NON-INTERVENTIONAL (NI) STUDY PROTOCOL

A POPULATION-BASED COHORT STUDY USING AN EXISTING DATABASE TO EVALUATE THE ASSOCIATION BETWEEN LATANOPROST USE AND PRIMARY MALIGNANT OCULAR MELANOMA AND FACIAL CUTANEOUS MELANOMA

Compound number:	PN-174912
Compound name:	XALATAN® (LATANOPROST)
Study number:	A6111157
Version and date:	Final 15 September 2013
Marketing Authorisation Holder (MAH)	Pfizer Limited Ramsgate Road, Sandwich, Kent CT130NJ United Kingdom

ABSTRACT

A POPULATION-BASED COHORT STUDY USING AN EXISTING DATABASE TO EVALUATE THE ASSOCIATION BETWEEN LATANOPROST USE AND PRIMARY MALIGNANT OCULAR MELANOMA AND FACIAL CUTANEOUS MELANOMA

Final, 15 September 2013

Wanning Xu, MD PhD, Director, Epidemiology, Pfizer Inc. New York, NY, USA

Helle Kieler, MD PhD, Associate Professor, Karolinska Institute, Stockholm, Sweden

Background: Latanoprost is a prostaglandin $F_{2\alpha}$ analogue that has been developed for the reduction of intraocular pressure (IOP) in patients with glaucoma. A potential signal of increased risk of malignant melanoma with latanoprost was raised at the 2009 Pharmacovigilance Working Party (PhVWP) of the Committee for Medicinal Products for Human Use (CHMP) meeting. Presently, there is insufficient evidence that establishes a causal link between latanoprost and ocular or cutaneous melanoma. This study is designated as a Post-Authorization Safety Study (PASS) committed to the Medicines and Healthcare products Regulatory Agency (MHRA) to further evaluate the potential association between latanoprost and melanoma.

Research aim: The primary research aim is to examine the potential association between latanoprost and primary malignant ocular melanoma (OM) and facial cutaneous melanoma (CM), respectively. The secondary research aim is to examine the potential association between prostaglandin analogues (PGAs) and primary malignant OM and facial CM, respectively.

Methods: A population-based cohort study will be conducted based on secondary use of existing data. The study population will include patients with recorded glaucoma or ocular hypertension (OH) in the Swedish national health care registers from July 1st, 2005 to December 31st, 2011 and with no previous malignant melanoma. Exposure groups (latanoprost, other topical PGAs and topical non-PGAs) will be categorized based on drug exposure data collected from the Swedish Prescription Drug Register (SPDR). Primary malignant OM and facial CM of each patient will be identified from the Swedish Cancer Register (SCR). Cox regression models will be developed to evaluate independent effects of having OM and facial CM, respectively, in association with use of latanoprost and topical PGAs, adjusting for potential confounding variables.

Milestones: The draft protocol (dated 06 December 2012) was submitted to the MHRA on 19 December 2012. The current protocol (dated 15 September 2013) is the final protocol. Data acquisition will be finalized within 6 months from the date that the final protocol is endorsed by the MHRA. Data analysis and study report will be completed within 12 months after the completion of data acquisition.

ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Definition
AE	Adverse event
ACME	Automated Classification of Medical Entities
ATC	Anatomical Therapeutic Chemical
CDR	Causes of Death Register
CHMP	Committee for Medicinal Products for Human Use
CM	Cutaneous Melanoma
DDD	Defined Daily Doses
e-HRD	Electronic Health Related Databases
EMA	European Medicines Agency
ICD-9/10	The International Classification of Diseases Ninth/Tenth Revision
ICD-O-2/3	The International Classification of Diseases for Oncology Second/Third Revision
EU	European Union
ICH GCP	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use – Good Clinical Practice
IQR	Interquartile Range
IOP	Intraocular Pressure
IRB/IEC	Institutional Review Board/Independent Ethics Committee
IRR	Incidence Rate Ratio
LPREIW	Longitudinal Population Register on Education, Income and Work
MAH	Marketing Authorisation Holder
MHRA	Medicines and Healthcare products Regulatory Agency
NI	Non-Interventional
NPR	National Patient Register
OH	Ocular Hypertension
OM	Ocular Melanoma
OTC	Over-the-counter
PASS	Post-Authorization Safety Study
PhVWP	Pharmacovigilance Working Party
PGA	Prostaglandin analogue
PV	Pharmacovigilance
RR	Relative Risk
SAP	Statistical Analysis Plan
SOP	Standard Operating Procedures
SCR	Swedish Cancer Register
SNOMED	Systematized Nomenclature of Medicine
SPDR	Swedish Prescribed Drug Register

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1. RATIONALE AND BACKGROUND

1.1. Background

Medical treatment of glaucoma includes use of oral and topical intraocular pressure-lowering medications. Xalatan® (latanoprost ophthalmic solution 0.005%) is a prostaglandin $F_{2\alpha}$ analogue that has been developed for the reduction of intraocular pressure (IOP) in patients with glaucoma and is delivered as a single liquid drop to the surface of the eye, with a serum half-life of 17 minutes. Xalatan was approved for the reduction of elevated IOP in adult patients with glaucoma and ocular hypertension (OH) in the European Union (EU) in 1996 and was approved for similar indications worldwide. It was also approved for the reduction of elevated IOP in pediatric patients with glaucoma or elevated IOP in the EU in 2010. Xalatan is losing patent exclusivity in European countries in 2012 and non-branded latanoprost is becoming available to patients. Xalacom® is the fixed combination of latanoprost and timolol maleate (beta-blocking agents). It was approved for the reduction of IOP in adult patients with open angle glaucoma and OH who are insufficiently responsive to topical beta-blockers or prostaglandin analogues (PGA) in the EU in 2000. Other topical PGAs available in the majority of EU markets include bimatoprost, travoprost, tafluprost and their fixed combinations with timolol.

