Protocol

Study ID: VAG-4602

Vaginal estradiol tablets (Vagifem®) and endometrial cancer risk in the treatment of postmenopausal vaginal atrophy: A register-based cohort study in postmenopausal women

Redacted protocol
Includes redaction of personal identifiable information only.

Non-interventional post-authorisation safety study (PASS)

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Title	Vaginal estradiol tablets (Vagifem®) and endometrial cancer risk in the treatment of postmenopausal vaginal atrophy: A register-based cohort study in postmenopausal women
Protocol version identifier	2.0
Date of last version of protocol	06 May 2021
Active substance	Estradiol, ATC code: G03CA03
Medicinal product	Vagifem® 10 mcg, Vagifem® 25 mcg
Product reference	NDA 020908
Marketing authorisation holder(s)	Novo Nordisk Inc., P.O. Box 846, Plainsboro, NJ 08536
Research question and objectives	The aim of this study is to evaluate whether exposure to Vagifem® increases the rate of endometrial cancer in postmenopausal women. The primary objective is to investigate the hypothesis that there is no difference in risk of endometrial cancer between women using low dose vaginal estrogens (LDVE) (split into Vagifem® and other LDVE products) and women using no hormone replacement therapy to manage symptoms related to the postmenopausal phase.
Country(-ies) of study	Denmark United States of America

Marketing authorisation holder(s)

Marketing authorisation holder (s) (MAH (s))	Novo Nordisk Inc., P.O. Box 846, Plainsboro, NJ 08536

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2 List of abbreviations

ATC	Anatomical Therapeutic Chemical (Classification System)
BMI	Body Mass Index
CI	Confidence Interval
CPR	Central Person Register
DDD	Daily Define Dose
EU	European Union
GSM	Genitourinary Syndrome of Menopause
GPP	Good Pharmacoepidemiological Practice
HR	Hazard Ratio
HRT	Hormone Replacement Therapy
ICD	International Classification of Diseases
IR	Incidence Rate
IUD	Intrauterine Device
LDVE	Low dose vaginal estrogens
MAH	Marketing Authorisation Holder
mcg	microgram
NIS	Non-interventional Study
PE	Primary Exposure
SE	Secondary Exposure

SAP	Statistical Analysis Plan
UTN	Universal Trial Number

3 Responsible parties

The present study will collect third party data from nationwide Danish patient registries. The data management and analyses will be conducted at [redacted]. Furthermore, the study includes a cohort study conducted using the US-based database [redacted] where data management and analysis will be conducted at Novo Nordisk A/S.

4 Abstract

4.1 Title

Vaginal estradiol tablets (Vagifem®) and endometrial cancer risk in the treatment of postmenopausal vaginal atrophy: A register-based cohort study in postmenopausal women.

4.2 Rationale and background

Vulvovaginal atrophy is part of the genitourinary syndrome of menopause (GSM) which occurs in up to 45% of women in mid or later life. It adversely impacts physical and sexual health. If untreated, it progresses over time. Local estrogen therapy restores the vaginal epithelium and normalizes the vaginal acidic pH, which then restores the normal flora and increases vaginal moisture. Despite the established safety and efficacy of vaginal estrogen treatment, GSM represents an under recognized and undertreated condition [1, 2]. Current product labelling for low-dose vaginal estrogens do not distinguish between route of administration and comprise several “black box” warnings in US prescribing information. One of the “black box” warnings is an increased risk of endometrial cancer which has been established for oral HRT [2-4]. Endometrial cancer is the most common cancer of the female reproductive system and comprises 4.8% of all cancers in women. Women have a cumulative risk of 1% of developing the disease by age of 75 years [5]. In a Danish study of a cohort of postmenopausal women, an incidence rate of 55 per 100,000 person-years was found [3]. Multiple risk factors have been identified: overweight/obesity, long-lasting endogenous or exogenous hyperestrogenism (due to polycystic ovary, tamoxifen therapy, anovulation, nulliparity), hypertension, and diabetes mellitus [5, 6].

Three papers have in the period of 2016-2018 published results from studies of the association between endometrial cancer and vaginal estrogen therapy with conflicting results [3, 7, 8]. Mørch et al [3] concluded that vaginal estrogen therapy increased the risk of endometrial cancer in post-menopausal women with a relative risk of 1.96 (95% CI 1.77- 2.17). In contrast two studies concluded that vaginal estrogen therapy does not increase the risk of endometrial cancer [7, 8]. They reported a relative risk of 1.47 (0.75-2.90)[8] and a hazard ratio (HR) of 1.62 (CI 95%: 0.88-2.97) [7].

Novo Nordisk holds the NDA 20908 for Vagifem® (25 and 10 mcg), a low dose vaginal estrogen product. The NDA 20908 for Vagifem® 25 mcg was approved by FDA on March 26, 1999 and sNDA 20908/S-013 for 10 mcg was approved on November 25, 2009. The sale of Vagifem® 25 mcg in the US was discontinued as of July 30, 2010. There is a potential increased risk of endometrial cancer in women exposed to vaginal estrogens. The FDA has requested an observational study to evaluate the risk of endometrial cancer in postmenopausal women with a uterus who use low-dose vaginal estrogen unopposed by a progestogen. Novo Nordisk is committed to patient safety and to address the request from FDA to provide more information on the safety of Vagifem®. The Vagifem® 10 mcg product is manufactured for different markets using the same formulation, facility, and process, and data collected globally should be applicable to the US

population. In addition, the patient population in the global registries and the medical practices for this patient population are generally considered similar between the US and rest of world which would allow these data to be used to evaluate the theoretical risk of endometrial cancer.

The study will include data from a nationwide Danish cohort of postmenopausal women and a US cohort of postmenopausal women. The Danish nationwide cohort will be established through linkage of Danish national patient registries. The US cohort will be established based on data from the United States of America (US) claims database, [redacted]. The results for the US cohort will be used as supportive to the Danish one.

4.3 Research question and objectives

The aim of this study is to evaluate whether exposure to Vagifem® increases the rate of endometrial cancer in postmenopausal women.

4.3.1 Primary objective

The primary objective is to investigate the hypothesis that there is no difference in risk of endometrial cancer between women using low dose vaginal estrogens (LDVE) (split into Vagifem® and other LDVE products) and women using no hormone replacement therapy to manage symptoms related to the postmenopausal phase.

