PGRX

STUDY OF CERVARIX®
& IDIOPATHIC THROMBOCYTOPENIC PURPURA

USING THE PGRx INFORMATION SYSTEM

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PGRx
Centre for Risk Research Inc.
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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 idiopathic thrombocytopenic purpura ("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' 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.

[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).

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 idiopathic thrombocytopenic purpura, 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 idiopathic thrombocytopenic purpura

Idiopathic (or immune) thrombocytopenic purpura (ITP) is an acquired disease related to the presence of platelet auto-antibodies. ITP is characterized by a decrease of the platelet count and in its more severe forms by bleeding symptoms.

Idiopathic thrombocytopenic purpura has two different presentations: one acute form more frequent in childhood and one chronic form predominant in adults (Fogarty, 2007):

Childhood ITP

- Acute form predominant;
- Often follows a viral infection;
- In 68–100% of cases the disorder resolves spontaneously within 6 months;
- Some 15–20% of children develop a chronic form;
- Incidence of acute ITP range between 4.0–5.3/ 100 000 children-year;
- Incidence of chronic ITP is 0.46/100 000 children-year;
- Prevalence of chronic ITP is 4.6/100 000 children-year;
- Mean age at diagnosis: 5.7 years.

Adult ITP

- Chronic form predominant;
- With an insidious onset;
- Rate of fatal hemorrhage < 5%;

- incidence of adult chronic ITP range between 5.8–6.6/100 000 person-year;
- 5-11% of adult ITP present a remission (after a median time of 4 years);
- Female/male ratio: 3/1;
- More frequent in young adults but can be observed in elderly.

Current diagnostic standards of Idiopathic thrombocytopenic purpura

The diagnosis of immune thrombocytopenic purpura is a process of elimination of other immune and nonimmune causes of thrombocytopenia (Geddis, 2007).

Guidelines from the British Society for Haematology

Recommendation for adults (Guidelines, 2003):

- The diagnosis of ITP is based principally on the exclusion of other causes of thrombocytopenia using the history, physical examination, blood count, peripheral blood film, autoimmune profile and other investigations. Further investigations are not indicated in the routine work-up of patients with suspected ITP if the history, examination, blood count and film are typical of the diagnosis of ITP and do not include unusual features that are uncommon in ITP, or suggestive of other causes.
- A bone marrow examination is unnecessary in adults unless there are atypical features, or the patient is over the age of 60 years, or the patient relapses following complete remission, on or off therapy, or splenectomy is being considered.
- PAIg is elevated in both immune and non-immune thrombocytopenia and therefore has no role in the diagnosis of uncomplicated ITP.
- It is worth determining the presence of H. pylori in patients' refractory to therapy since some patients have shown improvement in platelet counts following eradication therapy.

Compulsory elements for the diagnosis of ITP

Clinical history and physical examination may define if differential diagnosis is present:

- Post-surgery bleeding, post-traumatic bleeding;
- Post-transfusion purpura;
- Excess alcohol consumption;
- Family history of thrombocytopenia;
- Type IIB van Willebrand's disease;
- Autoimmune disorders;
- Lymphoproliferative disease and other malignancy.

The physical examination

Physical examination can find general symptoms like fever, nausea, vomiting. They can precede or be concomitant of bleeding symptoms. Purpura and bleeding from the nose, gums, and gastrointestinal or urinary tract can be seen.

Clinical severity depends on the localization (intracranial), extension and duration of bleeding.

At diagnosis the grade of disease severity is based on the clinical signs. is possible to see different clinical levels of severity with the same level of platelet count.

Blood count

Diagnosis of ITP is based on a platelet count lower than 150×10^9/L. The confirmation of thrombocytopenia in two separate platelet assays is required (Ruggeri, 2008).

The platelet count thresholds to define severe, moderate and mild ITP are:

- severe platelet count $\leq 5\times10^9$/L
- moderate platelet count between 5 to 30×10^9/L
- mild platelet count upper 30×10^9/L

Facultative elements for the diagnosis of ITP

Bone marrow examination

When atypical findings are found with the compulsory elements for diagnosis, a bone marrow examination could be done to precise the origin of thrombocytopenia.

Anti-platelet antibodies

Research of platelet auto-antibodies can be useful in case of atypical form of ITP but is not mandatory in routine.

1.4. Drugs allegedly associated with ITP

1.4.1. All drugs

Drug-induced thrombocytopenia has an acute onset and a platelet count $\leq 20\times10^9$/L. They are supposed to be caused by platelet destruction secondary to drug-induced antibodies.

Heparin-induced thrombocytopenia is the most common drug-related cause of thrombocytopenia. Other drugs are likely to trigger drug-induced thrombocytopenia (Aster, 2007):

- Cinchona alkaloids (Quinine, quinidine);
- Platelet inhibitors (Abciximab, eptifibatide, tirofiban);
- Antirheumatic agents (Gold salts D-penicillamine);
- Antimicrobial agents (Linezolid, rifampin, sulfonamides, vancomycin) ;
- Sedatives and anticonvulsant agents (Carbamazepine, phenytoin, valproic acid Diazepam);
- Histamine-receptor antagonists (Cimetidine, Ranitidine);
- Analgesic agents (Acetaminophen, diclofenac, naproxen Ibuprofen);
- Diuretic agents (Chlorothiazide, Hydrochlorothiazide);
- Chemotherapeutic and immunosuppressant agents (Fludarabine, oxaliplatin Cyclosporine, rituximab).

After exposure to the supposed trigger drug, the period before presenting with clinical signs of thrombocytopenia could be about 1 week or longer. In some cases, symptoms could develop within 1 or 2 days after the exposure.

After the exposure stop time before symptoms disappear and the platelet count return to normal depend on the type of exposure and the age of the patient.

Some times hemolytic–uremic syndrome or thrombotic thrombocytopenic purpura can complicate the initial ITP with the occurrence of disseminated intravascular coagulation or renal failure.

1.4.2. Time windows at risk used in studies

In the above mentioned studies, time-windows varying from 1 month to six months have been used for the study of the relation between idiopathic thrombocytopenic purpura and vaccines.