A potential signal of an increased risk of malignant melanoma with latanoprost treatment was raised at the June 2009 Pharmacovigilance Working Party (PhVWP) of the Committee for Medicinal Products for Human Use (CHMP) meeting. In order to assess the potential association between the use of latanoprost and malignant melanoma, the Marketing Authorisation Holder (MAH) submitted a cumulative review to the European Medicines Agency (EMA) in August 2009 (Pfizer 2009), including *in vitro* and *in vivo* data, data from MAH-sponsored clinical trials (November 1992 to November 2007), and spontaneous non-trial-related clinical reports from MAH's safety database (13 years for latanoprost and 9 years for latanoprost/timolol).

Among 12,880 latanoprost-treated patients in clinical trials, no cases of ocular melanoma and three cases of cutaneous melanoma were identified. Of 19,940 spontaneously reported adverse events among latanoprost users outside of clinical trials recorded in the safety database, eleven were ocular melanomas and six were cutaneous melanomas. Possible association with latanoprost use could not be excluded in three ocular and one periorbital melanoma events, but was excluded for all six reports of cutaneous melanoma. In a 5-year randomized open-label study of latanoprost and usual care (Goldberg, Li et al. 2008)and a 5-year open-label uncontrolled safety study of latanoprost (Alm, Schoenfelder et al. 2004)conducted to address long-term safety including possible malignant transformation within the ocular structures due to its administration, no ocular or cutaneous melanoma cases were observed. Non-clinical data supports the hypothesis that latanoprost is not carcinogenic and does not promote proliferation of melanocytes (Pfizer 2009). The increase in iris pigmentation observed with latanoprost treatment results from stimulation of melanin synthesis by induction of tyrosinase transcription without increasing mitotic activity (Lindsey, Jones et al. 2001; Pfeiffer, Grierson et al. 2003; Alm, Grierson et al. 2008), and appears to be a class effect for PGAs (Alm, Grierson et al. 2008). Further, published

literature showed no correlation between induced iris darkening or periocular skin pigmentation and the length of time a patient was exposed to latanoprost and PGAs (Alm, Grierson et al. 2008). In sum, presently, there is insufficient evidence to support a causal link between latanoprost or latanoprost/timolol use and ocular or cutaneous melanoma.

1.2. Ocular and cutaneous melanoma

Malignant ocular melanoma (OM) is a rare type of cancer with an estimated annual incidence of 2 to 9 per million in European general populations (Virgili, Gatta et al. 2007) and approximately 5 per million in the US general population (Singh, Turell et al.). Annual incidence of OM among patients with glaucoma is unknown; however one study suggested that the annual incidence may be lower than 30 per million (Huerta and Rodriguez 2001). Uveal melanoma, which is located in the iris, ciliary body and choroid of the eye, accounts for the majority (85%) of OM in adults (Singh, Bergman et al. 2005). Other less common locations include the orbit, lacrimal gland, and conjunctiva, cornea or other unspecified ocular sites (Singh, Bergman et al. 2005) .

Malignant cutaneous melanoma (CM) is uncommon but deadly (de Vries and Coebergh 2004). A wide range of incidence rates are reported due to varying geography and ultraviolet (UV) exposure patterns (Garbe and Leiter 2009). In Europe, the highest incidence rates have been reported in Scandinavia (about 15 cases per 100,000 inhabitants per year) and the lowest in the Mediterranean countries (about 5-7 cases per 100,000 inhabitants per year (Garbe and Blum 2001). The incidence is higher in the US (10-20 cases per 100,000 inhabitants per year) and in Australia (40-60 cases per 100,000 inhabitants per year) (Garbe and Blum 2001). If there were a causal relationship between the topical administration of latanoprost (eye drops) and cutaneous melanoma, the resultant lesions would be most likely in or around the skin associated with the eyes. From 1973 to 1981, the estimated incidence of CM on the face in the US general population was 2.0 per 100,000 person-years in males and 1.3 per 100,000 person-years in females (Newell, Sider et al. 1988). The incidence of CM on the face among glaucoma patients has not been reported in the literature.

