- south decreasing gradient of MS frequency has been observed (Kurtzke 1979, Visscher 1977, Baum 1981, Minden 1993, Rosati 1994, Miller 1990, Mcleod 1994). High prevalence areas (about 100 per 100 000 inhabitants) such as north of Europe and north of the USA, medium prevalence areas (about 50 per 100 000 inhabitants) such as Eastern Europe and Western and Southern USA, and low prevalence areas (less than 20 per 100 000 inhabitants) such as Mediterranean countries were identified. MS is exceptional in black people in Africa (Kurtzke Ann Neurol 1980, Van der Mei et al Neuroepi 2001, Zivadinov et al Neuroepidemiology 2003).

The incidence of MS varies from country to country, and changes with time. In several countries, the incidence seems increase either because diagnoses are more easily done with MRI or because of environmental or socioeconomic factors such as improved living conditions, development of health care or because of better epidemiological census. For

example, in Germany the mean annual incidence has increased from 2.6/10^5/year to 4.6/10^5/year between 1969 and 1989 (Poser, Kurtzke et al. 1989). In Spain, the incidence has increased between 1984-1993 and 1994-2003 from 3/10^5/year to 4.6/10^5/year (Modrego and Pina 2003). In Padova Italy, the incidence has increased from 2.2/10^5/year to 3.9/10^5/year and to 4.2/10^5/year over the period 1980-1989, 1990-1994, 1994-1999 respectively (Ranzato, Perini et al. 2003).
In France, several studies (Alperovitch et al, 1982; Berr et al., 1989; Confavreux et al., 1987; Gallou et al. 1983; Spieser-Stoecklin, 1987; Kurtzke et Delasnerie-Lauprêtre, 1996) have estimated a prevalence of MS about 40 per 100.000 inhabitants that seems to increase from south-western to north-eastern. The prevalence in Chalon sur Saone, in a 1984 study by Confavreux was estimated at about 58.5 while in Avignon it was 48.6 per 100000 inhabitants (Confavreux, Darchy et al. 1987). To estimate the prevalence of MS more precisely using the same methodology all over France, a national study was carried out in 1986 in which MS patients were invited to take part in a survey after a television programme. The Prevalence was evaluated at between 30 and 40 / 100000. In the same period, the national public health insurance system inventory retrospectively censored patients declared as having MS in 1994. The prevalence of MS among patients affiliated to the CPAM was between 37 and 47 / 100000. Incidence rate has been estimated at 4.3 per 100.000 inhabitants per year (Moreau et al, 2000).

1.3.2. Risk factors associated with central demyelination

From these data, at least two series of risk factors have been suggested: environmental factors (Debouverie et al Neurology 2007, Cabre et al Brain 2005, Dean et al J Neurol 1997, Gale et al Prog Neurobiol 1995, Hammond et al Brain 2000, Kurtzke et al Ann Neurol 1980, Delasnerie-Lauprêtre et al Neuroepidemiology 1990) and genetic factors (Debouverie et al Neurology 2007, Broadley et al. Brain 2000, Midgard et al. Acta Neurol Scand 1996, Poser et al 2006 Clin Neurol Neurosurg). Differences in the distribution of MS through the world and the latitude gradient observed in certain countries have not yet been explained. Genetic factors alone cannot explain this phenomenon. Indeed, concordance between monozygotic twins is 25 % and 5 % for dizygotic twins (Sadovnick, Armstrong et al. 1993). Thus several environmental factors have been studied. Among them an inverse relationship between hours of sunshine and the prevalence of MS has been found (Acheson, Bachrach et al. 1960; van der Mei, Ponsonby et al. 2001). Exposure to toxic substances such as organic solvents and cigarette smoking (Riise, Nortvedt et al. 2003; Hernan, Jick et al. 2005) may play a role. Rural residence seems to correlate with a higher risk of MS (Warren, Cockerill et al. 1991). There is also a hygiene hypothesis in that infection with Epstein Barr Virus at a late age increases the risk of MS (Bach 2002; Ascherio and Munger 2007). Socioeconomic factors that correlate with a higher risk of MS such as a high education level (Russell 1971), a large number of children, consumption of diets rich in animal saturated fats (Esparza, Sasaki et al. 1995) have also been studied. Another factor that is still under discussion is the role that vaccinations in peculiar hepatitis B vaccine could play.

1.4. Drugs allegedly associated with central demyelination

1.4.1. All drugs

Some studies and case reports have questioned the relationship between MS, exacerbation of MS or central demyelination and drugs including therapeutics and pharmaceuticals. Drugs studied are vaccines and essentially anti-hepatitis B vaccine, anti-TNFα and anecdotal others. Rare observations of acute disseminated encephalomyelitis after diphtheria, pertussis, tetanus vaccination or influenza vaccination have also been reported (see Disease-specific references page 34).

1.4.2. Time windows at risk used in studies

In the above mentioned studies, time-windows varying from 60 days to several years have been used for the study of the relation between central demyelination and vaccines.

Table 1 summarizes the main features stemming from the literature review.

Table 1: Epidemiology of Central Demyelination and data stemming the literature review

Socio-demographics (age, gender)	20 - 40 years old 2 women / 1 man
Incidence	France: $4.3/10^5$ inhabitants / year Germany: $4.6/10^5$ inhabitants /year Spain : $4.6/10^5$ inhabitants /year Italy: $4.2/10^5$ inhabitants /year
Prevalence	France: from 30 to 58.5 / 100000
Time to event tested	≥60 days, 61 to 180 days, ≤6 months, ≤1 year, ≥1 year, ≤2 years, ≥2 years, ≤ 3 years, ≥3 years

2. Cases

2.1. Populations for case recruitment

2.1.1. Source population

The source population for the study is made of patients who are:

- Hospitalised for the occurrence of the disease in one of the centres participating in the PGRx Network for AID;
- Or addressed to a centre participating in the PGRx Network for the diagnosis or the management of the disease.

2.1.2. Study population for cases

The study population is made of patients from the source population above who are:

- Incident cases patients presenting with the set of symptoms and signs retained for the diagnosis of the disease defined further below;
- Reported in PGRx by the specialist participating in PGRx;
- Recruited within 12 months after the date of the occurrence of the first clinical sign identified by a physician;
- Meeting all inclusion and exclusion criteria for the study.

2.2. Identification of cases

2.2.1 PGRx Centres for the recruitment of cases

Centres eligible to participate to the PGRx Network for the recruitment of contemplated events are [redacted] and [redacted] that have a specialized unit or a health care network for the management of this disease. These units are selected on the volume of incident cases of the disease that they treat per year.

2.2.2 Recruitment of cases

Participation must be proposed to all consecutive patients who respond to inclusion and exclusion criteria for the event in the PGRx participating centres.

2.2.3. Web entry

Each specialist recruiting a case fills out a medical data form directly on a secured Internet data entry system on which they have been individually provided with a login and a password.

2.3. Information collected

2.3.1. Medical form[4]

General information

When the case is included the following data are collected by the recruiting specialist:

- Date of the consultation;
- First and last name, date of birth and gender of the patient;
- Inclusion and exclusion criteria;
- Name and address or phone number of the usual treating general practitioner of the case recruited.

Medical information

The following sections of the medical form are used for case ascertainment:

- Date of the first symptoms evocative of the disease
- Description of the symptoms and signs of the first evocative episode
- Description of biological and imaging findings (if appropriate and/or available)
- Current and previous chronic diseases

[4] The web-based Clinical Research Forms are available for consultation to interested parties upon request.

- Familial history (1st degree) of autoimmune disorders.
- Recent pregnancy or surgery
- Elements of differential diagnosis

2.4. Case definition

Cases for the study are *incident cases* (i.e. newly diagnosed patients) reported as having occurred in the previous twelve months before the recruitment consultation.

2.4.1 Case ascertainment

Cases will be validated by an independent expert review panel blind to the medications and vaccinations status. The panel will review the medical forms of all the cases recruited. At the end of their review of each case, the expert review panel will qualify the cases as:

a) Definite
b) Possible
c) Rejected

Definite cases only will be used in the main analysis. Possible cases may be used for potential "unplanned analysis" (see further below). Rejected cases are used for the identification of biases (see special section "Identification of biases" further below). The diagnostic criteria to classify the patients are described below; they have been adapted from internationally accepted definitions to allow for the recruitment of cases at the early stages of the disease at hand and to better take into account the age groups concerned by the vaccination.

Every year, PGRx centres are contacted to assess the potential evolution of the diagnosis of the cases reported previously. Any change in the diagnosis of the case is recorded and the case is reclassified as definite, possible or rejected. .

2.4.2 General definition of cases for the study

Central demyelination cases are defined as patients:

- ✓ With a neurological episode evocative of central demyelination involving the optic nerve and / or the spinal cord and / or the brain and / or the brainstem and / or the cerebellum, lasting more than 24 hours and lacking any alternative explanation;
- ✓ With no reported previous history of neurological or visual event suggestive of possible central demyelination.

Other possible diagnosis or conditions producing clinical, biological or imaging abnormalities that may mimic central demyelination, including traumatic brain injury, meningoencephalitis and tertiary Lyme disease are reminded in the forms. The patient presenting with these conditions are excluded.

2.4.3. Definition of definite possible and rejected cases

Central demyelination of the optic nerve

Cases of central demyelination of the optic nerve are ascertained by the following algorithm, simplified in table 2A:

a) Definite optic nerve cases:

A case is considered as definite for the study of the association between Cervarix® and central demyelination of the optic nerve when there is:

- An optic neuritis defined as a visual loss and a fundoscopic examination normal or evidencing a minor papillary oedema
- With:
  - an encephalic MRI showing T2-weighted hyper intense lesions or a T1-weighted gadolinium enhancing lesion
  - OR a typical CSF findings

b) Possible optic nerve cases

A case is considered as possible when there is:

- an optic neuritis defined as visual loss and a fundoscopic examination normal or evidencing a minor papillary oedema
- and an encephalic MRI reported as normal, excluding another diagnosis

Central demyelination of the spinal cord

Cases of central demyelination of the spinal cord will be ascertained by the following algorithm, simplified in table 2B:

a) Definite spinal cord cases:

A case is considered as definite for the study when there is:

- A myelitis defined as a clinical medullar syndrome
- With:
  - A spinal or an encephalic MRI reported as showing a T1-weighted gadolinium enhancing lesion or T2-weighted hyperintense lesions
  - OR typical CSF findings

b) Possible spinal cord cases

A case is considered as possible when there is:

- A myelitis defined as a clinical medullar syndrome
- A spinal or encephalic MRI reported as showing hyperintense lesions.

Central demyelination involving the brain, the brainstem and the cerebellum

Cases of events suggestive of central demyelination involving the brain will be ascertained by the following algorithm, simplified in table 2B:

a) Definite brain, brainstem or cerebellum cases:

A case is considered as definite when there are:

- Monofocal or multifocal neurologic symptoms and signs

- An encephalic MRI reported as showing:
  - a T1-weighted gadolinium enhancing lesion
  - or T2-weighted hyperintense lesions

b) Possible brain, brainstem or cerebellum cases:

A case is considered as possible when there are:

- Monofocal or multifocal neurologic symptoms and signs
- An encephalic MRI reported as showing hyperintense lesions

2.4.4. Summary tables for case definition

Table 2A: Case definitions for the study of central demyelination of the optic nerve

	Clinical presentation		MRI or CSF findings
Definite cases	Optic neuritis	**AND**	MRI showing T2-weighted hyperintense lesions OR a T1-weighted gadolinium enhancing lesion on the affected optic nerve **OR** typical CSF findings
Possible cases	Optic neuritis	**AND**	Encephalic MRI normal (excluding another diagnosis)

Table 2B: Case definitions for the study of central demyelination of the spinal cord or the brain, the brainstem and the cerebellum

	Clinical presentation		MRI or CSF findings
Definite cases	**Spinal cord**: Myelitis	**AND**	Spinal or encephalic MRI with T1-weighted gadolinium enhancing lesion or T2-weighted hyperintense lesions **OR** typical CSF findings
	Brain, brainstem or cerebellum: monofocal or multifocal neurologic signs of progressive evolution	**AND**	Encephalic MRI as above
Possible cases	Myelitis or monofocal or multifocal neurologic signs	**AND**	A spinal or encephalic MRI reported as showing hyperintense lesions

3. Referents and matching rules

3.1. Definition of referents

Referents to the cases are patients selected from the pool of potential referents reported by physicians in general practice, who meet the same general inclusion and exclusion criteria as the cases.