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

Table 1: Epidemiology of idiopathic thrombocytopenic purpura and data stemming the literature review

Socio-demographics (age, gender)	Children: Mean age at diagnosis 5.7 years Female/male ratio: 3/1
Incidence	Children: - Incidence of acute ITP: 4.0–5.3/ 100 000 children-year; - Incidence of chronic ITP: 0.46/100 000 children-year; Adults: - Incidence of chronic ITP 5.8–6.6/100 000 person-year;
Prevalence	Children: Prevalence of chronic ITP 4.6/100 000 children-year
Time to event tested	1 month, 6 months (acute ITP)

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
- 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

[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

A case of idiopathic thrombocytopenic purpura is defined as the association of:

- Acute onset of symptoms due to thrombocytopenia, or fortuitous discover of thrombocytopenia with a previous history of a normal platelet count.
- Possibly presence of typical clinical signs: petechiae, bruising or bleeding.

2.4.3. Definition of definite possible and rejected cases

Cases of idiopathic thrombocytopenic purpura are ascertained by the algorithm in table 2.

Table 2: Case definitions for the study

	Elements for definition		
	Normal platelet count in the previous 12 months	**Clinical signs**	**Platelet count at inclusion**
Definite cases	Documented	Acute hemorrhage syndrome	$\leq 50.10^9$/L
	Documented	Fortuitous discover of thrombocytopenia	$\leq 50.\ 10^9$/L
Possible cases	Not documented	Acute hemorrhage syndrome	$\leq 50.\ 10^9$/L
Rejected cases	Not documented	Fortuitous discover of thrombocytopenia	whatever the platelet count

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.
- 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

[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 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

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 ITP

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

Risk factors considered a priori
- Family history of autoimmune disorder (1st degree) - Geographical origin - 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 thrombocytopenic disorders (co-morbidities, personal histories, symptoms spontaneously reported, drug use). Any referent with a possible or definite antecedent or presence of thrombocytopenia 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 idiopathic thrombocytopenic purpura 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 6 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 ITP
** Primary time-window for the study of ITP
*** Secondary time-window for the study of ITP: Rate exposure in referents too small

.3. Odds ratios detectable

7.3.1. Direction of effect

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 idiopathic thrombocytopenic purpura 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

[6] Gardasil®

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.

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
Finalization of PGRx idiopathic thrombocytopenic purpura -Cervarix® protocol	May 2009
1st Annual Descriptive report and blind analysis	
2nd Annual Descriptive report and blind analysis	
Final PGRx idiopathic thrombocytopenic purpura -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] and Dr [redacted].

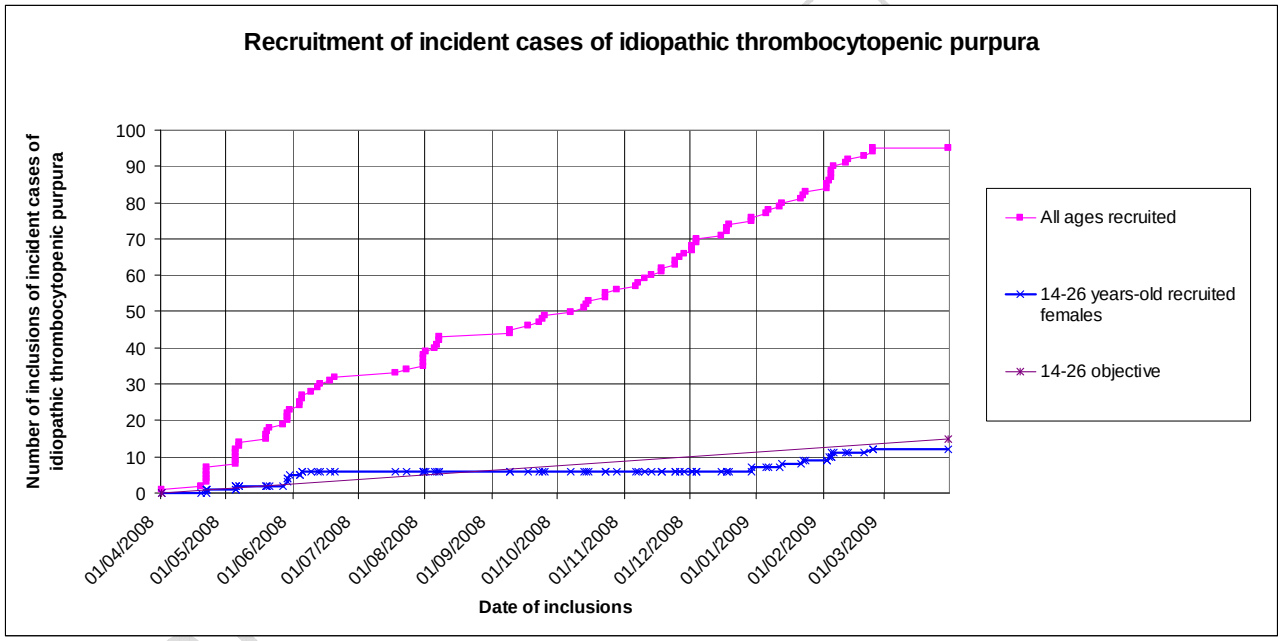
Appendix 1: Exhibit 1A: PGRx Information System General Methodology

Appendix 2: Recruitment of idiopathic thrombocytopenic purpura in PGRx

Table A2.1 Recruitment of idiopathic thrombocytopenic purpura cases 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
Idiopathic thrombocytopenic purpura	01/04/2008	13	95	12	15	45

Figure A2.1 Recruitment of idiopathic thrombocytopenic purpura cases in the PGRx System as of March 2, 2009



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PGRx

STUDY OF CERVARIX® & INFLAMMATORY ARTHRITIS

USING THE PGRx INFORMATION SYSTEM

VERSION 2

February 26, 2009

PGRx
Centre for Risk Research Inc.
LA-SER sarl

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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 inflammatory arthritis ("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 inflammatory arthritis, 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 inflammatory arthritis

Worldwide, rheumatoid arthritis (RA) is the most common form of chronic inflammatory arthritis. In France, RA prevalence rate in adult has been estimated at 0.4% (0.6% among women, 0.1% among men) approximately corresponding to 150 000 cases in France (Kahn MF, from the national public health insurance database, 2004). Kahn has also estimated the RA incidence rate of 20/100 000 person/year (>20 years) that seems to be constant over the past 20 years; the female to male ratio is 2 or 3:1, and decreases with age. The peak age specific incidence rate by sex is 55-64 years for women and 65-75 years for men, with an important standard deviation (about 25 years). The prevalent rates vary between regions with a north-south gradient in Europe: 0.6% in Finland, 0.32% in France and 0.13% en Italy (Symmons, 2002).