The developing consensus shows considerable differences between cutaneous and ocular melanoma in terms of etiology, molecular biology and clinical behavior, and these two cancers require distinct consideration. Cutaneous melanocytes are derived from a different cell line than are uveal melanocytes. The former are derived from neuro-ectoderm and the latter from neural crest (Pfizer 2009). Another significant difference is that the periocular darkening process involves the production of many new melanin granules, whereas in induced iris darkening the increase in granule size is the predominating factor (Alm, Grierson et al. 2008). Additionally, the metastatic behavior of the two melanomas and tumor cytogenetics are distinctly different (Young and Seddon 2005). Epidemiological findings also support the distinction between CM and OM as separate disease entities. The incidence of OM has been notably stable over the last five decades (Bergman, Seregard et al. 2002; Isager, Osterlind et al. 2005; Tressler, Wiseman et al. 2011), while there has been a continuous increase of CM incidence rates over the last 60 years in white populations, primarily due to increased UV exposure (de Vries and Coebergh 2004; Isager, Osterlind et al. 2005; Young and Seddon 2005; Garbe and Leiter 2009). In contrast to the known

contribution of UV radiation exposure to CM (Isager, Osterlind et al. 2005; Young and Seddon 2005), the etiological role of UV radiation exposure is equivocal in the case of OM (Dolin, Foss et al. 1994; Cree 2000; Hurst, Harbour et al. 2003; Young and Seddon 2005). OM more often affects older aged individuals whereas CM is often a disease of younger aged individuals (Young and Seddon 2005).

1.3. Rationale for a cohort study using an existing database

Based on a feasibility assessment that was submitted to the MHRA (Pfizer Inc. 2011), the requested case-control study with primary data collection from oncology centers is not feasible. An existing database (ie, Swedish national health care register) may be used for an adequately powered study for OM and facial CM associated with latanoprost use (Pfizer Inc. 2011). The associations can be evaluated either by a cohort or a case-control study using the database. As compared to a case-control study, a cohort study based on an existing data is advantageous (Pfizer Inc. 2011) because it will enable direct estimation of the incidence rates of OM and CM among latanoprost users and non-latanoprost users, and compare the incidence rate between two groups. Additionally, this study design will allow for the assessment of potential risk factors for OM and CM (such as demographics and concomitant medications) in patients who received latanoprost in comparison to patients who did not receive latanoprost. Finally, the cohort design will enable evaluation of both endpoints (eg, OM and CM) in one study.

1.4. PASS designation

This study is designated as a Post-Authorization Safety Study (PASS) committed to the Medicines and Healthcare products Regulatory Agency (MHRA) to further evaluate the potential association between latanoprost use and the risk of malignant OM and CM in patients with glaucoma or OH.

2. RESEARCH AIMS AND OBJECTIVES

2.1. Research aims

The primary research aim is to evaluate whether use of latanoprost increases the risk of primary malignant OM and facial CM.

The secondary research aim is to evaluate whether use of topical PGAs in general (latanoprost or other topical PGAs) increases the risk of primary malignant OM and facial CM.

2.2. Study objectives

The primary objectives are to estimate the hazard ratios of primary malignant OM and facial CM, respectively, among patients with glaucoma or OH:

- by comparing use of latanoprost with use of topical non-PGAs; and
- by comparing use of latanoprost with use of other topical PGAs.

The secondary objectives are to estimate the hazard ratios of primary malignant OM and facial CM, respectively, among patients with glaucoma or OH:

- by comparing use of other topical PGAs with use of topical non-PGAs; and
- by comparing use of topical PGAs (including latanoprost and other topical PGAs) with use of topical non-PGAs.

An additional objective is to describe, over time, the clinical characteristics and the use of latanoprost, other topical PGAs and topical non-PGAs in glaucoma/OH patients.

3. RESEARCH METHODS

3.1. Study design

This is an observational, population-based cohort study using existing data from the Swedish national health care registers.

3.2. Study population

3.2.1. Inclusion criteria

The study population includes all patients diagnosed with glaucoma or OH identified from the Swedish national health care registers between 1 July 2005 (ie, the start date of the Swedish Prescribed Drug Register) and 31 December 2011 (ie, the most recent date that data in the Swedish Cancer Register was updated) (defined as "study period") with no previous malignant melanoma history. To ensure the melanoma cases arising during the study represent incident malignant melanoma cases, patients with a previous diagnosis of malignant melanoma between 1958 (when the cancer register was established) and the date of first dispensing of latanoprost, other topical PGAs or topical non-PGAs will be excluded from the study.

Patients with glaucoma or OH will be identified using the English version of the International Statistical Classification of Diseases Tenth Revision (ICD-10) diagnosis codes, Anatomical Therapeutic Chemical (ATC) classification codes for glaucoma /OH medications, or glaucoma procedure codes recorded in the Swedish health care register. Patients seen by private eye specialists only and not reported to the Patient Register by diagnosis codes may be captured by glaucoma procedure and/or medication codes. In the following text, "diagnosis of glaucoma or OH" is equivalent to a recorded diagnosis of glaucoma or OH, a procedure code for glaucoma surgery or filling a prescription with a medication approved exclusively for glaucoma or OH. All codes and algorithms will be included in the SAP.

The study population for primary and secondary objectives (ie, the main study population) includes glaucoma/OH patients with a dispensing of at least one of the following drugs: latanoprost, other topical PGAs or topical non-PGAs.

Patients enter the main study population on the date of first dispensing of latanoprost, topical PGAs or topical non-PGAs (defined as "entry date").