Patients with no reported previous history of the disease considered for the cases, as reported by themselves or their physician will be selected from the pool of potential referents in the PGRx system to serve as referents to cases.

3.2. Recruitment of referents

3.2.1. PGRx Pool of Potential Referents

A network of *ca.* two hundred and fifty (250) general practitioners (GPs) enrols a pool of *ca.* 2,000 referents each year in the PGRx database and Information system. Each GP in the network is asked to recruit 1 male and 1 female in the following age categories: 18-34, 35-49, 50-64, 65-79 (age strata may be more detailed or doubled if needed).

For the purpose of the study of autoimmune disorders in younger age groups, voluntary GPs have been asked to also recruit patients 14 to 17 y.o (2 males and 2 females per year of age and by physician).

Physicians who recruit potential referents are requested to fill an electronic medical data form that includes medical information on the patient (current prescriptions with their motives and diagnoses, chronic diseases, medical risk factors and some biological data).

Physicians obtain consent of eligible patients to participate and transfer the coordinates of the patients to the PGRx staff for the telephone interview, through a secured Internet connection.

PGRx GPs are enrolled for the recruitment of referents in all telephone regions of the country. Physicians are randomly selected from a general list of practicing physicians in a given region. In order to be enrolled, they must have access to Internet and use computerized prescriptions. Those who agree are provided with a secured access to the PGRx system on Internet and are instructed on recruitment of consenting patients, on filling the medical data form and the electronic transfer of their computerized drug prescriptions over the previous two years.

Participating physicians are asked to recruit a set of potential referents patients one to three times a year on a rotating basis so that recruitment is not interrupted in a given region over the year. This recruitment spread out overtime facilitates matching of selected referents to cases on calendar time.

3.2.2. Referents selected for the study of autoimmune disorders

The selection of referents from the PGRx pool of potential referents proceeds in order to apply the same inclusion and exclusion criteria as in cases.

3.3. Matching

To each case is matched at least one referent. As many referents as possible meeting the criteria for the study and allowing proper matching to case are retained. It is estimated than an average of 4 referents will be available per case with the following priority rules:

1) Date of recruitment of the cases and referents: Cases and referents are organised by trimester of recruitment in a given year (Q1 to Q4): for each matching criteria below, a

referent is looked for in the same quarter of recruitment as the case or, if none is found, in the next adjacent quarter of recruitment, and then the next one again. If no matched referent is found, the case is not retained.

2) Age: matching will be done with the following order of priority: ± 1 month, then ± 3 months; then ±6 months, then ±1 year (for age ≤ 17), then ±2 years (for age ≥ 18); if no matching referent is found to a case, the case is not retained.

3) Number of visits to a physician in the previous year (0-5, >5). If no matching referent is found to a case, this matching criterion is dropped.

4) Place of residence (region or telephone zone): cases will be match to referents of the same region, if necessary matching will be performed with referents from contiguous regions; if necessary, referents from all France are considered.

4. Drug exposure ascertainment

The ascertainment of exposure follows 3 steps:

1 – Identifying and ascertaining drugs and vaccines used in the last 2 years

2 – Defining the index date for exposure

3 – Defining the relevant time window at risk for the exposure before that index date.

A subject is considered as 'exposed' whenever a vaccine use is ascertained during the time window at risk.

4.1. Identifying drug and vaccine use

4.1.1. Sources of information

Information on drug exposure is obtained from:

A) A structured telephone interview of the patient (cases and referents) or of one of the patient's parent (see below)using:
   - an interview guide,
   - a list of 19 General Health Conditions,
   - a list of up to 20selected drugs for each General Health Condition (see below)
   - and visual photographic displays of up to 10 drug packages per General Health Conditions
   - a list of all vaccines (with up to 10 visual displays of packages)

B) Medical records obtained from the Treating Physician[5] of the cases and the PGRx GPs reporting referents:
   - Either copies of computerized medical prescriptions
   - And/or medical prescription forms filled by the treating physician

[5] To obtain reimbursement of certain health services, including drug prescribed, from the national health insurance, French patients must identify a so-called 'Treating Physician'.

For cases, the name of the treating physician and consent to contact him/her is obtained from the patient. They are contacted by the PGRx research team

Exposure is defined by a combination of the information from these two sources (see further below).

The interview is conducted by trained telephone interviewers belonging to the PGRx Call Centre specialised in pharmacoepidemiology. Patients are conducted through a list of questions. The duration of the interview is recorded. Interviews may be taped for quality control (with the information of the patient).

Consent is confirmed from the patient (case or referent), or from the patient' parent at the beginning of the interview. If the patient is minor (under 18 y.o in France), both the parent and the minor are asked to be present during the interview. The person actually interviewed is decided by the parent.

4.1.2. Drug list and drug visual display for the guided interview

The drug list used in the interview contains roughly 325 brand drug names (including *ca.* 50 vaccines, see below), with up to 20 drug names in each of the 19 General Health Conditions categories (see Exhibit 1A) ; they are selected with the following criteria (in order of selection):

- Drugs containing new active principles that have been on the market for 3 years or less.
- Drugs targeted in risk management or surveillance plans under study.
- Drugs that are used by at least 250,000 patients per year (selected in order of sales' figures)

Up to 10 photographic visual displays of drug packages are provided in the interview guide for each General Health Condition and for the vaccines (same order of selection as above).
The drug lists and drug visual displays are systematically reviewed with the patient.

The drug list and drug visual displays are renewed three times a year using the criteria mentioned above.

4.1.3. Ascertainment of vaccine use

4.1.3.1. Vaccines in the guided interview

A list of ca. 50 vaccines is provided in a special section of the interview guide and used during the telephone interview. Cervarix® is one of these vaccines.

For each Cervarix® use reported by the patient, the following information is sought for:

- The number of shots received with their date
- The availability at the patient's of evidences of the vaccination: medical prescription, health record, the vaccine package or other, and the possibility to obtain the copy of the evidence if needed
- The batch number of the reported vaccine (if the package is available to the patient or if this number is available in the health record)

- The settings of the vaccination (general practice, specialised physician settings, vaccination centres or other).

4.1.3.2. Confirmation of Cervarix® use

Reported use of Cervarix® will be considered as 'confirmed' when: reported by the patient as used with at least one of the following source of confirmation obtained:

- Vaccine batch number reported by the patient (from the drug package or his/her health record)
- Copy of the doctor's vaccine prescription or of the health record or of other evidence sent by the patient
- Record of the vaccine prescription sent by the treating physician or the GP of the referent

Only confirmed vaccines reported by the patient are considered for 'definite exposure' (see further below) in the main analysis of the study. Thus 100% of definite exposure to vaccines used in the main analysis will be confirmed by at least one objective source.

4.1.4. Spontaneously reported drugs

Patients are instructed to report all drugs taken in the two years previous to the index date, whether they were obtained by prescription, over-the-counter or from the family pharmacy, even if they do not appear in the drug list of the interview guide.

- Patients are invited to remember OTC, homeopathic, phytotherapeutic, traditional medicines, pharmacists' preparations and other types of medications that they may have been taking.
- Hospital medications spontaneously reported by the patient are recorded.

4.1.5. Records of medical prescriptions

AID Cases: The treating physician of cases recruited is tentatively identified by the specialist who recruits the patient into PGRx. Or during the interview of the case Attempts are made (with the consent of the patient) to contact this physician and to obtain information on prescriptions and chronic health conditions of the patients over the previous two years. This is usually successful for 50% of the cases in PGRx.

Referents: The PGRx GPs are asked to transmit extracts of the patients' electronic records for the drug prescriptions over the previous two years. Approximately 90% of them usually do so in an exploitable way.

4.2 Index date

4.2.1. Definition of index date

The index date is the date before which drug use may be considered as exposure and after which drug use is considered as non exposure.

Within a given case-referent set, the index date is the reported date of the first clinical sign evocative of the disease in the case; it is applied to all matched referents of the set.

4.2.2. Ascertainment of the index date

The index date is ascertained by:

- The date of the first symptoms reported by the recruiting physician in the medical form of the case;

- The date of the first symptoms which led to a contact with a physician (GP, specialist or hospital), reported by the case patient during the telephone interview. During this interview, it is tempted to trace back the history of the event with the patient.

The earliest of these dates will be used as the principal index date for the study if they are not more than 1month apart. If the difference is longer the expert review panel will decide of the retained index date of the case, blind on exposure.

4.3. Time windows at risk

4.3.1. Cervarix® vaccination

- The full vaccination with Cervarix® requires 3 shots over a period of 6 months (T0 and ideally T1 and T6, with 1 month minimum between any two shots).
- Each shot is considered as a 'vaccine use'.
- Exposure is defined as the presence of a vaccine use during the time-window considered at risk for developing the event (see below).

4.3.2. Risk associated with each shot

The following assumptions have been retained for the main analysis:

a) A user may be a person receiving any one shot or the entirety of the Cervarix® vaccination during the at risk time window.
b) The risk does not vary according to the number of shots received.
c) The risk does not vary according to the rank of the shot.
d) After a given shot, and during the time considered at risk, the instantaneous risk or 'hazard' is constant.

4.3.3. Mortal & immortal times

Table 3 presents the time-windows considered at risk or not at risk for the study. It is based on the following definitions or mortal and immortal times:

1) *The initial 'immortal' time window*: the time following a contemplated shot during which an event, if it occurred, could not be considered as resulting from this contemplated use and should consequently be considered as "unexposed" if no relevant previous shot (as described just below) had occurred.

2) *The time at risk after vaccination or "mortal time"*: the time after the initial immortal time window, during which an event, if it occurred, could theoretically be attributable to a contemplated shot of the vaccination and should consequently be considered as "exposed". This period of time applies to each vaccine use (shot)

Mortal times of 24 months, 6 months and 2 months are considered for the study of autoimmune diseases and Cervarix® using the PGRx system. Table 3 identifies which have been retained as the primary, secondary and exploratory time-windows in this study according to the Scientific Committee. These different time-windows have been selected by consensus in the absence of definitive biological or epidemiological data on this respect.

3) *The final 'immortal' time window after last drug use*: After the last of the mortal time windows defined above, the time will be considered as at no risk or "immortal".

Table 3: Time considered potentially at risk after each individual shot of the vaccine for the study of Central Demyelination

	1st 24 Hours	2 months*	6 months*	24 months*
Risk	Immortal	Exploratory Mortal	Secondary Mortal	Primary Mortal

* After the first 24 hours

It is considered that there is no final 'immortal' time window after last drug use.

4.4. Definite and uncertain exposure

Exposure to Cervarix® will be considered as 'Definite' only if:

- The reported use is confirmed by an objective source
- The index date for the event (in case and referents) occurred during one of the time-windows at risk (or "mortal" time windows) following of the reported shots

Other reported use of Cervarix®, including reported uses not confirmed by an objective source, confirmed reported uses occurring in one of the immortal time windows and vaccine prescription records not reported by patients, whatever the time window, will be considered as "uncertain exposures to Cervarix®" and controlled for in the analysis (no odds ratios to be published).

5. Co-morbidities and risk factors

Information is recorded for the control of confounding as well as for performing interaction analyses:

5.1. Comorbidities

The following comorbidities are recorded:

- Chronic co-morbidities: documented with the list described with Exhibit 1A (Appendix 1). Co-morbidities reported spontaneously are systematically organised. Both sources allow classification that is consistent with the International Classification of Diseases 9th revision. Further coding is performed by trained medical archivists at PGRx when necessary.
- Past medical history in the previous two years
    - Review of 19 categories of morbid conditions
    - Number of visits to a physician in the previous year
    - Hospitalisations

5.2. Risk factors

Table 4 lists the risk factors considered *a priori* for the study.