The paediatric forms of chronic inflammatory arthritis are heterogeneous and are named Juvenile Rheumatoid Arthritis (JRA). In France JRA incidence rate ranges between 1.3 and 3.2/100 000/ year. In Norway incidence rate ranges between 0.8 and 22.6/100 000/ year in children less than 16 years old. Cooper (2003) averages incidence rates to 17/100 000 person-

year (USA 11.7/100 000 between 1960 and 1993, and Norway 22.6/100 000 between 1985-1994) and prevalence rates to 148/100 000. In France, prevalence lies between 10 and 20/100 000. In Australia prevalence was estimated between 8 and 400/100 000 for children less than 16 years. Problems with the case definition and their identification may explain such results. The most frequent form is oligoarthritis (40% of JRA), followed by polyarthritis with negative rheumatoid factor (22%), spondylarthropathy (18%), and the systemic forms (9%). Age of onset and sex ratio are different between these diseases (for instance, oligoarthritis concerns young girls of 4-6 years old). Anti-nuclear antibodies are present in 80% of patients with oligoarthritis.

1.3.2. Risk factors associated with inflammatory arthritis

Risk factors for RA are genetic (60% contribution) and environmental (Symmons, 2002; Aho, 2004):

- Hereditary predisposition: brother of a homozygote twin with a RA has a risk of 15% of developing RA. Genes from the HLA-DR group are involved in this predisposition; they are more frequent among homozygote twins.
- Rheumatoid Factor (RF) is an IgM type auto-antibody. Its specificity is lower than the anti-cyclic citrullinated peptide (CCP) antibodies. Patients with RF antibodies have worst prognostic than patients without RF, and RA patients are categorized between these two groups.
- Estrogens may have a protective effect because patients treated with oral contraceptive slow down the evolution of the disease, and remission of RA is induced during pregnancy.
- Tobacco consumption is a risk factor for all inflammatory arthritis with positive rheumatoid factor. It could be associated with the severity of the disease.

1.4. Drugs allegedly associated with inflammatory arthritis

1.4.1. All drugs

Bannwarth (2007) reviewed the literature on drugs and vaccines potentially associated with the occurrence of rheumatoid arthritis. There is no evidence of a link between vaccines and the occurrence of this disease. A case-control analysis in the GPRD studied the association between hepatitis B vaccination and arthritis and did not observe any association.

1.4.2. Time windows at risk used in studies

In the above mentioned study, time-windows varying several months to several years have been used for the study of the relation between inflammatory arthritis and vaccines.

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

Table 1: Epidemiology of inflammatory arthritis and data stemming the literature review

Socio-demographics (age, gender)	Adults: 55-64 years for women and 65-75 years old for men 2 or 3 women / 1 man Children: Young girls of 4-6 years old
Incidence	France: - Adults: $20/10^5$ inhabitants / year - children: 1.3 to 3.2/100 000/ year Children: - USA:11.7/100 000 (1960-1993) - Norway: 22.6/100 000 (1985-1994)
Prevalence	France: 0.4%
Time to event tested	≤1 year, ≥1 years, ≥5years

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
- 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

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

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 inflammatory arthritis reported as having occurred in the previous twelve months before the recruitment consultation.
Patients presenting with a spondylarthritis or intermittent or palindromic rheumatism or psoriasis are excluded.

2.4.4. Summary table for case definition

Cases of inflammatory arthritis are ascertained by the algorithm simplified in table 2:

Table 2: Case definition for the study

	Clinical presentation	Auto antibodies and imaging
Definite cases	At least 3 of the following criteria : ▪ Inflammatory pain chronology ▪ More than 2 joints with synovitis and/or pain ▪ Involvement of joints of the hand ▪ Bilateral disorders **AND** disorder present for at least 6 weeks	With or without anticitrullinated peptide antibodies With or without Rheumatoid factor With or without radiological articulation destruction
Possible cases	At least 2 of the 4 criteria above **AND** disorder present for at least 6 weeks	With or without anticitrullinated peptide antibodies With or without Rheumatoid factor With or without radiological articulation destruction

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.
- 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.

[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 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

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 inflammatory arthritis

	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 inflammatory arthritis

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 chronic inflammatory arthritis 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 rheumatologic disorders (co-morbidities, personal history, symptoms spontaneously reported, drug use). Any referent with a possible or definite antecedent or presence of chronic inflammatory arthritis 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 inflammatory arthritis 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
10	30	120	July 2009	July 2009

7.2. Exposure estimation

7.2.2. Expected rates of exposure

For the time-window of 24 months, the mean expected rate of exposure in the referents is estimated at 5%.

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 inflammatory arthritis 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
30	120	xxxx%		

* 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 inflammatory arthritis 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 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 inflammatory arthritis Centres	Done, and on-going for paediatricians' centres
Recruitment of 1st case	April 2008
Recruitment of potential Referents	On-going
Finalisation of PGRx inflammatory arthritis -Cervarix® protocol	May 2009
1st Annual Descriptive report and blind analysis	
2nd Annual Descriptive report and blind analysis	
Final PGRx inflammatory arthritis - 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].

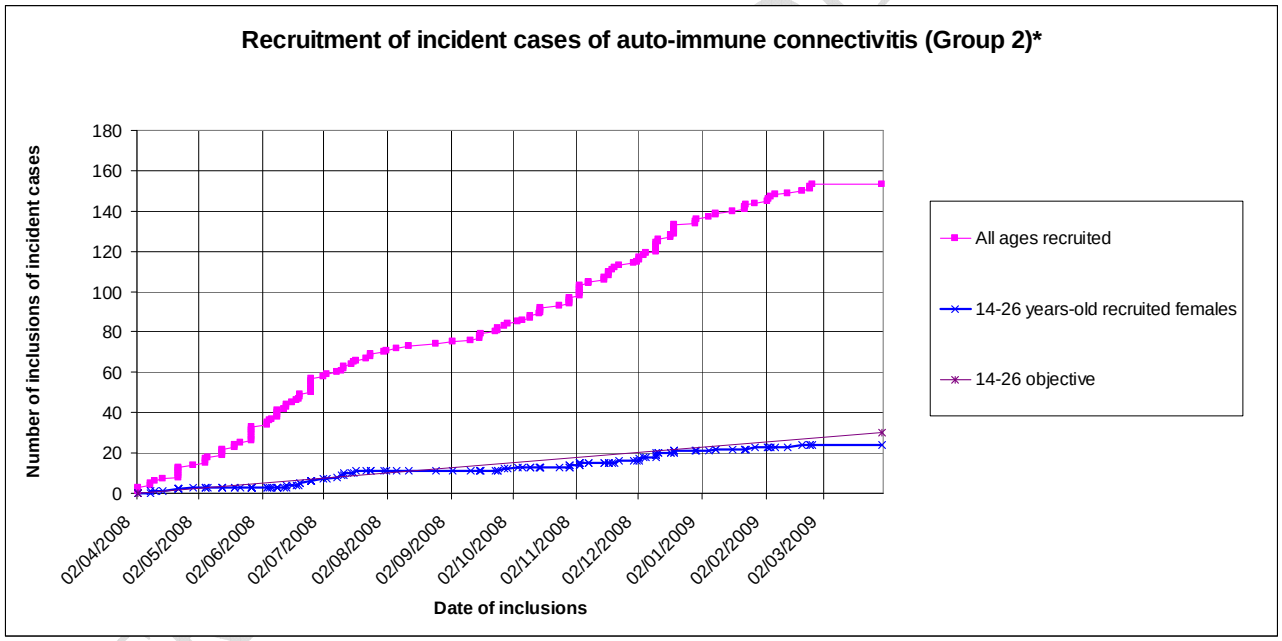
Appendix 1: Exhibit 1A: PGRx Information System General Methodology