Patients leave the main study population on the earliest of the following dates (defined as "exit date"):

- at the end of the study (31 December 2011);
- on the date of diagnosis of primary malignant OM or primary malignant facial CM;
- on the date of death; or
- on the date of discontinuation from the coverage by the register (eg, emigration).

The time period between the entry date and the exit date is defined as the "follow-up period" of each patient.

The induction time for the occurrence of malignant OM and facial CM associated with the use of latanoprost and other topical PGAs is unknown and it usually takes years for cancer occurrence. In main analyses, we will assume a *minimum* lag time of 6 months between the start of treatment and the occurrence of the melanoma cases. This implies that any cases that occur within the interval of the first 6 months since start of treatment will be excluded. Sensitivity analyses will be conducted to estimate the impact of different induction periods.

If a patient develops both primary malignant OM and facial CM, the patient will be censored at the date of the first malignancy.

3.2.2. Comparison groups

Patients may contribute person-time to one or more of the exposed or unexposed groups as follows:

Exposed groups

- **Exposure to latanoprost:** dispensing of latanoprost during the study period.
- **Exposure to other topical PGAs:** dispensing of a non-latanoprost topical PGA during the study period.

Unexposed group (ie, control group)

- **Exposure to topical non-PGAs:** dispensing of a topical non-PGA during the study period.

As the possible pathogenesis of cancer in connection with latanoprost and other topical PGA therapy is unknown, two different exposure definitions (***ever exposure*** and ***real-time exposure***) will be used to further categorize the exposure as follows. The lag time of 6 months will be used in each definition.

- ***Ever exposure:*** In this definition, we assume that exposure to latanoprost or other topical PGAs will result in a lifetime change in risk associated with the drug. That is, if a patient is exposed to latanoprost or other topical PGAs, the status of exposure is maintained throughout the whole follow-up even after stopping the treatment. The first drug (latanoprost or other topical PGAs) used during the study period will define to which study drug group the ever exposure will be assigned. The person-time of ever exposure to latanoprost or another topical PGA starts 6 months after the date of the first dispensing of the drug and continues to the end of the follow-up period.

  Relevant time on topical non-PGAs will be counted as unexposed time (ie, contribute to the control group). If topical non-PGAs are the only drugs being dispensed for a patient, the time of exposure to the topical non-PGA starts 6 months after the date of the first dispensing of the topical non-PGA and continues to the end of follow-up period. If the first drug being dispensed is a topical non-PGA then followed by a topical PGA, the time of exposure to the topical non-PGA starts 6 months after the date of the first dispensing of the topical non-PGA and continues to 6 months after the date of the first dispensing of the topical PGA. Time on topical non-PGAs after a dispensing of a topical PGA will be ignored.

  Based on the ever exposure definition, the comparisons below correspond to the primary and secondary objectives, respectively.

  In analyses for the primary objectives, the hazard ratios of primary malignant OM and facial CM, respectively, will be estimated for:

  - Ever exposure to latanoprost compared with non-exposure (ie, exposure to topical non-PGAs); and
  - Ever exposure to latanoprost compared with ever exposure to other topical PGAs.

  In analyses for the secondary objectives, the hazard ratios of primary malignant OM and facial CM, respectively, will be estimated for:

  - Ever exposure to other topical PGAs compared with non-exposure (ie, exposure to topical non-PGAs); and
  - Ever exposure to topical PGAs (ie, latanoprost or other topical PGAs) compared with non-exposure (ie, exposure to topical non-PGAs).

- ***Real-time exposure:*** Time varying exposure will be used in this definition for latanoprost, other topical PGAs, and topical non-PGAs (ie, the control group). That is, only the time on a specific drug (latanoprost, other topical PGAs or topical non-PGAs) will contribute to the time at risk of having malignant melanoma for that specific drug. The person-time at risk starts 6 months after the date of the first dispensing of the drug and continues to the earliest of the following dates: 6 months after the date of the last dispensing of the drug or to the end of follow-up period.

  Based on this definition, the comparisons below correspond to the primary and secondary objectives, respectively.

In analyses for the primary objectives, the hazard ratios of primary malignant OM and facial CM, respectively, will be estimated for:

- Real-time exposure to latanoprost compared with non-exposure (ie, real-time exposure to topical non-PGAs); and
- Real-time exposure to latanoprost compared with real-time exposure to other topical PGAs.

In analyses for the secondary objectives, the hazard ratios of primary malignant OM and facial CM, respectively, will be estimated for:

- Real-time exposure to other topical PGAs compared with non-exposure (ie, real-time exposure to topical non-PGAs); and
- Real-time exposure to topical PGAs (ie, latanoprost or other topical PGAs) compared with non-exposure (ie, real-time exposure to topical non-PGAs).

Concomitant use is defined as the dispensing of more than one drug (ie, latanoprost, other topical PGAs and topical non-PGAs) within one week in the Prescribed Drug register.

For the ever exposure definition, concomitant use will be classified according to the hierarchy: latanoprost and other topical PGAs > topical non-PGAs. Patients exposed to latanoprost or other PGAs, with concomitant exposure of a topical non-PGA, will only contribute person-time to the latanoprost or the other PGAs group, and the exposure of the topical non-PGAs will be ignored. For concomitant use of latanoprost and other topical PGAs, person-times will be exclusively contributed to the first dispensed group (latanoprost or other PGAs), and the exposure to the other drug will be ignored.