Table 4: Risk factors considered a priori for the study of Central Demyelination

Risk factors considered a priori
- Family history of autoimmune disorder (1st degree)
- Geographical origin
- Geographical mobility and age of mobility
- Recent pregnancy
- Recent surgery
- Smoking
- Alcohol use
- Social and professional status
- Use of Contraceptives
- Recent or prevalent Infections: (Flu-like syndromes, URTI infections, hepatitis (A, B & C), use of antibiotics and antiviral drugs, others)
- Seasonality
- Number of vaccines received

6. Procedures for the minimization of biases in data collection and management

6.1. Practices and Procedures

PGRx complies with the Good Pharmacoepidemiological Practices (GPP) issued by the International Society for PharmacoEpidemiology (ISPE) revised in 2004 (http//www.pharmacoepi.org/resources/guidelines_08027.cfm). The PGRx Standard Operating Procedures are applied, both to data collection and data management.

6.2. Minimisation of selection bias

Several techniques are used to limit and/or assess the extent of this potential bias:

Recruiting centres are instructed to report all cases to PGRx, whatever their exposure, during their time of participation in the system. External sources of information on the recruitment of patients are sought for in each centre. The number of patients included is

compared to the expected number in each centre and reasons for deviations are discussed with investigators. The sites recruiting autoimmune disorders are visited very frequently (on a bi-monthly basis on average) by trained clinical research assistants to elicit reporting and try and document non reported cases.

6.3. Minimisation of information bias

6.3.1. Classification of case/referent status

- The exclusion of the occurrence of a previous central demyelination diagnosis in cases and referents is achieved through 2 sources (physician and patient). The data collected on the selected referents will further be checked for the presence of elements in favour of neurologic disorders (co-morbidities, personal histories, symptoms spontaneously reported, drug use). Any referent with a possible or definite antecedent or presence of central demyelination will be excluded from the set of referents.

6.3.2. Classification of exposure status

- 100% of exposure considered in the study is uses confirmed with an objective source as described in section 4.4.2.

- Index date: two sources of information are used to define the index date (the medical form filled by the physician and the interview of the patient).

6.4. Information collected on potential confounders

Information on family history of AID is especially collected for this study, as patients with a family history of auto-immune disease may be at a lower probability of being vaccinated while having a higher probability of developing the disease and/or the vaccine may interact with a familial predisposition to develop the disease. It is however anticipated that the frequency of this risk factor in referents is expected to be very low.

7. Statistical issues

7.1. Sample size

7.1.1. Recruitment expected in PGRx

Table 5 identifies the number of female cases 14-26 years old with the disease expected per year and for 3 years in PGRx and the corresponding number of referents on average. This number was first derived from the declarations of the investigators of the first centres entered in the PGRx system and is consistent with the actual recruitment reported in Appendix A2.

Table 5 also reports the date of first case recruitment and the expected date of termination (3 years after).

Table 5: Expected number of cases and referents for central demyelination in PGRx and dates of start and of expected end of the study

Females 14-26 y.o Cases/.y. N	Females 14-26 y.o Cases/. 3 y. N	Matched Referents 3 y. N	Date 1st effective surveillance	Expected Date end
25	75	300	July 2008	July 2011

7.2. Exposure estimation

7.2.1. Expected rates of exposure

For the time-window of 24 months, the mean expected rate of exposure in the referents is estimated at xxx%.

Table 6: Estimated exposure to the vaccine used for power calculation according to the time window considered

	24 months
Expected % of referents exposed in the time-window	xxxx%

7.3. Odds ratios detectable

7.3.1. Direction of effect

The scientific committee has considered that some vaccines may as well decrease or increase the risk of auto-immune disease. Statistics are consequently presented as two-sided.

Tables 7 presents the odds ratio ascertainable as different from unity with 80% power and 95% confidence (2-sided) using the expected sample of cases and referents expected to be recruited over 3 years according to Table 5, and using the exposure rate displayed in Table 6 for the primary mortal time defined in Table 4 for this study.
Estimates have been made using StatCalc® in EpiInfo®, Version 6 and verified with the formula provided in Schlesselman[6]. Both estimates are close enough.

Table 7. Odds ratio (OR) detectable in the primary analysis for the risk of Central Demyelination in vaccine users

14-26 y.o Expected Female Cases* N	14-26 y.o Expected Referents N	Expected exposure of referents†	OR detected # StatCalc®	OR detected # Schlesselman formula
75	300			

* 3 years recruitment
# With 95% 2-sided confidence and 80% power
†Primary time window at risk of 24 months after each shot (mortal time),

[6] Case-control studies: Design, Conduct, Analysis. New-York: Oxford University Press, 1982. 354pp

8. General Analytical Plan

Analysis will be performed with the SAS 9.1.3 Service Pack 4, Windows version 5.1.2600 (copyright © 2003 SAS Institute Inc. Cary, NC 2713, USA) or a more recent version if it becomes available.

8.1. Descriptive Analysis

Cases and referents will be described for the variables listed in the previous sections of this protocol, including socio-demographics (age, region, ethnicity, socio-economic status) clinical features (according to Table 2); presence of severe co-morbidities; individual risk factors (see below); exposure to Cervarix® vaccine (by time-windows), separately by age (<18; ≥ 18 y.o) and case/referent status.

8.2. Univariate comparisons

8.2.1. Risk factors to be considered a priori

The distribution of the risk factors listed in Table 4 plus other risk factors that may arise in the literature and are retained by the Scientific Committee before the analysis (if available in PGRx) will be described in cases and referents.

8.2.2. Risk factors to be listed *a posteriori*

Classes of drugs and categories of co-morbid conditions will be tested for their difference in distribution between cases and referents. Any of these variables associated with case/referent status with a $p<0.1$ will be retained for the main multivariate model analysis.

8.2.3. Assessment of potentially strong confounders or risk factors

Matched odds ratios for exposure will be compared between sets of subjects presenting with and without the confounders identified *a priori* and *a posteriori* The position of the observed odds ratios will be examined (within or outside the interval) and decision taken on the analysis. If the number of cases and referents with the potentially strong confounders do not allow for an adequate control of their influence through modelling, the sample of sets used in the modelling for the sensitivity analysis will be censored of those with at least one subject presenting with the confounder. The same approach will be applied by the comparison of odds ratios for exposure to the vaccine in strata of 25^{th}, 50^{th}, 75^{th}, 100^{th} percentile of 'multivariate confounding scores'.

8.3. Modelling and Analysis using Multiple variables

8.3.1. Main model

All retained risk factors identified will be used in a multiple modelling of the risk of central demyelination associated with exposure to Cervarix®. A priori suspected and risk factors

identified a posteriori from the univariate analyses will be controlled for. The analysis will be also controlled for the use of another HPV vaccine reimbursed in France[7]. The risk associated with the number of shots received will be assessed.

Results will be presented as adjusted odds ratios with their 95% confidence intervals (two-sided, estimated with 80% power).

The model considered is the conditional logistic regression for the assessment of relative risks through odds ratios.

8.4. Analysis performed for the identification of biases

A series of descriptive analyses will be performed to identify potential biases. No results will be reported as arising from these analyses. Statistical tests will be applied when possible to help in the interpretation of potential differences or interactions.

8.4.1. Selection bias

- Participant patients will be compared to non-participants on age, time and centre.

- Centres will be described for their recruitment, percentage of rejected cases, and the mean exposure to Cervarix® in the patients reported. Face comparisons between centres will be made on the mean exposure prevalence. Cases rejected and interviewed will be compared to retained cases and to referents for their use of Cervarix®

Decision will be taken by the Scientific Committee to retain or reject centres with obvious outlying results in the above analyses.

8.4.2. Information bias

- Diagnostic bias:

Referents identified with any elements in favour of a disorder consistent with or evocative of the disease, including its *forme fruste*, will be excluded from the set of referents. Exposure to vaccine reported in the patients' interviews will be compared to prescriptions recorded by the physicians. A separate study of the validity of exposure ascertainment in PGRx is conducted. Its results will be presented to the Scientific Committee and potential consequences for the study protocol considered before the final analysis

8.5. Timing of the analysis

8.5.1. Planned analysis

The main analysis will be performed at 36 months after the first index case included in the PGRx system. This delay may be extended if necessary to achieve the recruitment of the sample size displayed in Table 5.

[7] Gardasil®

8.5.2. Unplanned analysis

An unplanned analysis may be performed before the end of the study:

- At the request of the Health Authorities and with the formal agreement of the Cervarix Scientific Committee.
- Or at the request of the Cervarix Scientific Committee, justified by a possible alert identified in the literature or through pharmacoviligance reports.

This unplanned analysis will use all the methods described in the analytical plan and will be applied to the sets of cases and referents satisfactorily documented and to the data considered as consolidated at that time.

Whatever the results of this unplanned analysis, the study will be pursued until the planned completion since, according to the assumption of this study; cases may arise as far as 24 months after exposure.

9. Discussion of the general study methodology

9.1. Limits of observational research

Biases associated with medical practice

This study presents limitations associated with observational research such as possible indication bias for the vaccine and preferential diagnosis in exposed. While the first one is more likely to bias the results towards a lesser risk associated with vaccination in the present context, the second may act in the reverse direction. These two biases are associated with medical practice rather than with the study methods itself and may also be present in so-called 'record-linkage' or medical database research as they pertain to the nature of medical activity. Note than they are also present in unblinded cohort studies. Only double blind randomised clinical trials may completely eliminate their effect, when the blind is not actually broken in practice. The feasibility of such trials to assess the incidence of a rare disease like central demyelination is very low (published trials did not actually have the power to do so). The ethical justification of larger trials in this respect is debatable in the absence of any alert.

The very high specificity of the diagnosis and the potential comparisons between the various degrees of certainty in the diagnosis, as well as the medical information recorded for both cases and referents will provide useful information on this respect. Documenting for a number of potential confounders such as family history of disease or behavioural confounders will help in minimizing the effect of indication bias.

9.2. Limits of field case-referent studies

As opposed to studies nested in medical or prescription databases, the field case-referent nature of recruitment raises the question of potential selection bias, *i.e.* the preferential recruitment into the study of cases associated with exposure. The selection bias of concern here is notoriety

bias where cases exposed to Cervarix® would be more likely to be reported than other, non-Cervarix®, patients. This would bias the results away from the null. The PGRx methodology, by collecting cases systematically in the absence of any alert, and announcing the surveillance of *ca.* 300 drugs to clinicians, limits the potential extent of this bias as compared to ad hoc case-referent studies. Important efforts are devoted at minimising this bias (section 7.2) and assessing its potential magnitude (section 9.4.1).

Note that the case-referent methodology allows for a volume of recruitment which is possible only with very large databases, especially if only definite cases of the disease are considered.

9.3. Nature of referents

The use of physicians as the source of referents in PGRx is a compromise between population-based referents and hospital based referents. They have been successfully used in pharmacoepidemiology (Abenhaim, 1996). Sampling of population-based referents may provide more valid estimates of exposure and behavioural risk factors than sampling of patients visiting physicians, but they are less likely to provide valid information on co-morbidities, antecedents and medical risk factors than the data collected through physicians. Also, the objective source of information on vaccination through medical records may be of great help in this instance. Hospital-based referents are frequently used because of the convenience of sampling and on the assumption that they may help control for referential biases. They are however frequently associated with exposure and reporting biases, as well as with actual referential bias. The pool of potential referents recruited in PGRx is less subject to this later bias while offering a convenient source of sampling of referents to be matched to the cases.

The matching of referents to cases on the number of visits to physician limits the extent of a bias associated with increased opportunity to exposure which may be feared with physician-based referents as opposed to population-based referents (although this bias is less likely to play a role in the contemplated age groups here). Another, to a certain extent symmetrical, concern is the so-called ‘overmatching’. Overmatching is not a validity bias but may impair the efficiency of a study.

9.4. Information biases

For the case/referent status, the specificity achieved in PGRx for the diagnosis of cases and also for the exclusion of referents with history of the disease at hand is very high as compared to any systematic collection of data available, especially in comparison to so-called ‘record-linkage’ databases or usual medical databases.

The infamous ‘recall bias’ feared in studies using retrospective interviews is limited in this study as 100% of reported exposure will have to be based on objective information or documentation. The use of two sources of data on drug use (patients and physicians) helps in this process. A separate validation study of the validity of the ascertainment of exposure in

PGRx is planned. Its results will be made available to the Scientific Committee before the final analysis is conducted.