Appendix 2: Recruitment of inflammatory arthritis in PGRx

Table A2.1 Recruitment of inflammatory arthritis cases 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 2 (inflammatory arthritis, lupus, myositis)			153	24	30	90
Inflammatory arthritis	02/04/2008	15	63	5	-	-

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



* Group 2 : Incident cases of lupus erythematosus, inflammatory arthritis, myositis and undifferenciated connectivitis and scleroderma

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PGRx

STUDY OF CERVARIX® & MYOSITIS AND DERMATOMYOSISTIS

USING THE PGRx INFORMATION SYSTEM

February 26, 2009

PGRx
Centre for Risk Research Inc.
LA-SER sarl

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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 myositis and dermatomyositis ("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 myositis and dermatomyositis, 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 myositis

The incidence rate of myositis (polymyositis, dermatomyositis, inclusion-body myositis) ranges from 0.2 and 1 per 100 000 person-year (Dalakas, 2003 & Briani, 2006). Differences in case definition can explain the discrepancies between studies. The frequency of myositis as a stand-alone disorder or in association with other systemic diseases remains unknown. Few prevalence data is available. In the USA, myositis incidence rate is 1.8 per 100 000 person-year and prevalence rate is 5.1 per 100 000 (Jakobson, 1997).

The female to male sex ratio varies between the different forms of myositis. For all myositis, the female/male ratio is about 2/1, for juvenile forms the ratio is 1/1 and 9/1 in the forms associated with other connective tissue diseases. Polymyositis is seen after the second decade of life. Inclusion-body myositis is more common in men over the age of 50 than in other population groups. In all age-groups, dermatomyositis is the most common form and polymyositis the least common; inclusion body myositis is the commonest myopathy above the age of 50.

Dermatomyositis affects both children and adults, and women more than men. In children, dermatomyositis is the most frequent inflammatory myopathy whereas polymyositis is very rare.

1.3.2. Risk factors associated with myositis

Genetic factors may have a role, as suggested by rare familial occurrences and association with certain HLA genes (DRB1 and HLA DQA1) (Dalakas, 2003).
The association between myositis and the development of malignancy had been described (Benveniste, 2007 &Levine, 2006).

1.4. Drugs allegedly associated with myositis

1.4.1. All drugs

Drug-induced muscle disorders show a large spectrum of clinical presentations from asymptomatic elevated serum muscles enzymes levels, to severe myopathies. Drug-induced myopathy usually develops insidiously. The onset of clinical manifestations can occur from days to months after exposure to the causative agent. Commonly, patients present with nonspecific complaints of progressive, generalized muscle weakness, muscle pain (myalgia), or fatigue. The clinical and histopathological features depend on the causative agent and individual susceptibility.
Many drugs have been implicated as causes of myopathy, including lipid-lowering agents (particularly statins), antimalarials, colchicine, corticosteroids, penicillamine, zidovudine, and drugs associated with abuse, such as alcohol and cocaine.
For statins the risk of myopathies has been formally shown and warnings are issued by the manufacturers (Bannwarth, 2002).

1.4.2. Time windows at risk used in studies

No epidemiological study has been conducted for the relation between myositis and vaccines.
Table 1 summarizes the main features stemming from the literature review.

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

Socio-demographics (age, gender)	All ages Female/male ratio is about 2/1, For juvenile forms the ratio is 1/1
Incidence	USA 1963-82: $5{,}5.10^{-6}$ (Oddis, 1990); $1.8.10^{-5}$ person.year (Jakobson, 1997); $0.2\text{-}1.10^{-5}$ person.year (Dalakas 2003, Briani 2006)
Prevalence	USA: 5.1 per 100 000
Time to event tested	-

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, histopathological 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.

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

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 myositis and dermatomyositis:

- ✓ Reported as having occurred in the previous twelve months before the recruitment consultation.
- ✓ And with clinical and biological presentation compatible with the onset of myositis and dermatomyositis.

2.4.3. Summary tables for case definition

Table 2 presents the algorithm for the case definition for the study for incident myositis and dermatomyositis evocative disorders.

Table 2: Case definition for the study of incident myositis or dermatomyositis evocative disorders (Adapted from Hoogendijk, 2004)

	Arguments
Definite cases	Proximal Weakness OR Pain OR specific (eyelids heliotrope rash, hands Gottron papules) rash **AND** Raised CPK blood levels OR positive EMG **AND** Positive biopsy, inflammation OR necrosis **Accepted Diseases** - Polymyositis (pure) - Polymyositis associated with arthritis, interstitial lung disease, Raynaud phenomenon, lupus, or scleroderma anti-synthetase (OR anti-SRP OR anti-Mi2) antibodies - Dermatomyositis
Possible cases	Proximal Weakness OR Pain Biopsy with normal standard optic microscopy (AND Widespread muscle cells HLA-class I molecules hyperexpression OR C5b-9 muscle capillary deposits)
Rejected cases	Paraneoplastic myositis Cancer

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.
- 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.