4.3.2 Secondary objective

The secondary objective is to compare the rate of endometrial cancer for women exposed to Vagifem® 10 mcg and 25 mcg, respectively, with women that have been exposed to systemic cyclic HRT (defined as estrogen taken daily and progestogen taken in a cyclic pattern for 10 to 14 days of the month) or oral, transdermal and opposed injectable systemic HRT products.

4.4 Study design

This is a non-interventional registry-based study including data from two countries: Denmark and US during the period 2000-2019 and period of 2007-2019, respectively.

The study design contains two separate cohort studies, one for each country. The two cohort studies follow postmenopausal women over time. The cohort design is useful for studies seeking to evaluate comparative safety parameters.

The primary analysis is a new user design.

4.5 Population

The study period for the Danish cohort starts on 1 January 2000 and ends on 31 December 2019.

The study period for the US cohort starts on 1 January 2007 and ends on 31 December 2019.

The study population for both the Danish and the US cohort consists of new users of LDVE (split into Vagifem® and other LDVE products) in the study period, and a comparator group consisting of women using no hormone replacement therapy. The comparator group is identified through

propensity score matching. Risk estimates will be calculated separately for estradiol 25 mcg or lower or conjugated estrogen 0.45 mg or lower, and for estradiol 10 mcg or lower or conjugated estrogens 0.3 mg or lower. In addition, Vagifem® 25 mcg will be analysed separately.

To be included in either of the cohorts the women needs to comply with the following inclusion criteria :

1. Female
2. Age 50-75 years at entry

and with the following exclusion criteria:

1. Endometrial cancer prior to entry
2. Any use of vaginal estrogen products prior to entry
3. Hysterectomy prior to entry

4.6 Variables

- The exposure variables include:Vagifem® 10 mcg, and/or
- Vagifem® 25 mcg
- Non-Vagifem® LDVE, estradiol 25 mcg or lower or conjugated estrogen 0.45 mg or lower
- Non-Vagifem® LDVE, estradiol 10 mcg or lower or conjugated estrogen 0.3 mg or lower
- Systemic cyclic HRT and oral, transdermal and opposed injectable systemic HRT products.

The adjustment variables include:

- Demographic and sociodemographic variables
  - Age
  - Education
  - Country of origin
  - Income
- Comorbidities
  - Polycystic ovarian syndrome
  - Endometriosis
  - Family history of cancer
- Medication use (including proxies for comorbidities):
  - Drug index to define hypertension medication
  - Antidiabetic medication
  - Hormonal contraceptives including hormone IUD
  - Treatment for infertility if available
  - Prior use of systemic estrogens or other estrogen- or progesterone-containing products

- Other variables, measured in parous women:
  - Parity
  - BMI
  - Smoking (yes/no)

4.7 Data sources

4.7.1 Danish cohort

The national prescription-, cancer- and patient register in Denmark are used in this study. In Denmark, data in the health care registries are made available for research by Statistics Denmark, which is a government institution collecting electronic records for a broad spectrum of statistical and scientific purposes. The Danish Civil Person Register covers every Danish resident and contains data on vital status (date of birth and death) and migrations to and from Denmark.

4.7.2 US cohort

Claims data from [redacted], including the [redacted] and the [redacted] will be used in the study.

4.8 Study size

For the Danish cohort, the calculations are based on assumptions for the study period 1 January 2000 to 31 December 2019, based on the feasibility counts in Appendix A for the period 1995-2017. With an endometrial cancer rate of 55 per 100,000 person years, and assuming that around 80,000 women are new users of Vagifem® 10 mcg vaginal estradiol tablets in the study period with an estimated average follow-up time of 5 years, the detectable hazard ratio will be 1.2 with a power of 80%.

In the US cohort the calculations are based on data between 1 January 2007 and 30 September 2019. With an endometrial cancer rate of 71 per 100,000 person years, and with around 190,000 new users of Vagifem® 10 mcg vaginal estradiol tablets and total person years around 500,000, the detectable hazard ratio will be 1.2 with a power of 80%.

4.9 Data analysis

The main statistical analysis will compare patients initiating LDVE (new users), split into Vagifem® and other LDVE, in the study period in a 1:2 ratio with non-users, in an intention to treat fashion, i.e. the patient will be considered at risk after initiation of treatment regardless of treatment discontinuation. Propensity score matching methods will be employed involving the available risk factors, including previous use of HRT.

The IR and the 95% confidence intervals will be presented both for the exposure groups and the comparator group.

Hazard Ratio and 95% confidence interval will be estimated using a Cox proportion hazard rate model.

For the secondary objective, the same methods will be used, with users of systemic cyclic HRT and oral, transdermal and opposed injectable systemic HRT products in the comparator group rather than non-users.

A range of sensitivity analyses will be performed.

4.10 Milestones

Finalization of common Statistical Analysis Plan (SAP): 31 October 2021

Registration in the EU PAS Register: 15 January 2022

First data extraction: 15 January 2022

Last data extraction: 15 January 2022

Final report of study results: 31 December 2022

5 Amendments and updates

None

6 Milestones

Milestone	Planned date
Final protocol	31 March 2021
Finalization of common Statistical Analysis Plan (SAP)	31 October 2021
Registration in the EU PAS Register	15 January 2022
First data extraction	15 January 2022
Last data extraction	15 January 2022
Study completion	31 July 2022
Final report of study results	31 December 2022

7 Rationale and background

Vulvovaginal atrophy is part of the genitourinary syndrome of menopause (GSM) which occurs in up to 45% of women in mid or later life. It adversely impacts physical and sexual health. If untreated, it progresses over time. Local estrogen therapy restores the vaginal epithelium and normalizes the vaginal acidic pH, which then restores the normal flora and increases vaginal moisture. Despite the established safety and efficacy of vaginal estrogen treatment, GSM represents an under recognized and undertreated condition [1, 2]. Current product labelling for low-dose vaginal estrogens do not distinguish between route of administration and comprise several "black box" warnings in US prescribing information. One of the "black box" warnings is an increased risk of endometrial cancer which has been established for oral HRT [2-4].