A comparison of observed exposure of referents to expected exposures based on the data available at the end of the study on the reimbursement of vaccination will allow for the documentation of these biases if they exist. A crude case-population comparison of exposure will be done using these reimbursement data for the assessment of the exposure of the base population and the results compared with those obtained in this case-referent study.

9.5. Residual confounding

Few potentially strong risk factors are known for the diseases at hand (personal and familial history of auto-immune disorders, the existence of severe chronic co-morbidities, ethnicity, and some drugs). Whether they may interact with vaccination and/or represent potential confounders of an association is unknown. Personal or familial history of AID is thought to lower the probability of vaccination, but no data is available on this subject. All these variables are expected to have low or very low prevalence in the sample.
Despite the statistical procedures listed above, in addition to the matching of referents to cases, to minimize and control for the effect of potential confounders, it is always possible that some residual confounding may still exist at the end of the study. The potential magnitude of this residual confounding effect and its likelihood to explain any potential observation or association will be discussed based,

10. Timelines & Reports

Item	Date
Network of PGRx central demyelination Centres	Done, and on-going for paediatricians' centres
Recruitment of 1st case	December 2007
Recruitment of potential Referents	On-going
Finalisation of PGRx central demyelination -Cervarix® protocol	May 2009
1st Annual Descriptive report and blind analysis	
2nd Annual Descriptive report and blind analysis	
Final PGRx central demyelination - Cervarix® Study report	

Recruitment reports are issued every month. Descriptive reports provide data on all the variables listed in the document.

Persons in charge of the analysis and reports

The statistical analysis and reports will be conducted under the supervision of Profs. [redacted] and Dr [redacted]

Appendix 1: Exhibit 1A: PGRx Information System General Methodology

Appendix 2: Recruitment of Central Demyelination in PGRx

Table A2.1 Recruitment of Central Demyelination cases in the PGRx System as of March 2, 2009

	Date of first inclusion	Participating centres	Cases (all age groups)	Recruited female cases of 14-26 y.-o.	Target recruitment Females cases of 14-26 y.-o.	
					per year	3 years
Central demyelination	08/11/2007	28	170	39	25	75

Figure A2.1 Recruitment of Central Demyelination cases in the PGRx System as of March 2, 2009

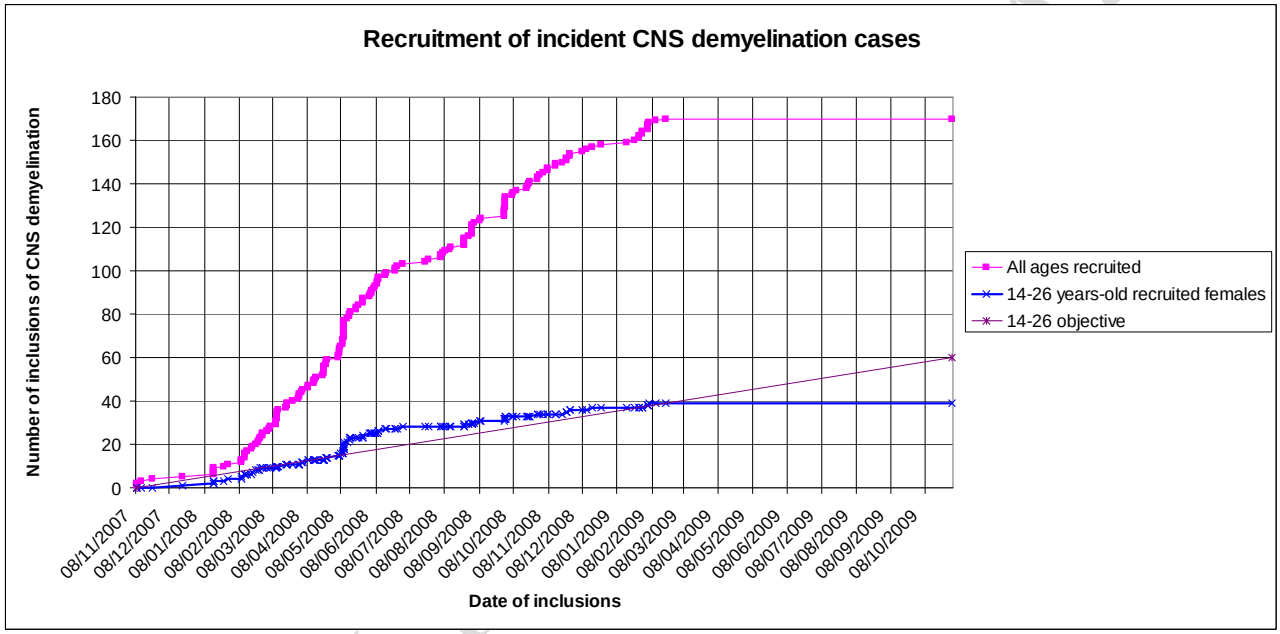

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Exhibit 1B-03B
PGRx information System
Standard Pathologies

Cutaneous Lupus
Case collection protocol

PGRx Information system
Standard pathologies
Cutaneous Lupus

Table of content

1. Composition of the Pathology scientific committee

Scientific Committee Lupus- Standard collection

[redacted] MD, PhD	Pharmacoepidemiology	[redacted]
[redacted], MD, PhD	Biostatistics	[redacted]
[redacted], MD	Dermatology	[redacted]
[redacted] MD	Dermatology	[redacted]
[redacted], MD	Dermatology	[redacted]
[redacted], MD, MSc	Pharmacoepidemiology	[redacted]

Scientific team

[redacted], PharmD, MSc	Pharmacoepidemiology	[redacted]
[redacted], MD, MPH	Pharmacoepidemiology	[redacted]

2. Elements of literature review

2.1. Epidemiology of Lupus

In France Lupus has an incidence rate of 5 per 100 000 person-year and a prevalence rate of 40 cases for 100 000 inhabitants (Danchenko, 2006). Before the age of 18, incidence rate ranges between 10 and 20 per 100 000 person-year (Quartier, 2003). The female to male ratio is 9:1 (Cervera, 2006). In the USA, Jakobson (1997) estimated Lupus incidence rate to 7.3 per 100 000 person-year and its prevalence rate of 23.8/100 000 inhabitants (review studies between 1965 and 1997).

Factors associated with Lupus occurrence have been reported:

- Genetic factors : (Tsao, 2003) :
    - Sisters and brothers of Lupus patients have a greater risk to have Lupus than the general population (Lawrence, 1987);
    - Homozygote twins have a higher frequency of Lupus than heterozygote twins;
    - 10% of Lupus patients have one case of Lupus in their family (Quartier, 2003);
    - Hereditary deficits of complement are described for some Lupus patients;
    - Blacks have a higher prevalence rate of Lupus.
- Exposures to sunlight and ultraviolet rays and viral infections (Epstein-Barr virus) have been reported to be trigger factors.

2.2. Current diagnostic standards of Cutaneous Lupus

Lupus is associated to the presence of auto-antibodies such as antinuclear antibodies. Lupus has various clinical presentations and its evolution can be long before the diagnosis of Lupus is made.

Four clinical categories are described in the litterature:

- A cutaneous and arthritic form;
- A systemic form;
- A pure cutaneous form;
- An incomplete systemic Lupus erythematosus also named “Undifferenciated connective tissue disease” (Mosca, 2006; Swaak, 2001).

The American Rheumatism Association (ARA) has defined diagnostic criteria for Lupus (appendix 1). The diagnosis of Lupus requires the association of four clinical and biological criteria of ARA classification.

Main cutaneous presentations of lupus are the following:

- *Discoid lupus*: chronic skin condition of sores with inflammation and scarring favoring the face, ears, and scalp. These lesions develop as a red, inflamed patch with a scaling and crusty appearance. The center areas may appear lighter in color with a rim darker than the normal skin. When lesions occur in hairy areas permanent hair loss can occur;

- *Lupus tumidus*: cutaneous lupus in which the infiltrate occurs primarily in the deeper portions of the dermis with rare epidermal changes. Firm, sharply demarcated nodules lying beneath clinically normal skin are seen;

- *Annular lupus*: pink to red papules and polycyclic plaques with thin scale and usually telangiectasia and dyspigmentation; occur in sun-exposed areas;

- *Chilblain lupus*: violaceous "pernio" plaques appear prominent over dorsal interphalangeal joints, often with positive antinuclear antibody (ANA) or rheumatoid factor (RF);

- *Lupus profondus*: the face, neck, shoulders and arms are affected. Hard, well-defined erythematous subcutaneous lesions are observed;

- Cutaneous signs reported in the ARA classification (Malar rash, Discoid rash, Photosensitivity, Oral ulcers).

Histologically, the skin of a patient with lupus may demonstrate a vasculitis and dermal chronic inflammatory infiltrates. If immunofluorescence microscopy using an antibody to complement or immunoglobulin is performed, then it is possible to see the brightly fluorescing band along the dermal epidermal junction that indicates the presence of immune complex deposits.

2.3. Drugs associated with Lupus

Antonov (2004) reviewed publications about drug associated with Lupus :

- 80 drugs have been described to be associated with Lupus;
- Relation between drug and the occurrence of Lupus is described in 10% of Lupus patients.

Lupus attributed to drugs is not clinically different from the general form of Lupus. They both present the cutaneous and systemic forms grouped under 3 clinical profiles:

- *Drug-Induced Systemic Lupus Erythematosus;*
- *Drug-Induced Subacute Cutaneous Lupus Erythematosus;*
- *Drug-Induced Chronic Cutaneous Lupus Erythematosus.*

The time between first clinical manifestations of Lupus and drugs have been reported in a wide range from 3 days to 8 years.

Criteria have been proposed to raise suspicion of drug-induced Lupus:

- Drug exposure between 3 weeks and 2 years before the occurrence of Lupus;
- Clinical signs stop when drug exposure ends;
- Biological profile as follow:
  - Anti-histone antibodies (anti-H2A and anti-H2B),
  - Normal complement.

Drugs frequently cited in related to drug-induced Lupus:

- Minocycline : can exacerbate Lupus; average time between exposure and first signs of Lupus is 19 months (3 days-6 years) (Antonov, 2003 ; Schlienger 2000) ;
- Oestrogens : difference between trigger and exacerbation mechanisms remains unknown (Antonov, 2003);
- Acebutolol (Wilson, 1980);
- Carbamazepine (Pelizza, 2006);
- Chlorpromazine (Price, 1995);
- Isoniazide (Siddiqui, 2002);
- Methyldopa (Price, 1995);
- Penicilline (Hernandez-Salazar, 2006);
- Quinidine (West, 1984);
- Sulfasalazine (Gordon, 1999 ; Gunnarsson, 2000);
- Anti-TNF α (De Bandt, 2005).

Vaccination and Lupus

Aron-Maor (2001) and Chen (2001) reviewed case reports and observational studies of vaccination and Lupus. The authors conclude that scientific evidence is insufficient to conclude on any association between vaccination and Lupus.

The association between hepatitis B vaccination and Lupus has been studied in a case-control study in Great-Britain (Sturkenboom, 2000) and showed no evidence of an association.

3. Case definition

3.1. PGRx general inclusion and exclusion criteria

Inclusion

- Male and female;
- Age between 18 and 79 years old (included);
- Patient does live in continental France;
- Patient can read and respond to a telephone interview;
- Patient has completed the participation form.

Exclusion

- Refusal to participate;
- Patient cannot be reached by phone.

3.2. Case definition of Cutaneous Lupus

Inclusion of cutaneous lupus cases is based on the clinical diagnosis made by dermatologists, the presence or not of auto-antibodies specific of lupus and on skin biopsy results when performed. Main cutaneous presentations of Lupus are the following:

- *Discoid lupus*;
- *Lupus tumidus*;
- *Annular lupus*;
- *Chilblain lupus*;
- *Lupus profondus*.

Inclusion and exclusion criteria specific to cutaneous lupus are:

Inclusion criteria:

- Clinical presentation compatible with a cutaneous lupus;
- Maximum delay of 12 months between the inclusion in the PGRx study and the first clinical symptom or sign evocative of lupus.

Exclusion criteria:

- Personal history of lupus.

3.3. Validation of cases

Cases will be validated by an independent expert review panel blinded to any medication received. The panel will particularly consider clinical information reported in the medical data form. They will also study the clinical evolution of the patient and the results of skin biopsy when performed.