For the real-time exposure definition, concomitant use will be classified according to the hierarchy: Latanoprost > other PGAs > non PGAs. Patients exposed to latanoprost or other PGAs, with concomitant exposure of a topical non-PGA, will only contribute person-time to the latanoprost or the other PGAs group, and the exposure of the topical non-PGAs will be ignored. For concomitant use of latanoprost and other topical PGAs, the exposure to other PGAs will be ignored and person-times will be exclusively contributed to the latanoprost group.

More details on exposure definitions, including handling of gaps, overlaps, concomitant use and switching will be given in the SAP.

3.3. Data sources

The Swedish national health care registers, including the Swedish Prescribed Drug Register (SPDR), the Swedish Cancer Register (SCR), the National Patient Register (NPR), the Causes of Death Register (CDR), and the Longitudinal Population Register on Education, Income and Work (LPREIW), will constitute the primary source of data.

The linkage between registers

A unique personal identity number is issued to all residents of Sweden upon birth or immigration and is used throughout life. The unique personal identity number will be used to link patients' data from different registers described below (Furu, Wettermark et al.).

The Swedish Prescribed Drug Register (SPDR)

Information about patients' drug exposure (exposure of interest and concomitant drugs) will be obtained from the Swedish Prescribed Drug Register (SPDR). The SPDR has been functioning since July 2005 and contains data with unique patient identifiers for all dispensed prescriptions to the whole population of Sweden (9.2 million inhabitants) (Furu, Wettermark et al. 2010; Wettermark, Hammar et al. 2007). The register is complete for the entire Swedish population (patient identity data are missing for <0.3% of all items). The data collection is administered by the National Corporation of Swedish Pharmacies, a state-owned company responsible for the provision of pharmaceutical services to the whole country. Information from all prescriptions dispensed is transferred monthly to the National Board of Health and Welfare, responsible for keeping the SPDR (Wettermark, Hammar et al. 2007). The register contains the following data on drugs prescribed and dispensed in ambulatory care: dispensed item (substance, brand name, formulation and package); dispensed amount, dosage, expenditure and reimbursement; age, sex and unique identifier (personal identification number) of the patient; place of residence of the patient (county, municipality and parish); date of prescribing and dispensing; the practice (primary healthcare centre or hospital clinic) that has issued the prescription; and the prescriber's profession (eg, general practitioner, internal medicine, psychiatry or pediatrics) (Wettermark, Hammar et al. 2007). All drugs are classified according to the ATC classification system (World Health Organization (WHO) Oslo 2005). Measurement units of utilization are prescriptions, Defined Daily Doses (DDDs) and expenditures. In general, the prescriptions are filled for a maximum of three months. The register holds data on dispensed prescriptions and not on prescriptions actually issued by physicians.

The register does not include data on over-the-counter (OTC) medications and drugs used in hospitals, and does not include complete data on drugs that are used in ambulatory care but administered in day-care at hospitals. The register is not complete with regard to drugs used in nursing homes. If a patient uses the study drug (eg, latanoprost) in hospital or nursing home only, the patient may be misclassified to the unexposed group. However, this case is expected to be rare as the exposures of interest in this study (latanoprost and other topical PGAs) are usually prescribed by ophthalmologists or GPs in out-patient settings.

The Swedish Cancer Register (SCR)

Information on patients' cancer status will be obtained from the Swedish Cancer Registry (SCR). The SCR was established in 1958 and covers the whole population of Sweden. All health care providers (public and private, and clinicians and pathologists/cytologist) are required by law to report newly detected cancer cases to one of the six regional center registries. A cancer report has to be sent for every cancer diagnosed at clinical,

morphological and other laboratory examinations, and those diagnosed at autopsy. The SCR is created annually at the National Board of Health and Welfare by merging data from six regional cancer registers. The relevant medical information available in the SCR comprises site, histological type, and stage of tumour. The SCR is generally considered to be of good quality as 99% of the cases are histologically verified and the completeness of the SCR is high (96.3% in 1998) (Barlow, Westergren et al. 2009; National Board of Health and Welfare. 2012).

Cases without a cancer notification according to the above requirements, but reported to the Cause of Death Register, ie, cases denoted as DCO (death certificate only) and DCN (death certificate notification), is not included in the CSR (Barlow, Westergren et al. 2009). These cases may be identified using The Cause of Death Register (see below).

The National Patient Register (NPR)

Information on glaucoma and OH diagnosis will be obtained from the Swedish National Patient Register (NPR). It was started in 1964, initially covering inpatients in six county councils in Sweden. From 1987 it covers all public inpatient care and since 2001 all outpatient visits. The medical data includes main and secondary diagnoses and surgical procedures (National Board of Health and Welfare. 2012). Main diagnosis, secondary diagnosis and procedures from public and private service providers are included in the NPR. A quality control of the NPR is performed on the register, and in 2006 the main diagnosis was missing in 1.0 percent. At present, the NPR is updated once a year (National Board of Health and Welfare. 2012).