Endometrial cancer is the most common cancer of the female reproductive system and constituting 4.8% of all cancers in women. Women have a cumulative risk of 1% of developing the disease by age of 75 years [5]. In a Danish study of a cohort of postmenopausal women, an incidence rate of 55 per 100,000 person-years was found [3]. Most cases of endometrial cancer are diagnosed in early stages when the disease is still restricted to the uterus. This is mainly because abnormal uterine bleeding is the presenting symptom in 90% of cases. Patients with advanced disease might have abdominal or pelvic pain and abdominal distension. Standard treatment consists of primary hysterectomy (removal of the uterus) and bilateral salpingo-oophorectomy (removal of the ovaries and fallopian tube). The 5-year overall survival ranges from 74% to 91% in patients without metastatic disease [5]. Multiple risk factors have been identified: overweight/obesity, long-lasting endogenous or exogenous hyperestrogenism (due to polycystic ovary, tamoxifen therapy, anovulation, nulliparity), hypertension, and diabetes mellitus. The latter is the strongest risk factor and body mass index (BMI) > 30 is associated with a 3-4-fold increase in the risk of developing endometrial cancer [5, 6].

Systemic estrogen exposure with 10 mcg E2 vaginal tablet is minimal. Clinical trial data up to 1 year indicated that systemic levels of estradiol remain within the normal untreated menopausal range (2.44 – 12.08 pg/ml) with the 10 mcg E2 vaginal tablet[9, 10]. Endometrial safety of 10-mcg E2 tablets was evaluated in a pooled population from two 52-week studies in which the incidence of endometrial hyperplasia and carcinoma was assessed [11]. Study 1 was a double-blind, randomized, parallel-group, placebo-controlled trial conducted in 309 postmenopausal women (205 treated with 10-mcg E2) in North America [12], and study 2 was an open-label endometrial safety trial conducted in 336 postmenopausal women in Europe [9]. Treatment regimens in the trials were identical. A 10-mcg E2 tablet was inserted vaginally once daily during the first two weeks and then twice weekly for an additional 50 weeks, resulting in a total annual estradiol exposure of 1.14 mg. Endometrial biopsies were performed at screening and at week 52, preceded by endometrial ultrasonography. In the 10-mcg E2 group, endometrial thickness remained unchanged from baseline at week 52 (LOCF). Across these two studies, the incidence rate for endometrial hyperplasia or cancer was 0.52% per year. These results suggested no increased risk for endometrial hyperplasia or carcinoma in postmenopausal women undergoing treatment with 10-mcg E2 vaginal tablets for one year.

Three papers have in the period of 2016-2018 published results from registry studies of the association between endometrial cancer and vaginal estrogen therapy. However, these studies have

shown conflicting results [3, 7, 8]. Mørch et al [3] concluded that vaginal estrogen therapy increased the risk of endometrial cancer in post-menopausal women with a relative risk of 1.96 (95% CI 1.77- 2.17). In contrast two studies concluded that vaginal estrogen therapy does not increase the risk of endometrial cancer [7, 8]. They reported a relative risk of 1.47 (0.75-2.90) [7] and a hazard ratio (HR) of 1.62 (CI 95%: 0.88-2.97) [8] for endometrial cancer in women exposed to vaginal estrogen therapy. This wider CI found by Crandall et al [7] and Bhupathiraju et al [8] compared to what was found by Mørch et al might be due to a lower number of exposed women included (3,003 users of vaginal estrogen and 13,166 person years of women exposed to vaginal estrogen therapy, respectively) than in the study by Mørch et al (335,362 person years on vaginal estrogen). All three studies showed an overall risk estimate across different type (e.g. tablets, creams, rings) and dosages of vaginal estrogens. None of the studies published today within this field have focused only on low dose vaginal estrogen where it is expected that there will be low to no systemic effect of the treatment. In a recent review article by Constantine et al [13], the authors reviewed studies that evaluated endometrial hyperplasia or cancer incidence with unopposed vaginal estrogens, including data for vaginal estradiol tablets, with doses ranging from 10 to 50 mcg, evaluated in 10 studies in 721 women with treatment durations ranging from 3 weeks to 2 years. The authors concluded that the overall data included in this review do not support increased endometrial hyperplasia or cancer risk with low-dose, unopposed vaginal estrogens.

Novo Nordisk holds the NDA 20908 Vagifem® (estradiol vaginal inserts). The NDA 20908 for Vagifem® 25 mcg was approved by FDA on March 26, 1999 and sNDA 20908/S-013 for 10 mcg was approved on November 25, 2009. The sale of Vagifem® 25 mcg in the US was discontinued as of July 30, 2010. In Denmark Vagifem® 25 mcg was discontinued on February 3 2014.

There is a theoretical increased risk of endometrial cancer in women exposed to vaginal estrogens. FDA has requested an observational study to evaluate the risk of endometrial cancer in postmenopausal women with a uterus who use low-dose vaginal estrogen unopposed by a progestogen. Novo Nordisk is committed to patient safety and to address the request from FDA to provide more information on the safety of Vagifem®. The Vagifem® 10 mcg product is manufactured for different markets using the same formulation, facility, and process, and data collected globally should be applicable to the US population. In addition, the patient population in the global registries and the medical practices for this patient population are generally considered similar between the US and rest of world which would allow these data to be used to evaluate the theoretical risk of endometrial cancer.

The study will include data from a nationwide Danish cohort of postmenopausal women, the results hereof will be supported by results from a US cohort of postmenopausal women. The Danish nationwide cohort will be established through linkage of Danish national patient registries (see section 9.4.1). The US cohort will be established based on data from [redacted].

The aim of the study is to assess if there is a long-term increased rate of endometrial cancer when postmenopausal women are exposed to Vagifem®.

8 Research question and objectives

The aim of this study is to evaluate whether exposure to Vagifem® increases the rate of endometrial cancer in postmenopausal women.

8.1 Primary objective

The primary objective is to investigate the hypothesis that there is no difference in risk of endometrial cancer between women using low dose vaginal estrogens (LDVE) (split into Vagifem® and other LDVE products) and women using no hormone replacement therapy to manage symptoms related to the postmenopausal phase.

8.2 Secondary objective

The secondary objective is to compare the rate of endometrial cancer for women exposed to Vagifem® 10 mcg and 25 mcg, respectively, with women that have been exposed to systemic cyclic HRT (defined as estrogen taken daily and progestogen taken in a cyclic pattern for 10 to 14 days of the month) or oral, transdermal and opposed injectable systemic HRT products.

9 Research methods

9.1 Study design

This is a non-interventional registry-based study including data from two countries: Denmark and US during the period 2000-2019 and period of 2007-2019, respectively.

The study design contains two separate cohort studies, one for each country. The two cohort studies follow postmenopausal women over time. The cohort design is useful for studies seeking to evaluate comparative safety parameters.

The primary analysis is a new user design.