At the end of their review of each case, the expert review panel will qualify the cases as:

a) Definite
b) Possible or uncertain
c) Rejected

The expert review panel can classify the cases in one of these categories by consensus.

Table 1 presents case definition of Cutaneous Lupus based on the American Rheumatism Association criteria (Appendix 1).

Table 1. Case definition of cutaneous lupus

	Clinical picture	Lupus specific auto-antibodies (AAb)	Skin biopsy
Definite cases	Characteristic skin disorders: discoid lupus, lupus tumidus, annular lupus, Chilblain lupus, lupus profondus with or without systemic(s) disorder(s) evocative(s) of lupus	**AND** presence or absence of lupus specific AAb	**AND** biopsy performed with characteristic elements for lupus diagnosis **OR** biopsy not performed
Possible cases	Non characteristic skin disorder **AND** presence of systemic(s) disorder(s) evocative(s) of lupus	**AND** Absence of lupus specific AAb	**AND** biopsy performed but without characteristic elements for lupus diagnosis **OR** not performed
Rejected cases	Non characteristic skin disorder **AND** no systemic disorder evocative of lupus	**AND** Absence of lupus specific AAb	Not performed **OR** performed but without characteristic elements for lupus diagnosis

3.4. Index date

The index date of cases is the date of first occurrence of cutaneous sign that led to a visit to a physician (general practitioner or specialist).

4. Recruitment of cases

4.1. Centers for recruitment of cases

Recruitment of cases of Cutaneous Lupus will take place in internal dermatology centers participating to the PGRx network. This network consists of physicians, trained to the PGRx System methodology and who regularly include patients corresponding to the disease surveyed.

Participating centers are public hospitals and Health centers from different regions of France.

For standard collection of cases of Lupus, it is expected that a number of 3 centers will participate. The number of recruiting centers will be adjusted after one year of recruitment.

4.2. Recruitment of cases

PGRx should begin to collect the cases of lupus by the beginning of 2008.

4.3. Medical data form

At inclusion, the recruiting specialist provides the following information:

- Date of the consultation;
- First and last name, date of birth and gender of the patient;
- Inclusion and exclusion criteria;
- Date of the first clinical sign related to Lupus;
- Current and previous chronic diseases;
- Name and address and phone number of the usual treating general practitioner of the case recruited.

Complete medical data form is available in appendix 3.
Data collected on cases by their usual treating general practitioner is described in Exhibit 1A.

5. Special options

For lupus, special cases aged between 14 and 17 y.o. could be included in PGRx for the need of a special surveillance. Medical data form for 18 to 79 y.o. cases of lupus is adapted for 14-17 y.o. cases according to our scientific committee.
These special cases can be recruited in centres of paediatry or in centres of adult internal medicine, rheumatology and dermatology with paediatric recruitment.
For cases under 14 years, a paediatric PGRx exhibit is needed.

6. Referents and matching rules

6.1. Definition of referents

Standard referents
Patients with no previous history of Lupus will be selected from the pool of referents in the PGRx database (see Exhibit 1A) to serve as standard referents for standard cases.

Special referents
For special cases, special referents aged 14-17 years old will be included in PGRx.

6.2. Matching rules

At least 4 referents are individually matched to each case on the following criteria:

- Gender;
- Age (within 1 year under the age of 18, within 5 years above 18);
- Place of residence (same recruitment region);
- Time of recruitment (closest time within 3 months as feasible);
- Has seen a physician in the previous year (yes/no).

The index date of referents is the date of visit to the general practitioner that led to the recruitment in PGRx.
For special referents matching rules will be the same as standard referents.

7. Crude analysis

7.1. Crude statistical analysis

The goal of the crude analysis is a general surveillance of adverse events and exposure to drugs or therapeutic classes. These analyses are performed periodically on a routine basis as a crude comparison between sets of cases and sets of referents for their exposure to therapeutic products. The association between an exposure and the occurrence of an adverse event is quantified through a crude Odds ratio and its 90% confidence interval. The crude Odds ratios are not adjusted for the various confounding variables and not subjected to particular risk curve modeling, and should be regarded as indicative only.

7.2. Estimation of power and minimum Odds ratio detectable

Table 2 presents the odds ratio detected with different sample sizes and relevant exposure rates. The 'relevant exposure rates' are those considered for study taking into account the time window of exposure and the age of the patient.

These tables display the odds ratios detectable with a 95% confidence and a power of 80%, using a one-sided test. Note that calculations are based on a pre-determined risk (odds ratio) and not the number expected under the null hypothesis.

Table 2. Odds ratios detectable in according to several hypotheses of relevant exposure

Relevant Exposure	5,00%	10,00%	15%	20%
Odds ratio detected*, 20 cases**	6.4	4.6	4.0	3.7
Odds ratio detected*, 30 cases**	4.9	3.7	3.2	3.0
Odds ratio detected*, 40 cases**	4.2	3.2	2.8	2.6
Odds ratio detected*, 60 cases**	3.4	2.6	2.4	2.2
Odds ratio detected*, 90 cases**	2.8	2.3	2.1	1.9

*One sided, with 95% Confidence and 80% power, 4 controls per case
** Number of cases observed under the contemplated hypothesis

8. Appendixes

8.1. Appendix 1: American Rheumatic Association criteria of Lupus

Malar rash
Discoid rash
Photosensitivity
Oral ulcers
Non erosive arthritis
Serositis: pleuritis or pericarditis
Renal disorder: persistent proteinuria, > 500 mg per 24 hours (0.5 g per day or > 3+) or cellular casts
Neurologic disorder: seizures or psychosis occurring in the absence of offending drugs or known metabolic derangement
Hematologic disorder: hemolytic anemia with reticulocytosis; or leukopenia, < 4,000/mm^3; or lymphopenia, < 1,500/mm^3; or thrombocytopenia, < 100,000/mm^3
Antinuclear antibodies
Antibody to double-stranded DNA antigen (anti-dsDNA); or antibody to Sm nuclear antigen (anti-Sm); or positive finding of antiphospholipid antibody

8.2. Appendix 3 : Cutaneous Lupus medical data form

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PGRx

STUDY OF CERVARIX® & GUILLAIN-BARRE SYNDROME

USING THE PGRx INFORMATION SYSTEM

February 26, 2009

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Table of content

NOTE

This protocol is provided with the *Exhibit 1A: The general methodology of PGRx (Appendix 1),* which applies to all studies conducted with the PGRx Information System.

The Exhibit 1A is up-dated on a yearly basis by the International Scientific Board of PGRx, taking into account evolution of the System resulting form the actual conduct of data collection and studies. For the purpose of the study of Cervarix®, in the case of any difference or apparent discrepancies between the Exhibit 1A and the present Protocol, it is this Protocol that prevails at any time.

1. Introduction

1.1. Overview of the study

1.1.1. Study Objective

The objective of the study is to assess whether the use of Cervarix® is associated with a modified risk of Guillain-Barre syndrome ("the disease").

1.1.2 .General inclusion & exclusion criteria for the cases and referents in the study

Study subjects are cases and referents from the PGRx system satisfying with the following criteria:

Inclusion criteria

- Female gender
- Age 14 to 26 years-old
- Patient residing in France (continental)
- Patient accepting to participate in the study

Exclusion criteria

- Prior reported history of the disease;
- Patient or Patient's parent cannot read the interview guide or answer a telephone interview questionnaire in French.

1.1.3. Study design

1.1.3.1. Case-control (or case-referent) methodology

This study is a systematic case-referent study. It consists in using the PGRx information system to:

a) Monitor a large number of neurology centres for the occurrence of the disease,
b) Match general practice-based controls to these cases, selected from the pool of PGRx potential referents
c) Document the previous vaccination by Cervarix® in both cases and controls,
d) Estimate the relative risk of the disease in Cervarix® vaccinated females by the odds ratio (adjusted for a series of confounders and interaction factors, including other drug use).

1.1.3.2. Rationale for the choice of the case-control design using PGRx

The case-control (or case-referent) methodology is the design of choice for the study of rare events, such as autoimmune disorders in epidemiology. Its power is not affected by the small incidence of diseases and has proved efficient in pharmacoepidemiology (Abenhaim, 1996). When based on field collection of data, this design allows for the documentation of individual risk factors.

Ad hoc case-control studies in pharmacoepidemiology are however cumbersome and require a large amount of work and procedure to control for the various sources of biases (Wacholder, 1992).

The PGRx Information System (PGRx) has been developed to minimise these difficulties and biases.

PGRx is a systematisation of the case-control referent (or case-referent, Miettinen, 1976) methodology. It is available in France and Canada. It addresses most of the concerns usually raised with ad hoc case-control studies. Autoimmune disorders have been listed as conditions of interests for PGRx since the inception of the system.

1.2. Overview of the PGRx Information System (PGRx)

1.2.1. General Description and Methods of PGRx[1]

The PGRx general methodology is described in PGRx Database & Information System Exhibit 1 A – *General Methodology*.

In brief, PGRx has been developed in response to the paucity of databases or information systems available for the study of rare diseases and/or delayed adverse events associated to medicines, with sufficient power and specificity on disease diagnosis and individual risk factors. It operates since 2007.

The system prospectively and routinely collects information on:

1) Cases[2] of a dozen diseases[3] collected in more than two hundred specialized referral centres and validated through a series of procedures. The collection ensures for a control of selection bias;
2) A large pool of general practice-based potential referents from which controls or referents can be selected and matched to cases of diseases under study. Matching can be made on calendar time, age, gender, region and any other relevant parameter available and can be individual matching or frequency-matching. The selection of referents is performed in such a way to ensure a fair representation of the population-time experience with the drugs studied in the relevant source populations,
3) 300 drugs (including vaccines) documented through: (i) guided telephone interviews and (ii) medical prescription records (in a sample of either treating physicians'

[1] See Exhibit 1A attached

[2] In the PGRx DIS, cases are defined as adverse *events* and not necessarily adverse *reactions*. No hypothesis is made *a priori* on the causality of the event (as opposed to spontaneous reports of adverse reactions frequently reported in pharmacovigilance systems).

[3] The diseases routinely surveyed in the PGRx Information System are presently: myocardial infarction, multiple sclerosis (first central demyelination), Guillain-Barré syndrome, lupus erythematosus, cutaneous lupus, myositis and dermatomyositis, inflammatory arthritis, unspecified connectivitis, type I diabetes, thyroiditis, thrombocytopenia, suicide attempts, torsade de pointes and acute liver injuries. First results have been presented in various conferences (ICPE, 2008; ISOP, 2008).

computerized prescriptions or treating physician's reports). All new molecules, products targeted in risk management plans and up to 24 products used by more than 250 000 persons in the country are listed, including most vaccines. Cervarix® is one of the vaccines routinely studied. The lists of drug or vaccines specifically studied at the different dates are provided with the Exhibit 1A.

4) Individual behavioural, medical and family risk factors: smoking, alcohol use, physical activity, occupation, chronic co-morbidities, familial history of certain diseases, others.

For each AID a PGRx Scientific Committee, called PGRx Pathology Specific Scientific Committee (see Exhibit 1A), has been organised and the general methodology for the study of each AID in PGRx has been developed under the auspices of those committees. The collection of data in PGRx follows the criteria developed by these committees. Out of these collected data, the scientific committee for each individual study (e.g. the one for Cervarix® and autoimmune disorders assembled by the manufacturer) may select those that it considers appropriate for its study.

1.2.2. PGRx Network for Autoimmune disease

A network of centres treating patients for these diseases has been assembled to participate in the PGRx Database and Information System.

Table A2.1 and Figure A2.1 in the Appendix 2 reports the number of centres participating in the collection of cases of Guillain-Barre syndrome, the date of start of the surveillance of this disease in the system, the number of cases recruited so far by age group (l4-26 years old, all age groups) and the objectives of recruitment per year in the System.

1.3. Overview of the literature

1.3.1 Epidemiology of Guillain-Barre syndrome

Guillain-Barré syndrome (GBS) is the commonest cause of acute neuromuscular paralysis in most countries. It is an acute polyradiculoneuropathy marked by flaccid areflexic paralysis.