The Causes of Death Register (CDR)

Information on date and cause of death will be obtained from the Cause of Death Register (CDR), which was established in 1961. Causes of death are classified according to the English version of ICD-10. Since 1987 the Automated Classification of Medical Entities (ACME) system, developed by the National Center for Health Statistics in the United States, has been used to select the underlying cause of death. Automated coding of diagnostic terms reported on the death certificate was introduced in 1993 (Socialstyrelsen 2011). The main variables included in the mortality register are personal identification number, sex, date of birth, date of death, place of residence, underlying cause of death, main injury, multiple causes of death, autopsy or not and if so type of autopsy, death abroad, surgery within four weeks before death, intent in cases of injury or poisoning and place of death by broad categories. The quality of the statistics varies, mainly with the quality and thoroughness of the examination of the cause of death and the accuracy with which the physician has reported the findings on the death certificate. Changes in diagnostic methods, medical concepts and vocabulary, the classification system or processing methods may also influence time trends (Socialstyrelsen 2011).

The Longitudinal Population Register on Education, Income and Work (LPREIW)

The individual and contextual socioeconomic characteristics such as occupation will be obtained the Longitudinal Population Register on Education, Income and Work (LPREIW). Occupation may be classified by The Swedish Standard Classification of Occupations 1996 (SSYK 96)(Statistics Sweden 1996).

3.4. Variables

3.4.1. Exposure assessment

All topical drugs for glaucoma and OH and marketed in Sweden during the study period will be included in relevant exposure groups.

- Latanoprost will include: Latanoprost and Latanoprost/Timolol;
- Other topical PGAs will include: Bimatoprost, Bimatoprost/Timolol, Travoprost, Travoprost/Timolol, Tafluprost, and Tafluprost/Timolol;
- Topical non-PGAs will include: Apraclonidine, Brimonidine, Pilocarpine, Dorzolamid, Brinzolamid, Timolol and Betaxolol and combinations of topical non-PGAs.

For each patient, data on latanoprost and other PGA exposure will be obtained from the SPDR. For each prescription dispensed to the patient, the following information will be extracted: dispensed item (substance, brand name, formulation and package); dispensed amount, dosage, date of prescribing and dispensing, as well as treatment duration (a prescription is filled for a maximum of 3 months; the duration can also be estimated from DDDs and package size). The detailed information will be included in the SAP.

3.4.2. Outcome assessment

The outcomes of interest are:

- Newly diagnosed and histologically proven primary (ie, not recurrent or secondary) malignant OM;
- Newly diagnosed and histologically proven primary (ie, not recurrent or secondary) malignant facial CM.

All cases occurring within the study period (ie, between 1 July 2005 and 31 December 2011) will be identified from the SCR, using ICD-9/10, ICD-O-2/3 and SNOMED (Systematized Nomenclature of Medicine) codes (codes and algorithm will be specified in the SAP).

3.4.3. Confounder assessment

As appropriate, the study analyses will incorporate both information on risk factors for the outcomes of interest and information on variables that may be related to the exposures of interest in order to control potential confounding, including channeling. Details will be included in the SAP.

Established risk factors of OM include Caucasian race and older age. Potential risk factors include male gender, light skin color, blond hair, blue eye color and presence of naevi (dysplastic nevus syndrome, atypical ocular nevi), ocular and oculardermal melanocytosis and familial atypical mole and melanoma syndrome. Evidence for sunlight exposure in the etiopathogenesis of uveal melanoma is at best weak. Although several case-control studies have evaluated occupation as a risk factor for uveal melanoma, there is no consistent evidence indicating occupational exposure to UV light or other agents as a risk factor (Egan, Seddon et al. 1988; Singh, Bergman et al. 2005; Young and Seddon 2005).

Numerous risk factors for the development of cutaneous melanoma have been identified, including Caucasian race, older age, family history, high density of freckles, fair skin color, blue eyes, fair hair color, pre-malignant and skin cancer lesions, actinic damage indicators, increased numbers of common naevi or atypical naevi, and intermittent sun exposure and sunburn history (Gandini, Sera et al. 2005; Gandini, Sera et al. 2005; Gandini, Sera et al. 2005).

Most of risk factors for OM and CM are unlikely to be confounders in the associations of interest as there is no evidence that they are associated with the prescription and dispensing of latanoprost and PGs. However, information for some important risk factors that are recorded in the register databases (eg, age, sex, country of birth, geographical location and occupation) will be collected and their potential impacts on the associations of interest will be assessed and controlled for as appropriate in analysis.

The clinical characteristics (eg, diagnosis of glaucoma and duration of glaucoma), co-morbidities (eg, diabetes), and the use of other non-PG IOP-lowering drugs (such as beta-blocking agents, topical carbonic anhydrase inhibitors and alpha-agonists) for glaucoma or OH may affect the prescription of latanoprost and PGAs. Although there is no evidence that these conditions and medications are risk factors of OM or CM, information on these variables will be collected and their impacts on the associations of interest will also be assessed and controlled for as appropriate.

Information for the following variables will be extracted from the registers:

- Socio-demographic factors:
  - Age;
  - Sex;
  - Country of birth (as a proxy variable of light skin and eye color);
  - Geographical location of residence (as a proxy variable of recreational UV exposure);
  - Occupation (as a proxy variable of occupational UV exposure).
- Clinical characteristics and co-morbidities:
  - Diagnosis of glaucoma;
  - Duration of glaucoma;
  - Diabetes.