9.1.1 Primary endpoint

First time occurrence of endometrial cancer (defined based on relevant ICD10/9 codes, yes/no) registered during the time in the cohort from entry (start of treatment) to exit (end of study period, occurrence of any other cancer (except non-melanoma skin cancer), date of emigration, or date of death).

Please see section 9.3 for definition of relevant ICD10/9 codes.

9.2 Setting

This non-interventional study is based on health care registries from Denmark (nationwide data) which will be supported by data from US (Claims data from [redacted]).

9.2.1 Study Population

9.2.1.1 Danish study population

The study period starts on 1 January 2000 and ends on 31 December 2019.

The study population consists of new users of LDVE (split into Vagifem® and other LDVE products) in the study period, and a comparator group consisting of women using no hormone replacement therapy. The comparator group is identified through propensity score matching. Risk estimates will be calculated separately for estradiol 25 mcg or lower or conjugated estrogen 0.45 mg or lower, and for estradiol 10 mcg or lower or conjugated estrogens 0.3 mg or lower. In addition, Vagifem® 25 mcg will be analysed separately.

New users are followed from the entry date, defined as the dispensing date of the first prescription of each exposure of interest as defined above, until occurrence of endometrial cancer, exposure to any non-LDVE hormone treatment, exposure to any non-Vagifem® LDVE (women in Vagifem® group only), death, emigration, or end of study period, whichever comes first. Women in the comparator group are allocated the same entry date as their match in the new user group, and followed up until occurrence of endometrial cancer, death, emigration, or end of study period, whichever comes first.

9.2.1.2 US study population

The study period starts on 1 January 2007 and ends on 31 December 2019.

The study population consists of new users of LDVE (split into Vagifem® and other LDVE products) in the study period, and a comparator group consisting of women using no hormone replacement therapy. The comparator group is identified through propensity score matching.

New users are followed from the entry date, defined as the dispensing date of the first prescription of each exposure of interest as defined above, until occurrence of endometrial cancer, exposure to any non-LDVE hormone treatment, exposure to any non-Vagifem® LDVE (women in Vagifem® group only), or end of study period, whichever comes first. Women in the comparator group are allocated the same entry date as their match in the new user group, and followed up until occurrence of endometrial cancer, or end of study period, whichever comes first.

9.2.2 Inclusion criteria

To be eligible for the study, persons are required to meet the following inclusion criteria:

1. Female
2. Age 50-75 years at entry

9.2.3 Exclusion criteria

1. Endometrial cancer prior to entry
2. Any use of vaginal estrogen products prior to entry
3. Hysterectomy prior to entry

9.2.4 Rationale for selection criteria

The rationale for the inclusion and exclusion criteria is:

Age 50-75 years was chosen to capture postmenopausal women in an age group where HRT is prescribed.

No hysterectomy: women without a uterus cannot develop endometrial cancer.

9.3 Variables

See section 9.4 for a description of the data sources. The following variables will be included in the analysis: Outcome, exposure and adjustment variables. More information on these variables is included below.

9.3.1 Outcome

9.3.1.1 Danish cohort

The outcome is an incident endometrial cancer diagnosis in the Danish Cancer Register until 2018 and in the National Health Register until 2019 as defined by the following diagnostic codes according to the International Classification of Diseases 10th version (ICD-10):

- C54.1 Malignant neoplasm of endometrium

9.3.1.2 US Cohort

To identify cases of endometrial cancer the approach to identify endometrial cases proposed by the FDA's Sentinel [14]. The same ICD-10 codes as for the Danish cohort will be applied using [redacted] data. For older data where the ICD-9 codes are used, the following codes will be used to identify endometrial cancer cases:

- 182.0 Malignant neoplasm of corpus uteri, except isthmus

9.3.2 Exposure

9.3.2.1 Danish cohort

The exposure to treatment will be included in the analyses as defined below. Relevant ATC codes and product information will be used to identify the exposure variables.

- Vagifem® 10 mcg
- Vagifem® 25 mcg
- Non-Vagifem® LDVE, estradiol 25 mcg or lower or conjugated estrogen 0.45 mg or lower
- Non-Vagifem® LDVE, estradiol 10 mcg or lower or conjugated estrogen 0.3 mg or lower
- Systemic cyclic HRT and oral, transdermal and opposed injectable systemic HRT products.

It has previously been shown that a lag-time period of at least 6 months is enough to reduce bias from reverse causation in general [15]. The lag time is subject to sensitivity analyses. Considerations regarding lag-time and exposure definitions are revisited in sensitivity and supplementary analyses (see section 9.7.3 and 9.7.4).

In the analyses the daily exposure will be estimated as follows: For the pharmaceutical compounds of interest calculations of exposure will assume standard use of each compound. For each compound a recommended dose, a minimal dose and a maximal dose is recorded. Pharmacy purchases and these doses are used to calculate exposure over time with the aim of ensuring as far as possible a continuous use of therapy as has been done previously [16]. If therapy starts with a drug that makes it unlikely that another drug is still continued it will be assumed that the first drug is stopped at the time where the second drug is introduced.

9.3.2.2 US cohort

- Vagifem® 10 mcg
- Vagifem® 25 mcg
- Non-Vagifem® LDVE, estradiol 25 mcg or lower or conjugated estrogen 0.45 mg or lower
- Non-Vagifem® LDVE, estradiol 10 mcg or lower or conjugated estrogen 0.3 mg or lower
- Systemic cyclic HRT and oral, transdermal and opposed injectable systemic HRT products.

Exposure to LDVE will be defined via the number of days supplied registered for each individual prescription record.

It has previously been shown that a lag-time period of at least 6 months is enough to reduce bias from reverse causation in general [15]. The lag time is subject to sensitivity analyses. Considerations regarding lag-time and exposure definitions are revisited in sensitivity and supplementary analyses (see section 9.7.3and 9.7.4).

9.3.3 Adjustment variables

To adjust for potential confounding, a priori selected risk factors for endometrial cancer are included in the propensity score matching.

The list below provides an overview of the selected adjustment variables. The final list and corresponding definitions (diagnosis codes etc.) will be compiled in collaboration with research partners and included in the Statistical Analysis Plan.

9.3.3.1 Danish cohort

For disease covariates individuals are assumed to maintain a disease (such as hypertension and diabetes) for all time after the diagnosis.