The incidence of the GBS in the developed countries is 1.5 for 100 000 inhabitants per year on average. A variation of the incidence between 1 and 2 for 100 000 of the population was reported. The GBS prevalence rates are little described. Prevalence rate of chronic inflammatory demyelinating polyneuropathy lies between 0.8 and 1.9/100 000 inhabitants.
Men are affected slightly more often than women with a male/female ratio of 5/4.
The GBS is observed in all age groups. The incidence increases slowly and in a continuous way with the age. There are two peaks of incidence, the first one at 14-25 y.o and the second one at 65-74 y.o.

Different patterns of the GBS exist; this syndrome presents subtypes that are similar enough in terms of clinical and biological findings:

- The acute inflammatory demyelinating polyradiculoneuropathy, 85-90% of GBS cases in Western countries;
- The axonal patterns of GBS, most frequent in other regions of the world (China for example): acute motor axonal neuropathy and acute motor sensory axonal neuropathy.

1.3.2. Risk factors associated with Guillain-Barre syndrome

The pathogenesis of GBS remains incompletely defined, it is considered to be a postinfectious disease as approximately two-thirds of patients report some form of preceding infectious illness. Knowledge of the epidemiology of GBS is limited regarding preceding infections and prognostic factors. The main infectious agents reported associated with GBS are *Campylobacter Jejuni* (most frequently reported) and *Cytomegalovirus*. Numerous other associations have been suggested in case reports or small series as infections with the *Virus of Epstein-Barr* and the *Mycoplasma pneumoniae*. The temporal association between such infections is often suggestive in individual cases. Many of the identified infectious agents are thought to induce antibody production against specific gangliosides and glycolipids, such as GM1 and GD1b, distributed throughout the myelin in the peripheral nervous system.

1.4. Drugs allegedly associated with Guillain-Barre syndrome

1.4.1. All drugs

Peripheral neuropathy is a common neurotoxic of some medications as cisplatine and some antiretroviral (Peletier, 2002). Some medications as tracolimus can result in a demyelinating neuropathy than can mimic GBS or chronic inflammatory demyelinating neuropathy. In contrast we did not found cases of GBS induced by drugs apart from vaccinations.
There has been some concern that certain immunisations might trigger GBS in susceptible individuals. This fear arose because of a slightly increased incidence of the syndrome after “swine flu” vaccines were given in the USA in 1976.

Other influenza vaccines have not been associated with the same risk, and there has been a steady decline in the number of cases of Guillain-Barré syndrome associated with influenza vaccine in the USA between 1990 and 2003. A retrospective case study of the combined 1992–93 and 1993–94 vaccine campaigns in the USA identified a marginally significant, very small increase in the risk of Guillain-Barré syndrome, equivalent to about one case per million vaccines above background incidence. Despite many individual case reports, other conventional vaccines have not been associated with a significant risk (Hughes, 2005).

1.4.2. Time windows at risk used in studies

The CDC uses a 6 weeks time window for the assessment of cases of Guillain-Barre syndrome potentially associated with vaccines.

Table 1 summarizes the main features stemming from the literature review.

Table 1: Epidemiology of Guillain-Barre syndrome and data stemming the literature review

Age / Gender	14-25 and 65-74 years old male/female ratio : 5/4
Incidence	Developed countries: 1.5 /10^5 inhabitants / year in average
Prevalence	-
Time to event tested	6 weeks

2. Cases

2.1. Populations for case recruitment

2.1.1. Source population

The source population for the study is made of patients who are:

- Hospitalised for the occurrence of the disease in one of the centres participating in the PGRx Network for AID;
- Or addressed to a centre participating in the PGRx Network for the diagnosis or the management of the disease.

2.1.2. Study population for cases

The study population is made of patients from the source population above who are:

- Incident cases patients presenting with the set of symptoms and signs retained for the diagnosis of the disease defined further below;
- Reported in PGRx by the specialist participating in PGRx;
- Recruited within 12 months after the date of the occurrence of the first clinical sign identified by a physician;
- Meeting all inclusion and exclusion criteria for the study.

2.2. Identification of cases

2.2.1 PGRx Centres for the recruitment of cases

Centres eligible to participate to the PGRx Network for the recruitment of contemplated events are [redacted] and [redacted] that have a specialized unit or a health care network for the management of this disease. These units are selected on the volume of incident cases of the disease that they treat per year.

2.2.2 Recruitment of cases

Participation must be proposed to all consecutive patients who respond to inclusion and exclusion criteria for the event in the PGRx participating centres.

2.2.3. Web entry

Each specialist recruiting a case fills out a medical data form directly on a secured Internet data entry system on which they have been individually provided with a login and a password.

2.3. Information collected

2.3.1. Medical form[4]

General information

When the case is included the following data are collected by the recruiting specialist:

- Date of the consultation;
- First and last name, date of birth and gender of the patient;
- Inclusion and exclusion criteria;
- Name and address or phone number of the usual treating general practitioner of the case recruited.

Medical information

The following sections of the medical form are used for case ascertainment:

- Date of the first symptoms evocative of the disease
- Description of the symptoms and signs of the first evocative episode
- Description of biological, electrophysiological and imaging findings (if appropriate and/or available)
- Current and previous chronic diseases
- Elements of differential diagnosis

2.4. Case definition

Cases for the study are *incident cases* (i.e. newly diagnosed patients) reported as having occurred in the previous twelve months before the recruitment consultation.

2.4.1 Case ascertainment

Cases will be validated by an independent expert review panel blind to the medications and vaccinations status. The panel will review the medical forms of all the cases recruited. At the end of their review of each case, the expert review panel will qualify the cases as:

a) Definite
b) Probable
c) Possible

[4] The web-based Clinical Research Forms are available for consultation to interested parties upon request.

Definite cases only will be used in the main analysis. Possible cases may be used for potential unplanned analysis" (see further below). Rejected cases are used for the identification of biases (see special section "Identification of biases" further below). The diagnostic criteria to classify the patients are described below; they have been adapted from internationally accepted definitions to allow for the recruitment of cases at the early stages of the disease at hand and to better take into account the age groups concerned by the vaccination.

Every year, PGRx centres are contacted to assess the potential evolution of the diagnosis of the cases reported previously. Any change in the diagnosis of the case is recorded and the case is reclassified as definite, possible or rejected. .

2.4.2 General definition of cases for the study

Cases for the study are incident cases reported as having occurred in the previous twelve months before the recruitment consultation. They are defined as patients with clinical, electrophysiological and biological presentation compatible with the onset of GBS according to the Brighton collaboration case definition for GBS (Sejvar et al. CDC; Schonberger LB et al. 1979; Asbury et al. 1978; Asbury et al. 1990).

2.4.3. Summary table for case definition

Table 2 presents the algorithm for the definition of cases for the study.

Table 2: Case definition for the study according to the Brighton collaboration case definition

	Clinical presentation
Definite cases (Level 1)	Requires clinical, electrophysiologic, and CSF data consistent with the onset of GBS
Probable cases (Level 2)	Requires clinical data and either electrophysiologic, OR CSF data consistent with the onset of GBS
Possible cases (Level 3)	Requires clinical data consistent with the onset of GBS

Clinical, electrophysiologic and CSF data required by the Brighton collaboration case definition and their availability in PGRx database:

Brighton	**Available in PGRx**
A. CLINICAL CRITERIA	
Acute onset	YES
Bilateral	YES
symmetric	YES
flaccid weakness/paralysis of the limbs	YES
with or without involvement of respiratory	YES (proxy: intubation or not)
or with or without cranial nerve-innervated muscles	YES
AND	

decreased or absent deep tendon reflexes at least in affected limbs	YES
AND	
Weakness or paralysis nadir reached within 28 days	YES
And subsequent improvement	YES
or death	YES (if case reported)
B. ELECTROPHYSIOLOGIC CRITERIA	
Electrophysiologic findings consistent with GBS	YES
C. CEREBROSPINAL FLUID CRITERIA	
Presence of cytoalbuminologic dissociation	YES
(elevation of CSF protein level	YES
and CSF total white cell count <50 cells/mm3)	YES

3. Referents and matching rules

3.1. Definition of referents

Referents to the cases are patients selected from the pool of potential referents reported by physicians in general practice, who meet the same general inclusion and exclusion criteria as the cases.

Patients with no reported previous history of the disease considered for the cases, as reported by themselves or their physician will be selected from the pool of potential referents in the PGRx system to serve as referents to cases.

3.2. Recruitment of referents

3.2.1. PGRx Pool of Potential Referents

A network of *ca.* two hundred and fifty (250) general practitioners (GPs) enrols a pool of *ca.* 2,000 referents each year in the PGRx database and Information system. Each GP in the network is asked to recruit 1 male and 1 female in the following age categories: 18-34, 35-49, 50-64, 65-79 (age strata may be more detailed or doubled if needed).

For the purpose of the study of autoimmune disorders in younger age groups, voluntary GPs have been asked to also recruit patients 14 to 17 y.o (2 males and 2 females per year of age and by physician).

Physicians who recruit potential referents are requested to fill an electronic medical data form that includes medical information on the patient (current prescriptions with their motives and diagnoses, chronic diseases, medical risk factors and some biological data).

Physicians obtain consent of eligible patients to participate and transfer the coordinates of the patients to the PGRx staff for the telephone interview, through a secured Internet connection.

PGRx GPs are enrolled for the recruitment of referents in all telephone regions of the country. Physicians are randomly selected from a general list of practicing physicians in a given region. In order to be enrolled, they must have access to Internet and use computerized prescriptions. Those who agree are provided with a secured access to the PGRx system on Internet and are instructed on recruitment of consenting patients, on filling the medical data form and the electronic transfer of their computerized drug prescriptions over the previous two years.

Participating physicians are asked to recruit a set of potential referents patients one to three times a year on a rotating basis so that recruitment is not interrupted in a given region over the year. This recruitment spread out overtime facilitates matching of selected referents to cases on calendar time.

3.2.2. Referents selected for the study of autoimmune disorders

The selection of referents from the PGRx pool of potential referents proceeds in order to apply the same inclusion and exclusion criteria as in cases.

3.3. Matching

To each case is matched at least one referent. As many referents as possible meeting the criteria for the study and allowing proper matching to case are retained. It is estimated than an average of 4 referents will be available per case with the following priority rules:

1) Date of recruitment of the cases and referents: Cases and referents are organised by trimester of recruitment in a given year (Q1 to Q4): for each matching criteria below, a referent is looked for in the same quarter of recruitment as the case or, if none is found, in the next adjacent quarter of recruitment, and then the next one again. If no matched referent is found, the case is not retained.

2) Age: matching will be done with the following order of priority: ± 1 month, then ± 3 months; then ±6 months, then ±1 year (for age ≤ 17), then ±2 years (for age ≥ 18); if no matching referent is found to a case, the case is not retained.

3) Number of visits to a physician in the previous year (0-5, >5). If no matching referent is found to a case, this matching criterion is dropped.

4) Place of residence (region or telephone zone): cases will be match to referents of the same region, if necessary matching will be performed with referents from contiguous regions; if necessary, referents from all France are considered.

4. Drug exposure ascertainment

The ascertainment of exposure follows 3 steps:

1 – Identifying and ascertaining drugs and vaccines used in the last 2 years

2 – Defining the index date for exposure

3 – Defining the relevant time window at risk for the exposure before that index date.

A subject is considered as 'exposed' whenever a vaccine use is ascertained during the time window at risk.

4.1. Identifying drug and vaccine use

4.1.1. Sources of information

Information on drug exposure is obtained from:

A) A structured telephone interview of the patient (cases and referents) or of one of the patient's parent (see below)using:
   - an interview guide,
   - a list of 19 General Health Conditions,
   - a list of up to 20selected drugs for each General Health Condition (see below)
   - and visual photographic displays of up to 10 drug packages per General Health Conditions
   - a list of all vaccines (with up to 10 visual displays of packages)

B) Medical records obtained from the Treating Physician[5] of the cases and the PGRx GPs reporting referents:
   - Either copies of computerized medical prescriptions
   - And/or medical prescription forms filled by the treating physician

For cases, the name of the treating physician and consent to contact him/her is obtained from the patient. They are contacted by the PGRx research team

Exposure is defined by a combination of the information from these two sources (see further below).