[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 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

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 myositis evocative disorders

	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 incident myositis and dermatomyositis evocative disorders

Risk factors considered a priori
- Family history of autoimmune disorder (1st degree) - Geographical origin - 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 myositis 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 myositis evocative disorders (co-morbidities, personal histories, symptoms spontaneously reported, drug use). Any referent with a possible or definite antecedent or presence of myositis 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 myositis or dermatomyositis evocative disorders 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
5-10	25	100		

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	

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 myositis and dermatomyositis 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
25	100			

* 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 myositis and dermatomyositis evocative disorders 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 myositis 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 myositis and dermatomyositis Centres	Done On-going for paediatric centres
Recruitment of 1st case	
Recruitment of potential Referents	On-going
Finalisation of PGRx myositis and dermatomyositis -Cervarix® protocol	May 2009
1st Annual Descriptive report and blind analysis	
2nd Annual Descriptive report and blind analysis	
Final PGRx myositis and dermatomyositis -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] and Dr [redacted].

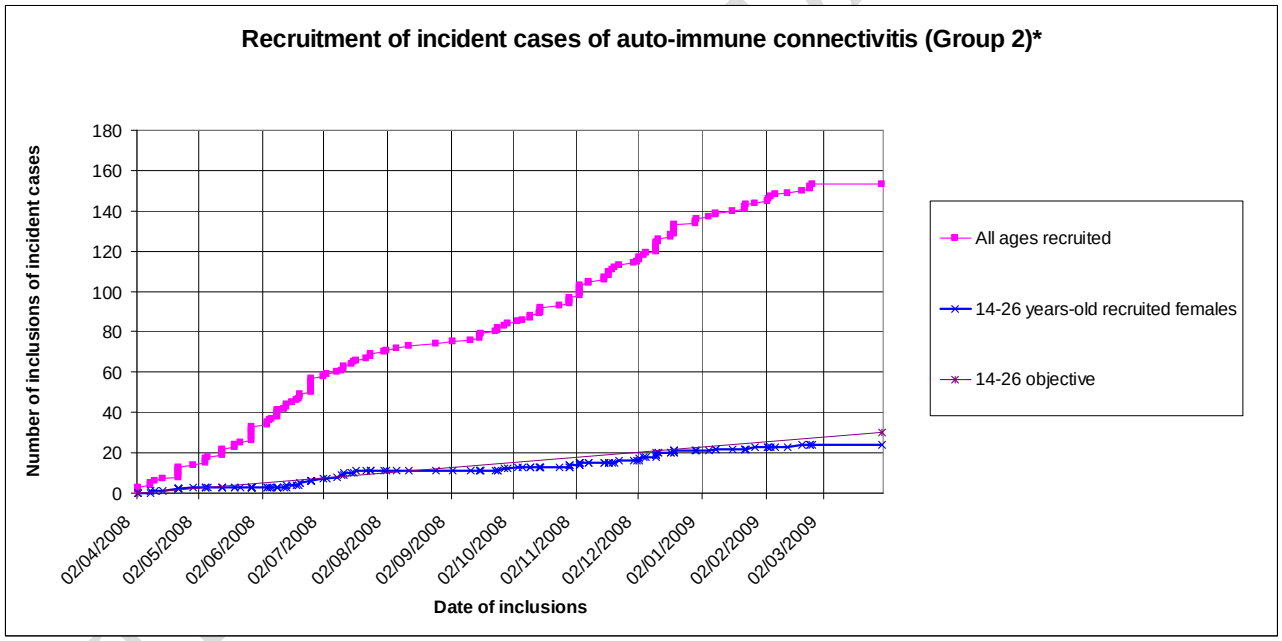
Appendix 1: Exhibit 1A: PGRx Information System General Methodology

Appendix 2: Recruitment of myositis and dermatomyositis in PGRx

Table A2.1 Recruitment of cases of myositis and dermatomyositis evocative disorders 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 2 (inflammatory arthritis, lupus, myositis)			125	20	30	90
Cases of systemic disorders evocative myositis	10/04/2008	20	25	-	-	-

Figure A2.1 Recruitment of of cases of myositis and dermatomyositis evocative disorders in the PGRx System as of March 2, 2009



* Group 2 : Incident cases of lupus erythematosus, inflammatory arthritis, myositis and undifferenciated connectivitis /incident scleroderma

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PGRx

STUDY OF CERVARIX®
& SYSTEMIC LUPUS ERYTHEMATOSUS

& ADDENDUM FOR CUTANEOUS LUPUS

USING THE PGRx INFORMATION SYSTEM

February 26, 2009

PGRx
Centre for Risk Research Inc.
LA-SER sarl

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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 lupus erythematosus ("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 lupus, 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 lupus erythematosus

In France lupus erythematosus 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).

1.3.2. Risk factors associated with lupus erythematosus

Factors that have been reported associated with lupus erythematosus occurrence or as trigger factors are exposures to sunlight and ultraviolet rays and viral infections, particularly Epstein-Barr virus (Tsao, 2003; Lawrence, 1987; Quartier, 2003). Also pregnancy, cigarette smoking, and oral contraception have been reported as potential risk factors for lupus. These factors may interact with multiple genetic factors in determining susceptibility to Lupus (Simard, 2007).

1.4. Drugs allegedly associated with lupus erythematosus

1.4.1. All drugs

Antonov (2004) reviewed publications about drugs associated with lupus: 80 drugs have been described to be associated with lupus. The time between first clinical manifestations of lupus and drugs intake have been reported in a wide range from 3 days to 8 years.
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 the GPRD and reported no evidence of an association.
Also influenza or anti-streptococcus vaccinations in lupus patients with quiescent disease seems to be safe as reported by Holvast (2006 & 2007), Abu-Shakra (2007) and Elkayam (2006).

1.4.2. Time windows at risk used in studies

In the above mentioned studies, time-windows varying from several months to several years have been used for the study of the relation between systemic lupus and vaccines.

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

Table 1: Epidemiology of lupus erythematosus and data stemming the literature review

Gender	9 women / 1 man
Incidence	France: $5/10^5$inhabitants / year <18years-old: 10-$20/10^5$ inhabitants / year USA: $2,4/10^5$ to $7,3/10^5$ inhabitants / year
Prevalence	France: 40 cases for 100 000 inhabitants
Time to event tested	≤1 year, ≥1 years, ≥5years

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

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

- 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
- 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

Case definition for the study of systemic disorder

Cases for the study are *incident cases* defined as patients with a clinical and biological picture evocative of lupus.
The presence or absence of the ACR (American College of Rheumatology) criteria is recorded:

- Skin disorder: malar rash, alopecia, photosensitivity, mucosal ulcers, discoid lupus, lupus tumidus, annular lupus, Chilblain lupus, lupus profondus;
- Musculo-skeletal disorder: non erosive arthritis, myositis;
- Serositis: pleuritis or pericarditis
- Fever;
- Renal disorder: proteinuria, hematuria, pyuria, cellular casts;
- Neurologic disorder: seizure, psychosis, Organic Brain Syndrome, visual disturbance, cranial nerve disorder, lupus headache, cerebrovascular accident;
- Vasculitis;
- Hematologic disorder: anemia, leukopenia, lymphopenia, thrombocytopenia;

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- Autoimmune disorder:
  - Antibody to double-stranded DNA antigen (anti-dsDNA),
  - Antibodies to anti-nuclear antigens (anti-ECT): anti-Sm, anti-SSA, anti-SSB, anti-RNP,
  - Antibody to histones,
  - Positive finding of antiphospholipid antibody,
  - Low complement.