<u>Demographic and sociodemographic variables</u>

- Age: years
- Education: measured prior to cohort entry date, categorized into groups e.g.:
  - Basic school
  - Vocational Training
  - Bachelor
  - Higher education
- Country of origin, categorized into groups (based on available data defined in the SAP)
- Income: measured 5 years back in time from cohort entry date, categorized into groups e.g.:
  - High
  - Medium
  - Low

Comorbidities

Relevant ICD codes and other information available in the data will be used to identify the comorbidities (will be defined in the SAP)

- Polycystic ovarian syndrome: measured prior to cohort entry date (yes/no)
- Endometriosis: measured prior to cohort entry date (yes/no)
- Family history of cancer (any cancer in mothers of women in the cohort), measured prior to cohort entry date (yes/no)
    - Not available in all patients, sensitivity analyses will be made without this information.

Medication use (including proxies for comorbidities):

Relevant ATC codes and product information will be used to identify the medication variables.

The medication variables will be included in the matching process.

- Drug index to define hypertension medication (yes/no)
- Antidiabetic medication (yes/no)
- Hormonal contraceptives including hormone IUD (yes/no)
- Prior use of systemic estrogens or other estrogen- or progesterone-containing products

The following medication variable will be measured prior to cohort entry date

- Treatment for infertility if available (yes/no)

Other variables, measured in parous women:

- Parity, measured prior to cohort entry date, categorized into following groups:
    - 0 childbirths
    - 1-2 childbirths
    - More than 2 childbirths
- BMI (kg/m2), measured in parous women, categorized:
    - underweight (<18.5)
    - normal (18.5-<25)
    - overweight (25-<30)
    - obese (>= 30)
- Smoking (yes/no)

9.3.3.2 US cohort

The US cohort will include the same variables if available.

9.4 Data sources

9.4.1 Danish cohort

The national prescription-, cancer- and patient register in Denmark are used in this study (Table 9-1). Denmark is chosen due to the high data quality, the long history of health care registrations, the possibility of linkage between registries using the unique person identifier and the nationwide coverage of the registries. It is possible to link the data through the Central Person Register (CPR) number. The CPR number is unique for individuals and the same number follows one person throughout their life (with very few exceptions, such as identity theft). The linkage enables follow-up of individuals through time in different registries. Thus, for individuals with permanent residence in Denmark, loss to follow-up is unlikely [17, 18]. The unique person identifier was introduced more than 50 years ago in the Denmark [19]. It is assigned to all residents and makes it possible to conduct accurate population-based register linkage studies with linkage of e.g., prescription and diagnosis data.

In Denmark, data in the health care registries are made available for research by Statistics Denmark and the Danish board of health (Sundhedsdatastyrelsen), which are government institutions collecting electronic records for a broad spectrum of statistical and scientific purposes. The Danish Civil Person Register covers every Danish resident and contains data on vital status (date of birth and death) and migrations to and from Denmark since 1968 [20].

For the current project all necessary data are made available in the research environment of Statistics Denmark where multiple registries can be combined using the CPR number. The data are provided with an encrypted CPR to protect individuals.

In Denmark, health care data are collected for administrative purposes. Retrospective analysis of registry data requires the study to be listed in institutional project lists. Informed consent to use such data is not required and ethical approval is also not required. The ethical committee system does not accept applications for such data according to the legislation for scientific ethical committees.

Table 9-1 Danish registries used in the analyses

Health care registries	Start of registry	Variables included in the analyses from the registry
Danish National Prescription Registry	1995	Exposure variables Hormonal contraceptive exposure Antidiabetic medications Treatment for infertility Information used in hypertension drug index Metformin exposure
The Danish Cancer registry	1942 (complete from 1943)	Endometrial cancer

		Family history of cancer (any cancer in mothers of women in the cohort) Other cancers (except non-melanoma skin cancer)
Danish Nation Patient Registry	1977 (hospital admissions) 1995 (outpatient contacts)	Polycystic ovarian syndrome Endometriosis
Statistics Denmark	1966	Education Income Country of origin
The Danish Civil Person Register	1968	Age Migration Death
The National Birth register	1973/2004*	Parity BMI Smoking

*BMI and smoking were included in the National Birth register in 2004

9.4.1.1 Danish National Prescription Registry

The Danish National Prescription Registry includes individual-level data on all prescriptions filled and redeemed by Danish residents at community pharmacies. The registry contains 46 variables that characterize each redeemed prescription, including those describing the patient, the drug dispensed, the health provider issuing the prescription and the dispensing pharmacy sold in primary care or purchased for use in Danish hospitals [21]. Of relevance to the present study, the national prescription registries in the Denmark contain data on the unique person identifier, date of dispensing, the product strength, the Anatomical Therapeutic Chemical (ATC) code of the dispensed drug, and the volume of the dispensed drug in Defined Daily Doses (DDD). The nationwide coverage of the national prescription registries makes it possible to conduct studies based on these data sources with no selection bias.

9.4.1.2 The Danish Cancer Register

The Danish Cancer Registry is a research register. The information includes personal information (e.g. CPR number, sex, and age at diagnosis) and tumor characteristics (e.g. ICD-10 code, (ICD-O-3 codes, morphology, topography, and basis for diagnosis) [18].

9.4.1.3 The National Health Register

The Danish National Patient Registry has collected data from all Danish hospitals with complete nationwide coverage since 1978. Information includes administrative data (e.g. CPR number, hospital information, admission type and date), diagnoses (e.g. primary and secondary diagnosis. complications in ICD-10 codes), treatments (surgery, anesthesia, intensive care), and examinations (e.g. radiological procedures) [22].

9.4.1.4 Statistics Denmark

Data obtained from Statistics Denmark will be social status (ongoing and finished education and employment) and income.

9.4.2 US Cohort

The [redacted] database is split into two parts, the [redacted] the [redacted]. The maximal age of a patient in the first database is 65 years. Older patients can be found in the [redacted], records for these patients start at 65 years, and it is possible to link between the two databases.

9.5 Study size

All women meeting the inclusion and exclusion criteria will be part of the analyses.

In order to calculate the power for the detectable Hazard Ratio for endometrial cancer, we assume a 2:1 ratio between the comparator group and the Vagifem® group. Further we are assuming that the statistical tests are performed as two-sided with a 5% significance level.

For the Danish cohort, the calculations are based on assumptions for the study period 1 January 2000 to 31 December 2019, based on the feasibility counts in Appendix A for the period 1995-2017, . With an endometrial cancer rate of 55 per 100,000 person years, and assuming that around 80,000 women are new users of Vagifem® 10 mcg vaginal estradiol tablets in the study period with an estimated average follow-up time for every use of 5 years, the detectable hazard ratio will be 1.2 in the Danish cohort with a power of 80%.