The interview is conducted by trained telephone interviewers belonging to the PGRx Call Centre specialised in pharmacoepidemiology. Patients are conducted through a list of questions. The duration of the interview is recorded. Interviews may be taped for quality control (with the information of the patient).

Consent is confirmed from the patient (case or referent), or from the patient' parent at the beginning of the interview. If the patient is minor (under 18 y.o in France), both the parent and the minor are asked to be present during the interview. The person actually interviewed is decided by the parent.

4.1.2. Drug list and drug visual display for the guided interview

The drug list used in the interview contains roughly 325 brand drug names (including *ca.* 50 vaccines, see below), with up to 20 drug names in each of the 19 General Health Conditions categories (see Exhibit 1A) ; they are selected with the following criteria (in order of selection):

- Drugs containing new active principles that have been on the market for 3 years or less.
- Drugs targeted in risk management or surveillance plans under study.

[5] To obtain reimbursement of certain health services, including drug prescribed, from the national health insurance, French patients must identify a so-called 'Treating Physician'.

- Drugs that are used by at least 250,000 patients per year (selected in order of sales' figures)

Up to 10 photographic visual displays of drug packages are provided in the interview guide for each General Health Condition and for the vaccines (same order of selection as above).
The drug lists and drug visual displays are systematically reviewed with the patient.

The drug list and drug visual displays are renewed three times a year using the criteria mentioned above.

4.1.3. Ascertainment of vaccine use

4.1.3.1. Vaccines in the guided interview

A list of ca. 50 vaccines is provided in a special section of the interview guide and used during the telephone interview. Cervarix® is one of these vaccines.

For each Cervarix® use reported by the patient, the following information is sought for:

- The number of shots received with their date
- The availability at the patient's of evidences of the vaccination: medical prescription, health record, the vaccine package or other, and the possibility to obtain the copy of the evidence if needed
- The batch number of the reported vaccine (if the package is available to the patient or if this number is available in the health record)
- The settings of the vaccination (general practice, specialised physician settings, vaccination centres or other).

4.1.3.2. Confirmation of Cervarix® use

Reported use of Cervarix® will be considered as 'confirmed' when: reported by the patient as used with at least one of the following source of confirmation obtained:

- Vaccine batch number reported by the patient (from the drug package or his/her health record)
- Copy of the doctor's vaccine prescription or of the health record or of other evidence sent by the patient
- Record of the vaccine prescription sent by the treating physician or the GP of the referent

Only confirmed vaccines reported by the patient are considered for 'definite exposure' (see further below) in the main analysis of the study. Thus 100% of definite exposure to vaccines used in the main analysis will be confirmed by at least one objective source.

4.1.4. Spontaneously reported drugs

Patients are instructed to report all drugs taken in the two years previous to the index date, whether they were obtained by prescription, over-the-counter or from the family pharmacy, even if they do not appear in the drug list of the interview guide.

- Patients are invited to remember OTC, homeopathic, phytotherapeutic, traditional medicines, pharmacists' preparations and other types of medications that they may have been taking.
- Hospital medications spontaneously reported by the patient are recorded.

4.1.5. Records of medical prescriptions

AID Cases: The treating physician of cases recruited is tentatively identified by the specialist who recruits the patient into PGRx. Or during the interview of the case Attempts are made (with the consent of the patient) to contact this physician and to obtain information on prescriptions and chronic health conditions of the patients over the previous two years. This is usually successful for 50% of the cases in PGRx.

Referents: The PGRx GPs are asked to transmit extracts of the patients' electronic records for the drug prescriptions over the previous two years. Approximately 90% of them usually do so in an exploitable way.

4.2 Index date

4.2.1. Definition of index date

The index date is the date before which drug use may be considered as exposure and after which drug use is considered as non exposure.

Within a given case-referent set, the index date is the reported date of the first clinical sign evocative of the disease in the case; it is applied to all matched referents of the set.

4.2.2. Ascertainment of the index date

The index date is ascertained by:

- The date of the first symptoms reported by the recruiting physician in the medical form of the case;

- The date of the first symptoms which led to a contact with a physician (GP, specialist or hospital), reported by the case patient during the telephone interview. During this interview, it is tempted to trace back the history of the event with the patient.

The earliest of these dates will be used as the principal index date for the study if they are not more than 1 month apart. If the difference is longer the expert review panel will decide of the retained index date of the case, blind on exposure.

4.3. Time windows at risk

4.3.1. Cervarix® vaccination

- The full vaccination with Cervarix® requires 3 shots over a period of 6 months (T0 and ideally T1 and T6, with 1 month minimum between any two shots).

- Each shot is considered as a 'vaccine use'.
- Exposure is defined as the presence of a vaccine use during the time-window considered at risk for developing the event (see below).

4.3.2. Risk associated with each shot

The following assumptions have been retained for the main analysis:

a) A user may be a person receiving any one shot or the entirety of the Cervarix® vaccination during the at risk time window :
b) The risk does not vary according to the number of shots received.
c) The risk does not vary according to the rank of the shot
d) After a given shot, and during the time considered at risk, the instantaneous risk or 'hazard' is constant

4.3.3. Mortal & immortal times

Table 3 presents the time-windows considered at risk or not at risk for the study. It is based on the following definitions or mortal and immortal times:

1) *The initial 'immortal' time window*: the time following a contemplated shot during which an event, if it occurred, could not be considered as resulting from this contemplated use and should consequently be considered as "unexposed" if no relevant previous shot (as described just below) had occurred.

2) *The time at risk after vaccination or "mortal time"*: the time after the initial immortal time window, during which an event, if it occurred, could theoretically be attributable to a contemplated shot of the vaccination and should consequently be considered as "exposed". This period of time applies to each vaccine use (shot)

Mortal times of 24 months, 6 months and 2 months are considered for the study of autoimmune diseases and Cervarix® using the PGRx system. Table 3 identifies which have been retained as the primary and secondary time-windows in this study according to the Scientific Committee. These different time-windows have been selected by consensus in the absence of definitive biological or epidemiological data on this respect.

3) *The final 'immortal' time window after last drug use*: After the last of the mortal time windows defined above, the time will be considered as at no risk or "immortal".

Table 3: Time considered potentially at risk after each individual shot of the vaccine for the study of Guillain-Barre syndrome

	1st 24 Hours	2 months*	6 months*	> 6months
Risk	Immortal	Primary Mortal	Secondary Mortal	Immortal

* After the first 24 hours

4.4. Definite and uncertain exposure

Exposure to Cervarix® will be considered as 'Definite' only if:

- The reported use is confirmed by an objective source
- The index date for the event (in case and referents) occurred during one of the time-windows at risk (or "mortal" time windows) following of the reported shots

Other reported use of Cervarix®, including reported uses not confirmed by an objective source, confirmed reported uses occurring in one of the immortal time windows and vaccine prescription records not reported by patients, whatever the time window, will be considered as "uncertain exposures to Cervarix®" and controlled for in the analysis (no odds ratios to be published).

5. Co-morbidities and risk factors

Information is recorded for the control of confounding as well as for performing interaction analyses:

5.1. Comorbidities

The following comorbidities are recorded:
- Chronic co-morbidities: documented with the list described with Exhibit 1A (Appendix 1). Co-morbidities reported spontaneously are systematically organised. Both sources allow classification that is consistent with the International Classification of Diseases 9th revision. Further coding is performed by trained medical archivists at PGRx when necessary.
- Past medical history in the previous two years
    - Review of 19 categories of morbid conditions
    - Number of visits to a physician in the previous year
    - Hospitalisations

5.2. Risk factors

Table 4 lists the risk factors considered *a priori* for the study.

Table 4: Risk factors considered a priori for the study of Guillain-Barré syndrome

Risk factors considered a priori
- Family history of autoimmune disorder (1st degree) - Social and professional status - Recent or prevalent Infections: (Flu-like syndromes, URTI infections, hepatitis (A, B & C), use of antibiotics and antiviral drugs, others) - Seasonality - Number of vaccines received - Place of residence

6. Procedures for the minimization of biases in data collection and management

6.1. Practices and Procedures

PGRx complies with the Good Pharmacoepidemiological Practices (GPP) issued by the International Society for PharmacoEpidemiology (ISPE) revised in 2004 (http//www.pharmacoepi.org/resources/guidelines_08027.cfm). The PGRx Standard Operating Procedures are applied, both to data collection and data management.

6.2. Minimisation of selection bias

Several techniques are used to limit and/or assess the extent of this potential bias:
Recruiting centres are instructed to report all cases to PGRx, whatever their exposure, during their time of participation in the system. External sources of information on the recruitment of patients are sought for in each centre. The number of patients included is compared to the expected number in each centre and reasons for deviations are discussed with investigators. The sites recruiting autoimmune disorders are visited very frequently (on a bi-monthly basis on average) by trained clinical research assistants to elicit reporting and try and document non reported cases.

6.3. Minimisation of information bias

6.3.1. Classification of case/referent status

- The exclusion of the occurrence of a previous Guillain-Barré syndrome diagnosis in cases and referents is achieved through 2 sources (physician and patient). The data collected on the selected referents will further be checked for the presence of elements in favour of neurologic disorders (co-morbidities, personal histories, symptoms spontaneously reported, drug use). Any referent with a possible or definite antecedent or presence of Guillain-Barré syndrome will be excluded from the set of referents.

6.3.2. Classification of exposure status

- 100% of exposure considered in the study is uses confirmed with an objective source as described in section 4.4.2.
- Index date: two sources of information are used to define the index date (the medical form filled by the physician and the interview of the patient).

6.4. Information collected on potential confounders

Information on family history of AID is especially collected for this study, as patients with a family history of auto-immune disease may be at a lower probability of being vaccinated while having a higher probability of developing the disease and/or the vaccine may interact with a familial predisposition to develop the disease. It is however anticipated that the frequency of this risk factor in referents is expected to be very low.

7. Statistical issues

7.1. Sample size

7.1.1. Recruitment expected in PGRx

Table 5 identifies the number of female cases 14-26 years old with the disease expected per year and for 3 years in PGRx and the corresponding number of referents on average. This number was first derived from the declarations of the investigators of the first centres entered in the PGRx system and is consistent with the actual recruitment reported in Appendix A2.

Table 5 also reports the date of first case recruitment and the expected date of termination (3 years after) under two scenarios of recruitment. According to the centres recruited into the study, only 9 female cases 14-26 years old are expected over three years (scenario A). This sample size is not sufficient to plan a case-control analysis. However these expectations are subjective. The actual recruitment seems a bit higher (7 cases over 11 months). If at least 15 cases are reported over the whole study period (Scenario B), a case-control assessment would be conducted (with 60 referents: scenario B) if the referents exposure allows it.

Table 5: Expected number of cases and referents for Guillain-Barré syndrome in PGRx and dates of start and of expected end of the study

Scenario	Females 14-26 y.o Cases/.y. N	Females 14-26 y.o Cases/. 3 y. N	Matched Referents 3 y. N	Date 1st effective surveillance	Expected Date end
A	3	9	NA*		
B	5	15	60	"	"

7.2. Exposure estimation

7.2.1. Expected rates of exposure

For the time-window of 2 months, the mean expected rate of exposure in the referents is estimated at xxxx%.

Table 6: Estimated exposures to the vaccine used for power calculation according to the time window considered

	24 months*	6 months**	2 months***
Expected % of referents exposed in the time-window			

* Not tested for the study of Guillain Barre Syndrome
** Secondary time-window for the study of Guillain Barre Syndrome
*** Primary time-window for the study of Guillain Barre Syndrome: Rate exposure in referents too small

7.3. Odds ratios detectable

7.3.1. Direction of effect

The scientific committee has considered that some vaccines may as well decrease or increase the risk of auto-immune disease. Statistics are consequently presented as two-sided.

7.3.2. Power to detect

8. General Analytical Plan

Analysis will be performed with the SAS 9.1.3 Service Pack 4, Windows version 5.1.2600 (copyright © 2003 SAS Institute Inc. Cary, NC 2713, USA) or a more recent version if it becomes available.

8.1. Descriptive Analysis

Cases and referents will be described for the variables listed in the previous sections of this protocol, including socio-demographics (age, region, ethnicity, socio-economic status) clinical features (according to Table 2); presence of severe co-morbidities; individual risk factors (see below); exposure to Cervarix® vaccine (by time-windows), separately by age (<18; ≥ 18 y.o) and case/referent status.