2.4.4. Summary table for case definition

Table 2A: Case definition for the study of systemic disorder

	Number of clinical and-biological criteria from the ACR classification (except immunological disorders)	Lupus specific auto-antibodies: Anti-Sm, anti-DNA.
Definite cases	≥ 2 criteria	**AND**: - Anti-Sm, - OR anti-DNA - OR FAN
Possible cases	1 criterion	**AND**: - Anti-Sm, - OR anti-DNA - OR FAN

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).

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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.

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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 systemic disorders evocative of incident lupus erythematosus

	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 potential risk factors or eventual triggers considered *a priori* for the study.

Table 4: Risk factors or eventual triggers considered a priori for the study of systemic disorders evocative of incident lupus erythematosus

Risk factors or eventual triggers considered a priori
- Family history of autoimmune disorder (1st degree) - Geographical origin - Recent pregnancy - Use of estroprogestatives - 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 lupus erythematosus 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 lupus (co-morbidities, personal histories, symptoms spontaneously reported, drug use). Any referent with a possible or definite antecedent or presence of lupus erythematosus will be excluded from the set of referents.

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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 systemic disorders evocative of incident lupus erythematosus 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		

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	

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 incident lupus erythematosus 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),

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 incident lupus erythematosus 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®

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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.

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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 lupus Centres	Done and on-going for paediatric centres
Recruitment of 1st case	
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 Lupus erythematosus - 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] and Dr [redacted].

ADDENDUM: Cutaneous lupus

1.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 criteria

- Male and female;
- Age between 14 and 79 years old (included);
- Clinical presentation compatible with a 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.

2. 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 or probable Lupus
b) Possible Lupus
c) Rejected cases

Definite cases or probable cases will be used as cases in the main analysis. Possible cases are used in the sensitivity analysis. Rejected cases are used for the identification of biases. The diagnostic criteria to classify the patients are described below.

After one year, PGRx centres are contacted to assess the evolution of the diagnosis of the definite cases

Table: Algorithm of selection of cases of cutaneous lupus

	Clinical picture	Lupus specific auto-antibodies (AAc)	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 AAc	**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 AAc	**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 AAc	Not performed **OR** performed but without characteristic elements for lupus diagnosis

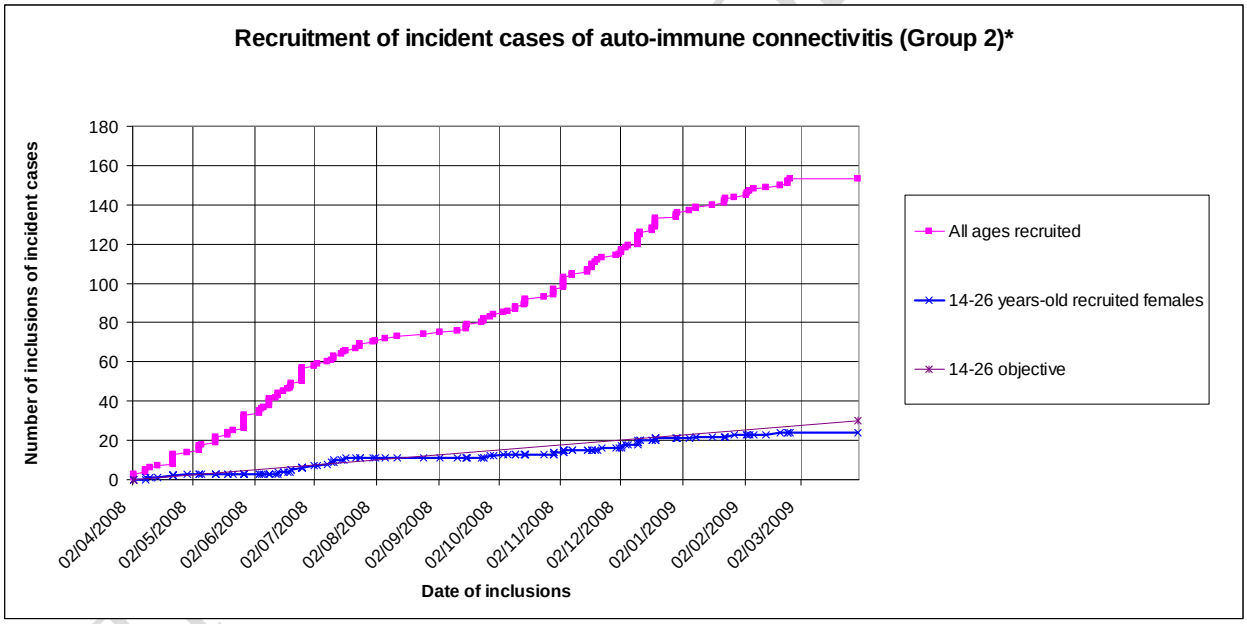
Appendix 1: Exhibit 1A: PGRx Information System General Methodology

Appendix 2: Recruitment of systemic disorders evocative of incident lupus in PGRx

Table A2.1 Recruitment of cases of systemic disorders evocative of incident lupus 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 2 (inflammatory arthritis, lupus, myositis)			125	20	30	90
Cases of systemic disorders evocative of incident lupus	10/04/2008	15	40	15	-	-

Figure A2.1 Recruitment of systemic disorders evocative of incident lupus cases in the PGRx System as of March 2, 2009.