In the US cohort the calculations are based on data between 1 January 2007 and 30 September 2019. With an endometrial cancer rate of 71 per 100,000 person years, and with around 190,000 new users of Vagifem® 10 mcg vaginal estradiol tablets and total person years around 500,000, the detectable hazard ratio will be 1.2 with a power of 80%.

9.6 Data management

The data extraction will follow the SAP which will fully detail the conduct of the study, including the analytical approach, operational definitions of variables, as well as shell tables and figures to be presented.

9.6.1 Danish cohort

All extraction of raw data will occur at servers at Statistics Denmark, where it will be available for analysis by the coordinating study entity.

The academic collaborators will retrieve as much information as possible about how data are generated and should be interpreted. This information will include level of coverage, a full dictionary for all variables, data on migration, data on co-prescribed medication, and co-morbidity.

9.6.2 US cohort

All extraction of raw data and data analysis will occur at Novo Nordisk A/S.

9.7 Data analysis

9.7.1 Definition of analysis sets

For both the Danish and US cohorts, the analysis set will include women fulfilling the inclusion and exclusion criteria. The exposed women are new users of LDVE, (split into Vagifem® and other LDVE products). Risk estimates will be calculated separately for estradiol 25 mcg or lower or conjugated estrogen 0.45 mg or lower, and for estradiol 10 mcg or lower or conjugated estrogens 0.3 mg or lower. In addition, Vagifem® 25 mcg will be analysed separately.

9.7.2 Statistical methods

9.7.2.1 The Danish cohort

The detailed steps for making the analytic dataset and generating the study results will be described in detail in the statistical analysis plan (SAP). The main statistical analysis will compare patients initiating LDVE (new users), split into Vagifem® and other LDVE, in the study period in a 1:2 ratio with non-users, in an intention to treat fashion, i.e. the patient will be considered at risk after initiation of treatment regardless of treatment discontinuation. The main obstacle for such an analysis is the fact that the exposures and especially exposures to other types of HRT products can make the interpretation of such a comparison very difficult. Propensity score matching methods will be employed involving the available risk factors, including previous use of HRT, in order to ensure that the comparison will be as robust as possible.

The incidence rate (IR) of endometrial cancer will be calculated both for the exposure groups and the comparator group. The IR and the 95% confidence intervals will be presented for the exposure groups and the comparator group.

Hazard Ratio and 95% confidence interval will be estimated using a Cox proportion hazard rate model.

For the secondary objective, the same methods will be used, with users of systemic cyclic HRT and oral, transdermal and opposed injectable systemic HRT products. in the comparator group rather than non-users.

A range of supplementary and sensitivity analyses will be performed, see sections 9.7.3 and 9.7.4.

9.7.2.2 US cohort

The analyses will be replicated in US cohort and adjusted according to availability of data using the methods described above.

9.7.3 Supplementary analyses

The following supplementary analyses are planned:

- The analyses will be repeated for all Low Dose Vaginal Estrogen (LDVE) products combined together. This also includes the sensitivity analyses in section 9.7.4 with alternative sets of ICD codes and with two alternative lag time definitions.
- The combined LDVE analyses using the most specific ICD codes (ICD-9 code 182.0 and ICD-10 code C54.1) and the most inclusive ICD codes (all ICD-9 182 codes and all ICD-10 C54 codes) will be performed to assess the risk of endometrial cancer by:
    1. Accumulated duration, categorized as <0.5 years, 0.5 to <1 year, 1 to <2 years, 2 to <3 years, 3 years and above.
    2. Accumulated dose.
    3. Average LDVE daily dose usage, categorized as high (estradiol >10mcg to ≤25mcg; conjugated estrogens >0.3mg to ≤0.45mg) and low (estradiol ≤10 mcg; conjugated estrogens ≤0.3 mg).
    4. Active ingredient (vaginal estradiol, vaginal conjugated estrogens).
    5. Formulation (vaginal cream, ring, tablet or insert) on the date of first dispensing.
- The analyses will be repeated for two on-treatment periods, where follow-up is censored after a gap of more than 90 and 180 days, respectively, after the completion of the last prescription. This also includes the sensitivity analyses in section 9.7.4 with alternative sets of ICD codes and with two alternative lag time definitions.
- The analyses will be repeated only including women 55-75 years. Restriction to the lower age 55 years is to explore a potential impact of premenopausal women included in the main analyses for the full age range (ages above 50 years).
- For the Danish cohort, the analyses will be repeated to include endometrial cancer diagnosis using the national patient register on top of the cancer register
- The analyses will be repeated where patients are censored on occurrence of non-endometrial cancer.
- The analyses will be repeated grouped on presence (yes/no) of vulvovaginal atrophy.

9.7.4 Sensitivity analyses

By use of the Danish data several sensitivity analyses are performed to check the influence from the analytical/design choices on the study findings. The following analyses are planned and will be further specified in the Statistical Analysis Plan:

- For the women that have not given birth or have given birth before 2004 some of the known risk factors (e.g. BMI and smoking) for endometrial cancer are not captured in the National Birth Registry. However, it is expected that this information can be estimated by multiple imputations based on the information available on these confounders among the younger women in the cohort.

- Including additional data from the cancer register with information on histology and restricting to Type I malignancy only. Type I tumors are considered hormone sensitive tumors. For this analysis, the tumors will be categorized according to the ICD-Oncology (ICD-O-3) coding system for topography and morphology codes into Type I or Type II tumors. Type I tumors include endometrioid (ICD-O-3 codes 8380, 8381, 8382, 8383), tubular adenocarcinoma (8210, 8211), papillary adenocarcinoma (8260, 8262, 8263), squamous adenocarcinoma (8570), mucinous adenocarcinoma (8480, 8481) and adenocarcinoma not otherwise specified (8140). Type II tumors include clear cell (8310), serous (8441), papillary serous (8460, 8461), squamous cell (8050, 8070, 8071, 8072), adenosquamous (8560), small cell carcinoma (8041) and mixed cell adenocarcinoma (8323). For these analyses of Type I tumours we also use cases with ICD10 code C55 if they have a histology defined as Type I according to the ICD-O-3 coding system. The definitions of type I and II tumours are continuously discussed in the scientific literature and therefore some minor adjustments may be made to the above in the SAP.