8.2. Univariate comparisons

8.2.1. Risk factors to be considered a priori

The distribution of the risk factors listed in Table 4 plus other risk factors that may arise in the literature and are retained by the Scientific Committee before the analysis (if available in PGRx) will be described in cases and referents.

8.2.2. Risk factors to be listed *a posteriori*

Classes of drugs and categories of co-morbid conditions will be tested for their difference in distribution between cases and referents. Any of these variables associated with case/referent status with a $p<0.1$ will be retained for the main multivariate model analysis.

8.2.3. Assessment of potentially strong confounders or risk factors

Matched odds ratios for exposure will be compared between sets of subjects presenting with and without the confounders identified *a priori* and *a posteriori* The position of the observed odds ratios will be examined (within or outside the interval) and decision taken on the analysis. If the number of cases and referents with the potentially strong confounders do not allow for an

adequate control of their influence through modelling, the sample of sets used in the modelling for the sensitivity analysis will be censored of those with at least one subject presenting with the confounder. – The same approach will be applied by the comparison of odds ratios for exposure to the vaccine in strata of 25th, 50th, 75th, 100th percentile of 'multivariate confounding scores'.

8.3. Modelling and Analysis using Multiple variables

8.3.1. Main model

All retained risk factors identified will be used in a multiple modelling of the risk of Guillain-Barré syndrome associated with exposure to Cervarix®. A priori suspected and risk factors identified a posteriori from the univariate analyses will be controlled for. The analysis will be also controlled for the use of another HPV vaccine reimbursed in France[6]. The risk associated with the number of shots received will be assessed.

Results will be presented as adjusted odds ratios with their 95% confidence intervals (two-sided, estimated with 80% power).

The model considered is the conditional logistic regression for the assessment of relative risks through odds ratios.

8.4. Analysis performed for the identification of biases

A series of descriptive analyses will be performed to identify potential biases. No results will be reported as arising from these analyses. Statistical tests will be applied when possible to help in the interpretation of potential differences or interactions.

8.4.1. Selection bias

- Participant patients will be compared to non-participants on age, time and centre.

- Centres will be described for their recruitment, percentage of rejected cases, and the mean exposure to Cervarix® in the patients reported. Face comparisons between centres will be made on the mean exposure prevalence. Cases rejected and interviewed will be compared to retained cases and to referents for their use of Cervarix®

Decision will be taken by the Scientific Committee to retain or reject centres with obvious outlying results in the above analyses.

8.4.2. Information bias

- Diagnostic bias:

Referents identified with any elements in favour of a disorder consistent with or evocative of the disease, including its *forme fruste*, will be excluded from the set of referents. Exposure to vaccine reported in the patients' interviews will be compared to prescriptions

[6] Gardasil®

recorded by the physicians. A separate study of the validity of exposure ascertainment in PGRx is conducted. Its results will be presented to the Scientific Committee and potential consequences for the study protocol considered before the final analysis

8.5. Timing of the analysis

8.5.1. Planned analysis

The main analysis will be performed at 36 months after the first index case included in the PGRx system. This delay may be extended if necessary to achieve the recruitment of the sample size displayed in Table 5.

8.5.2. Unplanned analysis

An unplanned analysis may be performed before the end of the study:

- At the request of the Health Authorities and with the formal agreement of the Cervarix Scientific Committee.
- Or at the request of the Cervarix Scientific Committee, justified by a possible alert identified in the literature or through pharmacoviligance reports.

This unplanned analysis will use all the methods described in the analytical plan and will be applied to the sets of cases and referents satisfactorily documented and to the data considered as consolidated at that time.

Whatever the results of this unplanned analysis, the study will be pursued until the planned completion since, according to the assumption of this study; cases may arise as far as 24 months after exposure.

9. Discussion of the general study methodology

9.1. Limits of observational research

Biases associated with medical practice

This study presents limitations associated with observational research such as possible indication bias for the vaccine and preferential diagnosis in exposed. While the first one is more likely to bias the results towards a lesser risk associated with vaccination in the present context, the second may act in the reverse direction. These two biases are associated with medical practice rather than with the study methods itself and may also be present in so-called 'record-linkage' or medical database research as they pertain to the nature of medical activity. Note than they are also present in unblinded cohort studies. Only double blind randomised clinical trials may completely eliminate their effect, when the blind is not actually broken in practice. The feasibility of such trials to assess the incidence of a rare disease like Guillain-Barré syndrome is very low (published trials did not actually have the power to do so). The ethical justification of larger trials in this respect is debatable in the absence of any alert.

The very high specificity of the diagnosis and the potential comparisons between the various degrees of certainty in the diagnosis, as well as the medical information recorded for both cases and referents will provide useful information on this respect. Documenting for a number of potential confounders such as family history of disease or behavioural confounders will help in minimizing the effect of indication bias.

9.2. Limits of field case-referent studies

As opposed to studies nested in medical or prescription databases, the field case-referent nature of recruitment raises the question of potential selection bias, *i.e.* the preferential recruitment into the study of cases associated with exposure. The selection bias of concern here is notoriety bias where cases exposed to Cervarix® would be more likely to be reported than other, non-Cervarix®, patients. This would bias the results away from the null. The PGRx methodology, by collecting cases systematically in the absence of any alert, and announcing the surveillance of *ca.* 300 drugs to clinicians, limits the potential extent of this bias as compared to ad hoc case-referent studies. Important efforts are devoted at minimising this bias (section 7.2) and assessing its potential magnitude (section 9.4.1).

Note that the case-referent methodology allows for a volume of recruitment which is possible only with very large databases, especially if only definite cases of the disease are considered.

9.3. Nature of referents

The use of physicians as the source of referents in PGRx is a compromise between population-based referents and hospital based referents. They have been successfully used in pharmacoepidemiology (Abenhaim, 1996). Sampling of population-based referents may provide more valid estimates of exposure and behavioural risk factors than sampling of patients visiting physicians, but they are less likely to provide valid information on co-morbidities, antecedents and medical risk factors than the data collected through physicians. Also, the objective source of information on vaccination through medical records may be of great help in this instance. Hospital-based referents are frequently used because of the convenience of sampling and on the assumption that they may help control for referential biases. They are however frequently associated with exposure and reporting biases, as well as with actual referential bias. The pool of potential referents recruited in PGRx is less subject to this later bias while offering a convenient source of sampling of referents to be matched to the cases.

The matching of referents to cases on the number of visits to physician limits the extent of a bias associated with increased opportunity to exposure which may be feared with physician-based referents as opposed to population-based referents (although this bias is less likely to play a role in the contemplated age groups here). Another, to a certain extent symmetrical, concern is the so-called ‘overmatching’. Overmatching is not a validity bias but may impair the efficiency of a study.

9.4. Information biases

For the case/referent status, the specificity achieved in PGRx for the diagnosis of cases and also for the exclusion of referents with history of the disease at hand is very high as compared to any systematic collection of data available, especially in comparison to so-called 'record-linkage' databases or usual medical databases.

The infamous 'recall bias' feared in studies using retrospective interviews is limited in this study as 100% of reported exposure will have to be based on objective information or documentation. The use of two sources of data on drug use (patients and physicians) helps in this process. A separate validation study of the validity of the ascertainment of exposure in PGRx is planned. Its results will be made available to the Scientific Committee before the final analysis is conducted.

A comparison of observed exposure of referents to expected exposures based on the data available at the end of the study on the reimbursement of vaccination will allow for the documentation of these biases if they exist. A crude case-population comparison of exposure will be done using these reimbursement data for the assessment of the exposure of the base population and the results compared with those obtained in this case-referent study.

9.5. Residual confounding

Few potentially strong risk factors are known for the diseases at hand (personal and familial history of auto-immune disorders, the existence of severe chronic co-morbidities, ethnicity, and some drugs). Whether they may interact with vaccination and/or represent potential confounders of an association is unknown. Personal or familial history of AID is thought to lower the probability of vaccination, but no data is available on this subject. All these variables are expected to have low or very low prevalence in the sample.

Despite the statistical procedures listed above, in addition to the matching of referents to cases, to minimize and control for the effect of potential confounders, it is always possible that some residual confounding may still exist at the end of the study. The potential magnitude of this residual confounding effect and its likelihood to explain any potential observation or association will be discussed based,

10. Timelines & Reports

Item	Date
Network of PGRx central demyelination Centres	Done On-going for paediatric centres
Recruitment of 1st case	
Recruitment of potential Referents	On-going
Finalisation of PGRx Guillain-Barré syndrome -Cervarix® protocol	May 2009
1st Annual Descriptive report and blind analysis	
2nd Annual Descriptive report and blind analysis	
Final PGRx Guillain-Barré syndrome - Cervarix® Study report	

Recruitment reports are issued every month. Descriptive reports provide data on all the variables listed in the document.

Persons in charge of the analysis and reports

The statistical analysis and reports will be conducted under the supervision of Profs. [redacted], [redacted], and Dr [redacted].

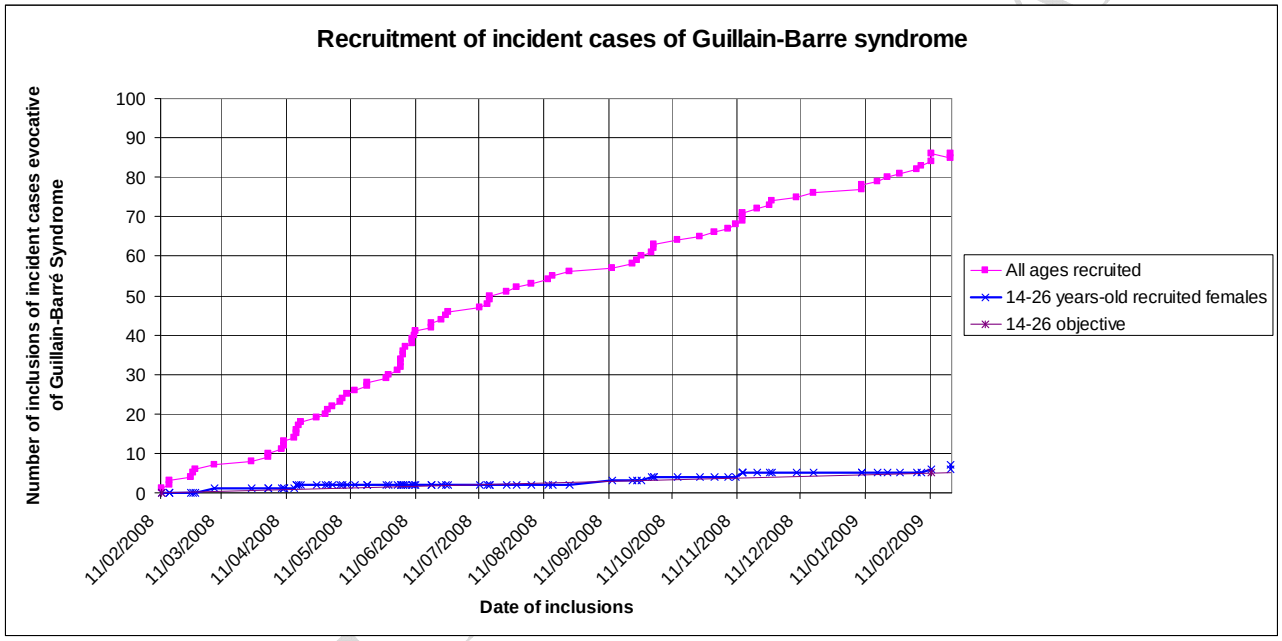
Appendix 1: Exhibit 1A: PGRx Information System General Methodology

Appendix 2: Recruitment of Guillain-Barré syndrome in PGRx

Table A2.1 Recruitment of Guillain-Barré syndrome cases in the PGRx System as of March 2, 2009

	Date of first inclusion	Participating centres	Cases (all age groups)	Recruited female cases 14-26 y.-o.	Target recruitment Females cases of 14-26 y.-o.	
					per year	3 years
Guillain-Barré syndrome	11/02/2008	20	86	7	3	9

Figure A2.1 Recruitment of Guillain-Barré syndrome cases in the PGRx System as of March 2, 2009



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