- Concomitant drugs (ie, other IOP-lowering drugs);
  - Nonselective and selective beta-blocking agents;
  - Topical and oral carbonic anhydrase inhibitors;
  - Alpha-agonists.

3.5. Power and sample size

Incidence rates of OM and facial CM in glaucoma population, the loss-to-follow up rate, and the true exposed vs. unexposed hazard ratios (HRs) are unknown and were estimated based on the data obtained from the preliminary feasibility assessment. For example, based on standard Cox proportional hazard model, assuming the incidence rate of a given melanoma in glaucoma population of 150 per million-year, a latanoprost exposed-to-unexposed ratio of 1:2, a study period of 6.5 years, a loss-to-follow rate of 3% per year and the minimum detectable HR of 2.0 with 80% power at a 5% significance level, the total number of glaucoma/OH patients required for the study would be 116,172. If the incident rate of melanoma in glaucoma/OH population is less than 150 per million-year, the loss-to-follow up rate is more than 3% per year, and/or the true HR of melanoma associated with latanoprost is less than 3.0, the number of patients required for the study will increase.

3.6. Data management and quality control

All data for this study will be collected through the routine data collection and quality control practices of the Swedish national health care registers. The National Board of Health and Welfare in Sweden will prepare the linked and anonymized databases. The academic lead investigator (Appendix 1) will independently create and manage the study database based on the databases prepared by the National Board of Health and Welfare in Sweden and will follow their own Standard Operating Procedures (SOPs) for quality control.

3.7. Data analysis

Detailed methodology for summary and statistical analyses of data collected in this study will be documented in a SAP, which will be dated, filed and maintained by the sponsor. The SAP may modify the plans outlined in the protocol; any major modifications of primary endpoint definitions or their analyses would be reflected in a protocol amendment.

3.7.1. Descriptive analysis

Descriptive statistics will be presented to describe patient characteristics such as age, sex, country of birth, occupation, geographical location of residence, glaucoma diagnosis, pharmacological and surgical treatments used in the latanoprost, other topical PGA and topical non-PGA groups. Frequencies and percentages will be used to describe categorical variables and mean and standard deviations (or median with inter quartile range (IQR), where appropriate) will be calculated for continuous variables. The patterns of drug use over time and the characteristics of users of latanoprost, other topical PGAs and topical non-PGAs will also be described.

3.7.2. Incidence rate estimates

Incidence rates of primary malignant OM and facial CM will be calculated by dividing the number of incident cases of melanoma by the person-time at risk for the appropriate exposure group (details included in the SAP). Incidence rates of OM and facial CM will be estimated in the latanoprost, other topical PGAs and topical non-PGAs exposed groups. If there are sufficient numbers of patients with each melanoma, the incidence estimates will be stratified by selected variables such as:

- Age;
- Sex;
- Country of birth (light skin color and blue eyes/dark skin and non-blue eyes);
- Occupation (outdoor/indoor);
- Geographical location of residence (coastal/non coastal);
- Diagnosis of glaucoma;
- Concomitant drug use.

3.7.3. Univariate and multivariate analyses

For primary and secondary objectives, standard Cox proportional hazard regression analysis will be performed for the ever use exposure definition, and Cox regression analysis with time varying covariates will be performed for the real-time use exposure definition.

3.7.3.1. Primary analyses

Comparison of latanoprost exposed group and unexposed group (ie, topical non-PGAs exposed), and Comparison of latanoprost exposed group and other topical PGAs exposed group (primary objectives).

A change-in-estimate procedure using Cox regression (standard or with time varying covariates) including only exposure and one potential confounder at a time will be conducted to assess the association between latanoprost and covariates and the development of primary malignant OM and facial CM.

Based on the results of the change-in-estimate procedure, a multivariable Cox regression model (standard or with time varying covariates) will be developed to evaluate the independent effect of latanoprost on the risk of primary malignant OM and facial CM while controlling for effects of potential confounding variables (as describe in Section 3.4.3). More details on the change-in-estimates procedure will be given in the SAP.

3.7.3.2. Secondary analyses

Comparison of other topical PGA exposed group and unexposed group (ie, topical non-PGAs exposed), and comparison of topical PGA (latanoprost or other topical PGAs) exposed group and unexposed group (ie, topical non-PGAs exposed) (secondary objectives)

The same analytical approach as described in section Section 3.7.3.1 will be used, replacing latanoprost with other topical PGAs and topical PGAs in general.

3.7.3.3. Additional and sensitivity analyses

In order to assess the potential associations of interest among incident users, the same analytical approach as described in Section 3.7.3.1 and 3.7.3.2, will be repeated among incident users. Incident users in the study population will be defined as users with no dispensing of any PGA between 1 July 2005 and 1 July 2006. The Swedish Prescribed Drug register includes all drugs dispensed since July 2005 and as treatment with PGAs is expected to be a continuous treatment; those with a first dispensing of PGAs after 1 July 2006 are considered to be incident users.