In both data sources the following sensitivity analyses will be performed:

- Changing lag time to 3 and 12 months.
- Repeating the analyses using the following ICD codes:
  - 182.0, 182.1 and 182.8 in ICD-9 or C54.1, C54.2, C54.3, C54.8 and C54.9 in ICD-10
- Repeating the analyses using the following ICD codes:
  - all C54 codes and 182 codes, except 182.1, C54.2, and C54.3
- Repeating the analyses using the following ICD codes:
  - all C54 codes in ICD-10 and 182 codes in ICD-9
- Repeating the analyses using the following ICD codes:
  - all C54 codes and 182 codes, except 182.1, 182.8, C54.2, and C54.3

9.7.5 Quantitative bias analysis

Potential bias due to incomplete information on BMI will be quantified by a quantitative bias analysis, which enables estimation of non-random errors in epidemiologic studies, assessing the magnitude and direction of biases and quantifying their uncertainties.

In the US database, additional bias analyses will be performed for the history of hysterectomy and prior hormonal product use.

9.8 Quality control

SAP and records of statistical programming performed to generate the results will be stored by the academic collaborators for the Danish cohort and by Novo Nordisk A/S for the US cohort. The academic collaborators will file the necessary documents that would allow a replication of all data

extractions and analyses performed during the study. The documentation should clearly show what was done at which time point, to meet the requirements for full transparency.

9.8.1 Danish registries

Data quality control is made within the setting of the Danish registries. The Danish national health registries are of high quality with a high level of completeness and validity. Validation of the registries includes both manual and automatic checks to ensure the high degree of quality [18, 21, 22].

Data extraction will be performed and transferred to a server at Statistics Denmark, where it will be available for analysis. Such centralized analysis is expected to provide the highest level of quality and accountability. Data will be structured according to a common data model that will be developed for the study.

The academic collaborators will ensure that the standard operating procedures applicable for this type of study in their research unit are followed.

9.8.2 US database

The [redacted] database contains de-identified records for more than 186 million patients to provide a patient-centric perspective reflecting real-world treatment patterns and the cost of care. Full integration, longitudinal strength and the deep cross-sectional detail of the claims databases provide a wide breadth of information for detailed research quality [23].

A major advantage of [redacted] data involves their comprehensive coding. Key examples include:

- Fully paid and adjudicated claims
- Complete outpatient prescription drug information, including injectables, specialty pharmacies, all carve-outs, manual and electronically submitted claims, and plan/formulary summaries [23].

Several quality checks are made within the data collection system and potential erroneous data is returned to the data originator for verification.

9.8.3 Critical documents

Before the academic collaborators perform the first data extract of the Danish data, the following documents must be available to Novo Nordisk:

- Signed and dated agreement on the final protocol
- Signed and dated SAP
- Signed and dated agreement on any amendment(s), if applicable
- Signed contract of research collaboration

9.8.4 Retention of study documentation

Novo Nordisk will comply with GPP and relevant national legislation related to archiving of study documentation.

The academic collaborators must agree to archive the documentation pertaining to the study in an archive for at least 5 years after final report/first publication of the study, whichever comes later. The academic collaborators should not destroy any documents without prior permission from Novo Nordisk.

Novo Nordisk will retain the documentation pertaining to the study according to company procedure and in accordance with national regulations if they require a longer retention period.

9.9 Limitations of the research methods

The indication for drug use and prescribed dose is not easily available for research purposes in the national prescription registries. Thus, this study relies on assumptions on how the HRT is routinely administered by the patient. However, there is good reason to assume that the study population to a large extent only includes postmenopausal women.

As this is a non-interventional study, potential confounding factors cannot be ruled out. Data collection will reflect routine clinical practice rather than mandatory assessments at pre-specified time points, which may have an impact on the amount of data and its interpretation. Control for confounding in the study is done by multiple regression analyses including adjustment variables.

However, confounding may still be present if covariates (e.g. unmeasured confounders) that are unevenly distributed between users of LDVE and non-users of HRT are not included in the model, or if they are included but only poorly measured in the health care registries. Lifestyle risk factors such as alcohol use and smoking are considered as risk factors for endometrial cancer. These lifestyle risk factors are, however, sparsely measured in the health care registries. Therefore, other variables are included as proxies (see section 9.3.3). However, they are only crude proxies. Importantly, however, there is no reason to suspect that users of LDVE have a markedly different smoking history or different alcohol consumption compared to users of active comparators or non-users of hormone therapy.

The inclusion criteria do not include the diagnosis of vulvovaginal atrophy due to the assumption that this condition is not always captured in the registries. The impact of this assumption is tested in a supplementary analysis.

9.9.1 Specific for the US data source

The [redacted] database includes records for more than 186 million persons. There is no continuous coverage as people often switch insurance when changing employers. The follow-up time in the [redacted] database is shorter than in the Danish data sources, and there may be limited information available for diseases, prescriptions, and procedure performed (hereunder hysterectomy) prior to registration in the database. The [redacted] database is mostly based on data from large employers; medium and small firms are not represented. However, these data can complement other datasets or be used as benchmarks against them.

The Danish registry data are considered the primary data source and the results of these analyses will be compared to the results from the [redacted] database in order to ensure the applicability to the US population.

10 Protection of human subjects

The present study is a non-interventional study based on already existing registry data. Anonymized data of relevance to the objectives of the study is extracted for analysis.

The study is conducted in accordance with GPP [24] and any local regulations. Special attention is paid to data privacy protection and data protection as reflected in Directive (EU) 2016/680 of 27 April 2016 and its implementation in the national legislation by May 2018. Novo Nordisk will get access to the results of the study, but not to the data used in the study.

10.1 Data handling

Data collected will be used as part of the statistical analysis.

In the Denmark, studies based solely on registry data do not require informed consent from individuals in the study population. Investigators involved in the study are governed by regional rules that guarantee the integrity of data and the privacy of individuals.

10.2 Institutional Review Boards/Independent Ethics Committee, health authorities and other relevant national institutions/bodies

Study specific documentation (study protocol, amendments, and the non-interventional study report) will be submitted to regulatory authorities as required by national requirements. Approval of the study protocol will be sought from FDA.

It is the responsibility of the academic collaborators to perform the required submissions and get the necessary approvals for the study from the relevant authorities, the national Data Protection Agencies and the relevant data owners. Ethical approval is not required in Denmark for purely registry-based studies.