* Group 2: Incident cases of lupus erythematosus, inflammatory arthritis, myositis and undifferenciated connectivitis /incident scleroderma

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PGRX

STUDY OF CERVARIX® & TYPE 1 DIABETES

USING THE PGRx INFORMATION SYSTEM

VERSION 2

February 26, 2009

PGRx
Centre for Risk Research Inc.
LA-SER sarl

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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 type 1 diabetes ("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 or referents 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 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 type 1 diabetes, 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 type 1 diabetes

Several epidemiological studies assessed the incidence rate of Type 1 Diabetes (T1D) in different countries; here is a summary of such studies and their results.
A French registry of T1D in childhood was set up between 1988 and 1995 with children aged less than 20 years (Lévy-Marchal, 1998). Standardized annual incidence rates were 7.17/100,000 person-year in 1988 and 9.28/100,000 person-year in 1995. Lora-Gomez (2005) recorded retrospectively all type 1 diabetes cases with onset <14 years of age between 1988 and 1999 to determine the incidence of type 1 diabetes in Spain in children less than 14 years of age. The incidence rate was 16.8/100,000 person-year ($CI_{95\%}$:14.1–19.8). There was no significant variation in incidence rates between 1988 and 1999. An increasing gradient in incidence was observed with age, peaking in the age group 10–13 years (19.1/100,000 person-year, $CI_{95\%}$:14.2–25.1). No significant differences between sexes were noted in the whole group. Analysis of seasonal trend confirmed that disease occurs significantly more often during autumn and winter and less in spring and summer. Zhao (1999) assessed the incidence of T1D in children aged 0-15 years in the south of England between 1975 and 1996. A children's

diabetes register was performed through two data sources: hospital and general practitioners. An overall crude incidence of 14.9/100,000 person-year was found. A significant increase of incidence was observed during the study period (2.49% per year). A significant peak incidence appeared in autumn and winter. Metcalfe (1991) made a register of all children diagnosed under the age of 15 years with T1D in the year 1988 in the British Isles. Regional incidence rates varied between isles: 19.8/100,000 person-year in Scotland and 6.8/100,000 person-year in Ireland. An overall incidence rate of 13.5/100,000 person-year was found ($CI_{95\%}$: 12.9-14.2) with a peak in the 10-14 years old group (17.8/100,000 person-year). Seasonal variations were observed. Cotellessa (2003) assessed the incidence of T1D in 0 to 14 year-old children in a Northwest region of Italy, between 1989 and 1998. The standardized incident ratio (IR) over the 10-year period was 12.6/100,000 person-year ($CI_{95\%}$: 11.0 –14.3). The sex-specific IR among men was 14.2/100,000 men-year and among women 10.9/100,000 women-year. The age-specific IR was higher in the 10-14 year-old group (15/100,000 person-year) than the 0-4 year-old group (9.01/100,000) and the 5-9-year-old group (13.03/100,000). Onkamo (1999) made a review of 37 studies between 1960 and 1996 to estimate the magnitude of the change in T1D incidence. The analysis of pooled data from all studies showed that the overall increase in incidence was 3.0% per year ($CI_{95\%}$: 2.6; 3.3).
In the French registry there was a significant variation in incidence rates with a 29% increase between 1988 and 1995 (Lévy-Marchal, 1998). This increase affected equally both sexes.

1.3.2. Risk factors associated with type 1 diabetes

Type 1 diabetes presents a high rate of familial transmission: the risk of becoming diabetic is approximately 5% for a sibling and 3-4% for a child of a diabetic.
The role of the MHC genes in genetic predisposition to T1D is predominant, as shown by the high disease concordance rate in HLA-identical siblings (12%, and even 15-17% in DR3/4 heterozygote). It was also confirmed in animal models where MHC genes were associated to the occurrence of T1D. T1D is positively associated in Caucasians with two sets of alleles: HLA DR3 and HLA DR4 (Bach, 1994).
A French Registry of type 1 diabetes in childhood was set up between 1988 and 1995 with children aged less than 20 years of age. HLA-DR3/DR4 phenotype was found in 32% of the children, and 11% carried none of these antigens, irrespectively of age and 8% of the children had a first-degree relative with T1D (Levy-Marchal, 1993).

The hypothesis for a role of environmental factors in the pathogenesis of T1D can be based on 4 arguments (Bach, 1994):

- more than 60% of identical twins are discordant for the disease ;
- disease frequency varies from country to country, a North/South gradient exist ;
- a number of apparently non immunological interventions can increase or decrease the disease rate in animal models: specific diets (low essential fatty acid or protein intake), and several viral infections can reduce or increase disease susceptibility in mice models;
- T1D incidence is on the increase in most countries, even in areas with a distinct genetic background.

1.4. Drugs allegedly associated with type 1 diabetes

1.4.1. All drugs

A link between childhood vaccinations and the occurrence of T1D has been presumed based on the results of ecological studies on a Danish cohort (Classen 1996, 97, 99 & 2002). It has been hypothesized that any vaccination after 2 months of age increases the risk of T1D while vaccination in the first month of life protects against T1D (Classen, 1996 & 1997). Classen (1999) had compared two vaccine schemes against *haemophilus influenzae b* (Hib) (3 doses at 3 months of age versus only one dose at 24 months of age). This study showed an association between the age of vaccination and the risk of T1D; early vaccination seemed to protect against this risk. Based on data of a large clinical trial on Hib vaccine, Classen (2002 & 2003) reported that the exposure to anti HiB vaccine was associated with an increased risk of T1D. A statistically significant cluster of extra cases of T1D was found and occurred between 3 and 4 years after immunization. Classen's publications are based on countries comparisons and on ecological methodology. When other methods were used such as case-control studies or cohort studies, the link between any childhood vaccination and T1D was questioned.
Several studies in different countries did not report any increase of risk of T1D with vaccination.
4 case-control studies (CCS):

- In Sweden, a CCS with children younger than 12 y.-o. vaccinated or not against tuberculosis, smallpox, pertussis, mumps, tetanos, rubella, did not report any significant effect (Blom, 1991) ;
- In Canada, a CCS on tuberculosis vaccination did not report a risk difference of T1D between vaccinated and unvaccinated children (Parent, 1997) ;
- In the USA, result of a CCS of children aged 12 y.-o. who have a first-degree relative with type 1 diabetes suggest that early vaccinations do not affect the risk of developing β-cell autoimmunity or T1D (Graves, 1999) ;
- Destefano (2001) made a CCS and did not found any link between childhood vaccinations and T1D ;

3 cohort studies:

- As part of the Vaccine Safety Datalink Project, Black (2002) defined a birth cohort of more than 350,000 live births from 1993 to 1998 and registered all deaths occurring under 29 days of age. This study found no significant difference in the proportion of HBV-vaccinated and unvaccinated neonates dying of unexpected causes.
- A 12 years follow-up cohort in Sweden was made to compare the cumulated incidence of T1D between vaccinated and unvaccinated children against pertussis. No difference was found between the 2 groups (Heijbel, 1997) ;
- A Finnish cohort of children vaccinated against Hib and followed 10 years did not found any increase risk of T1D (Karvonen, 1999);
- Hviid (2004) has followed a cohort of children born in Denmark from 1990 through 2000. The rate ratio for T1D among children who received at least one dose of vaccine, as compared with unvaccinated children, was not statistically different for all vaccines.