A lag time of 6 months between the start of treatment and the occurrence of the study endpoints will be applied in the main analyses. As the induction time for the occurrence of malignant OM and facial CM associated with the exposures is unknown, we will conduct sensitivity analyses to address the impact of different lag times on study associations.

More additional and sensitivity analyses will be included in the SAP.

3.8. Strengths of the research methods

Use of cohort study design with historical data: One of the major advantages of a cohort design over a case-control design in study using an existing database is that the incidence of primary malignant ocular and facial cutaneous melanoma in glaucoma/OH patients receiving latanoprost and PGs can be estimated and compared with glaucoma/OH patients who did not receive latanoprost and PGs. The characteristics of the two groups such as demographic variables and concomitant medication use can also be estimated. Further, the two endpoints (OM and facial CM) can be studied in the same study using a retrospective cohort study design.

Use of large, population-based and linked databases: Swedish national register database covers the entire population of Sweden (9.1 million) and provides a relatively large sample size to study rare events. Recall and misclassification bias will also be minimized due to the mandatory reporting and verification procedure of cancer cases in Sweden. In addition, the national register system allows for a continuous and long follow-up period for majority of patients. Finally, a unique personal identity number of each patient allows for the data linkage between the prescription drug, the outcome, and the covariates.

3.9. Limitations of the research methods

Control for potential confounders: A limitation of this study is that known and potential risk factors of OM and facial CM, for which information are not available in the database, cannot be adjusted for. This study will attempt to assess and control for the potential effect of age, sex, country of birth (a proxy of race/ethnicity), occupation, geographical location of residence (a proxy of sun exposure), co-morbidities and concomitant medications on the associations of interest. These are either known or potential risk factors of the outcomes or

the factors that may be associated with exposures of interest. Information on other risk factors of melanoma (eg, light skin and eye color) is not recorded in the database and therefore cannot be adjusted for. However, there is no evidence that these factors are associated with latanoprost prescription and thus are unlikely to confound the associations of interest in this study.

Misclassification of exposure: The primary analysis will be conducted among prevalent glaucoma/OH patients and latanoprost use before the Swedish Prescription Drug Database was available (1 July 2005) may not be captured. If a patient used latanoprost before 2005 but not thereafter, this patient may be misclassified as unexposed. This is likely to be a non-differential misclassification as there is no reason to believe that this misclassification is related to the outcomes of interest. Depending on the number of incident glaucoma patients identified from the database, subgroup analysis among incident glaucoma population may be conducted to address this limitation.

Generalizibility of study findings: This study will be conducted in the Swedish population and thus the findings may not be generalizable to populations in other geographical areas.

4. PROTECTION OF HUMAN PATIENTS

4.1. Patient Information and Consent

Not Applicable.

4.2. Patient withdrawal

Not Applicable.

4.3. Institutional Review Board (IRB)/Independent Ethics Committee (IEC)

Approval of use of the data from Swedish national health care register will be requested by the academic lead investigator (Appendix 1) from regional ethical board at Karolinska Institute as well as National Board of Health and Welfare. All correspondence with the IRB/IEC should be retained in the Investigator File. Copies of IRB/IEC approvals should be forwarded to Pfizer.

4.4. Ethical Conduct of the Study

The study will be conducted in accordance with legal and regulatory requirements, as well as with scientific purpose, value and rigor and follow generally accepted research practices described in *Good Pharmacoepidemiology Practices* (GPP) issued by the International Society for Pharmacoepidemiology (ISPE), Good Epidemiological Practice (GEP) guidelines issued by the International Epidemiological Association (IEA), Good Outcomes Research Practices issued by the International Society for Pharmacoeconomics and Outcomes Research (ISPOR), International Ethical Guidelines for Epidemiological Research issued by the Council for International Organizations of Medical Sciences (CIOMS), and European

Medicines Agency (EMA) European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP) Guide on Methodological Standards in Pharmacoepidemiology.

5. MANAGEMENT AND REPORTING OF ADVERSE EVENTS/ADVERSE REACTIONS

This study uses patient-level electronic health related databases (e-HRD), in which it is not possible to link (ie, identify a potential association between) a particular product and medical event for any individual. Thus, the minimum criteria for reporting an adverse event (ie, identifiable patient, identifiable reporter, a suspect product, and event) are not available and adverse events are not reportable as individual adverse event reports.

6. ANTICIPATED STUDY TIMELINE

The draft protocol (dated 06 December 2012) was submitted to the MHRA on 19 December 2012. This is the final protocol (dated 15 September 2013) submitted to the MHRA. Data acquisition will be finalized within 6 months from the date that the final approved protocol is endorsed by MHRA. Data analysis and study report will be completed within 12 months after the completion of data acquisition.

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Appendix 1. RESPONSIBLE PARTIES

Protocol Author/Principal Investigator of the Protocol

Role in the study	Name, degree(s)	Title	Affiliation	Address
Principal Investigator	Helle Kieler, MD PhD	Associate Professor	Karolinska Institute	T2, Karolinska University Hospital, SE 171 76 Stockholm , Sweden
Protocol Author	Wanning Xu, MD PhD	Director, Epidemiology	Pfizer Inc.	235 East 42nd Street, MS 219-9-1, New York, NY, USA 10017