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 type 1 diabetes and vaccines.

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

Table 1: Epidemiology of type 1 diabetes and data stemming the literature review

Socio-demographics (age, gender)	10-13 years old No difference between genders
Incidence	France: 7.17 to 9.28 /10^5 inhabitants / year South England: 19 [16.44-21.51]/10^5 inhabitants /year Spain: 19 [14.2-25.1]/10^5 inhabitants /year Italy: 15 [12.2-18.3]/10^5 inhabitants /year Europe: - For the 15-19 y.-o: Men: between 3.6 [0.3-13.4] and 20.4 [13.4-29.7] Women : between 5.3 [2.6- 9.8] et 11.9 [6.6- 19.6] - For the 20-24 y.-o. Men: between 5.7 [2.7 – 10.6] and 15,6 [11.8- 23.2] Women : between 3,3 [1.8- 5.4] and 13.6 [6.2- 26,0]
Time to event tested	≤60 days, ≤2 years, ≤ 4 years, ever –receipt (children)

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
- Familial history (1st degree) of type 1 diabetes.
- Description of biological and imaging findings (if appropriate and/or available)
- Current and previous chronic diseases
- Elements of differential diagnosis

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

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

Type 1 diabetes cases are defined as patients with a clinical and biological presentation compatible with the onset of type 1 diabetes. A personal history of type 1 diabetes excludes the patient

2.4.3. Definition of definite, possible and rejected cases

Table 2 presents the algorithm for the case definition of type 1 diabetes.

Table 2: Case definition for the study

	Clinical presentation	Biological tests	Auto-antibodies (AAb)
Definite cases	Abrupt onset with polyuro-polydipsia **AND/OR** weight loss **AND/OR** asthenia **AND/OR** acido-cetosis	**AND** Hyperglycemia > 2g/l Glycosuria	**AND** Presence of anti-insuline, or anti-GAD, or anti-IA2
Possible cases		Hyperglycemia > 2g/l Glycosuria	No AAb

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.
- 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.

[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 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.

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 Type 1 diabetes

	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 type 1 diabetes

Risk factors considered a priori
- Family history of autoimmune disorder (1st degree)
- Geographical origin
- Recent pregnancy
- 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 type 1 diabetes 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 endocrinal disorders (co-morbidities, personal history, symptoms spontaneously reported, drug use). Any referent with a possible or definite medical history or presence of type 1 diabetes 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.

26.02.2009
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
15	45	180	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 xxxxx%.

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	xxxxx%

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 type 1 diabetes 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),

[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 type 1 diabetes 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.

8.5.2. Unplanned analysis

[7] Gardasil®

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 type 1 diabetes 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 type 1 diabetes Centres	Done, and on-going for paediatricians' centres
Recruitment of 1st case	April 2008
Recruitment of potential Referents	On-going
Finalisation of PGRx type 1 diabetes - Cervarix® protocol	May 2009
1st Annual Descriptive report and blind analysis	
2nd Annual Descriptive report and blind analysis	
Final PGRx type 1 diabetes -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] and Dr [redacted].

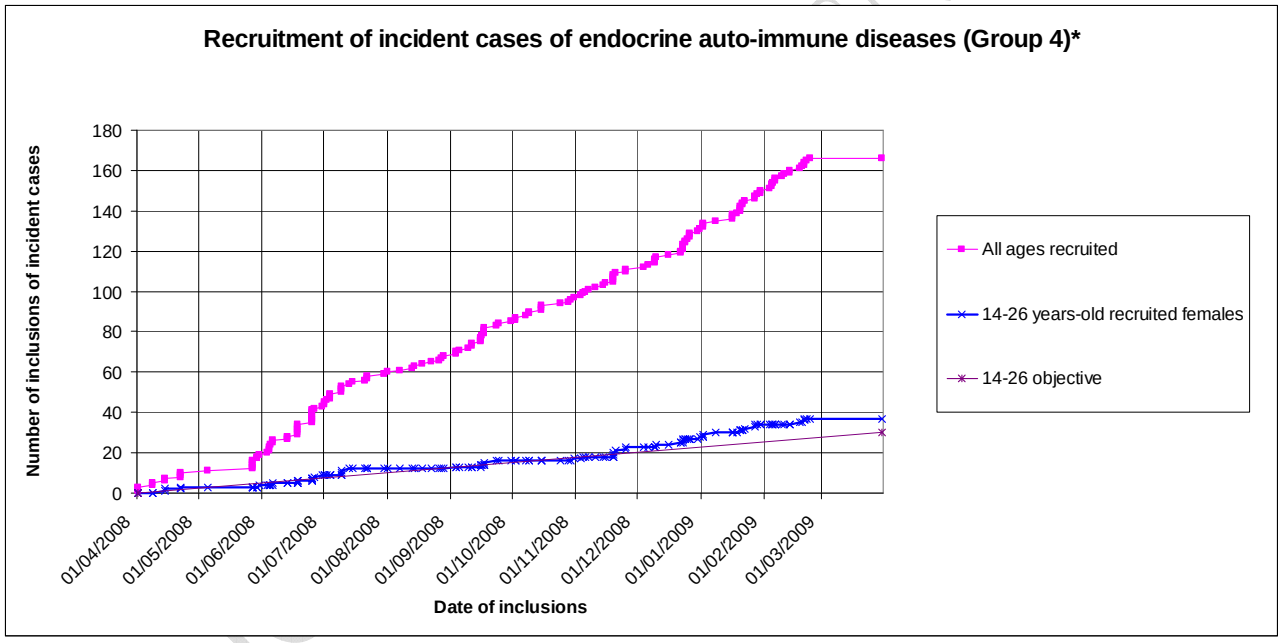
Appendix 1: Exhibit 1A: PGRx Information System General Methodology

Appendix 2: Recruitment of type 1 diabetes

Table A2.1 Recruitment of type 1 diabetes 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
Group 4		34	166	37	30	90
Type 1 Diabetes	08/04/2008	17	77	20	-	-

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



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