11 Collection and reporting of safety information

This study is based on data already available in existing databases (secondary data) and single case collection and reporting from such studies is not required according to the current European Pharmacovigilance Regulations (Module VI (rev.2)).

12 Plans for disseminating and communicating study results

A final study report will be submitted to the FDA upon study completion.

12.1 Registration of study information

In accordance with Novo Nordisk commitment to transparency in clinical activities, this study will be registered on www.clinicalTrials.gov and www.novonordisk-trials.com no later than first data extraction.

This study is subject to registration no later than the first data extraction according to Novo Nordisk requirement on non-interventional study disclosure. For studies that include data collected also retrospectively, the study is to be registered prior to the first capture of the data.

12.2 Communication and publication

Novo Nordisk commits to communicating or otherwise making available for public disclosure results of studies regardless of outcome. Public disclosure includes publication of a paper in a scientific journal, abstract submission with a poster or oral presentation at a scientific meeting, or by other means.

At the end of the study, one or more publication(s) may be prepared by the academic collaborators in collaboration with Novo Nordisk. Novo Nordisk reserves the right to postpone publication and/or communication for up to 60 days to protect intellectual property and reserves the right not to release interim results or data until a study report is available. The results of this study will be subject to public disclosure on external web sites according to international regulations, as reflected in the Novo Nordisk Commitment to share information about clinical studies.

In all cases, the study results must be reported in an objective, accurate, balanced and complete manner, with a discussion of the strengths and limitations of the study. All authors will be given the relevant statistical tables, figures, and reports needed to support the planned publication. In the event of any disagreement about the content of any publication, both the physicians' and Novo Nordisk's opinions must be fairly and sufficiently represented in the publication.

Novo Nordisk maintains the right to be informed of academic collaborators plans for publication and to review any scientific paper, presentation, communication or other information concerning the investigation described in this protocol. Any such communication must be submitted in writing to the Novo Nordisk study manager prior to submission for comments. Comments will be given within four weeks from receipt of the planned communication.

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Appendix A Feasibility counts for Danish cohort

The numbers provided in this feasibility counts are provided before the final cohort is built and purely created for the purpose of being included in the protocol. The numbers are created based on data available in the Danish registries. All numbers are subject to change for the final analyses. Datasets will be updated in time prior to analysis, and further considerations may also change numbers slightly.

The study sample for the feasibility count is selected by the following: The starting date is the date where the woman turns 50 years or 1 January 1995 whichever comes last. Exit from the analysis is the censoring data of 31 December 2017, date of death, date of hysterectomy or date of any cancer excluding non-melanoma skin cancer. Women where the exit date is before the starting date are excluded from analysis.

The number of women entering analysis is 1,794,550. The number of endometrial cancers is 16,007

Exposure to Vagifem® is calculated by assuming 10 weeks of exposure from each prescription. A more elaborate procedure will be used in final calculations. The total time of exposure is the sum of 10-week periods. Continuous exposure for each dose of Vagifem® is calculated as the maximal period with overlapping 10-week periods. Combined exposure to the two doses of Vagifem® is a simple sum.

Total exposure by dose is for each dose of Vagifem® calculated as the number of packages multiplied by the number tablets in packages multiplied by dose.

For an estimate of systemic use prior to Vagifem®, ATC groups G03D, G03F and G03C are included. If the compound is for vaginal local therapy is assumed, otherwise treatment is considered systemic. Prior or later use requires at least one prescription prior to or later than a Vagifem® prescription.

Gaps in treatment with Vagifem® are for power considerations simply maximal time between prescriptions minus 10 weeks. Maximal gap is calculated for each user.

Table 1 Usage patterns hormone replacement therapy

Variable	Values
Observation time [days]	4330.0 [1974.0, 7182.0] 4486.7 (2782.0)
Number of women received Vagifem®10 mcg (%)	83276 (4.7)
Continuous time – Vagifem® 10 mcg, days	139.0 [84.0, 182.0] 152.3 (85.2)
Total time - Vagifem® 10 mcg, days	280.0 [140.0, 630.0] 466.5 (524.4)
Total dose - Vagifem® 10 mcg, mcg	720.0 [360.0, 1620.0] 1199.5 (1348.4)
Number of women received, Vagifem® 25 mcg (%)	187105 (10.5)
Continuous time - Vagifem® 25 mcg, days	139.0 [81.0, 204.0] 156.5 (93.7)
Total time - - Vagifem® 25 mcg, days	280.0 [140.0, 770.0] 662.6 (1009.5)
Total dose - - Vagifem® 25 mcg, mcg	1500.0 [750.0, 4125.0] 3549.5 (5407.8)
Number of women received Vagifem® 10 or 25 mcg (%)	270381 (15.2)
Continuous time - Vagifem®10 or 25 mcg, days	140.0 [98.0, 270.0] 202.5 (149.1)
Total time - Vagifem®10 or 25 mcg, days	280.0 [140.0, 700.0] 602.2 (893.3)
Total dose - Vagifem®10 or 25 mcg, mcg	1125.0 [375.0, 3000.0] 2825.7 (4687.6)
Number of women received other vaginal estradiol	30136 (1.7)
Total time - other estradiol (vaginal ring), days	360.0 [90.0, 900.0] 721.2 (951.2)
Total dose - other estradiol, mcg	2700.0 [675.0, 6750.0] 5409.0 (7133.9)
Maximal gap in therapy	1714.0 [658.0, 3055.0] 2075.2 (1692.1)
Number of women used systemic HRT before Vagifem®, (%)	175455 (9.9)
Number of women used systemic HRT after Vagifem®, (%)	17298 (1.0)

All times are in days. For continuous variables results are: median [25%/75% percentiles] mean (standard deviation). For discrete variables numbers and (percent) is shown. Doses are micrograms

ANNEX. ENCePP Checklist for Study Protocols

Doc.Ref. EMA/540136/2009

ENCePP Checklist for Study Protocols (Revision 4)

Adopted by the ENCePP Steering Group on 15/10/2018

The European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP) welcomes innovative designs and new methods of research. This Checklist has been developed by ENCePP to stimulate consideration of important principles when designing and writing a pharmacoepidemiological or pharmacovigilance study protocol. The Checklist is intended to promote the quality of such studies, not their uniformity. The user is also referred to the ENCePP Guide on Methodological Standards in Pharmacoepidemiology, which reviews and gives direct electronic access to guidance for research in pharmacoepidemiology and pharmacovigilance.

For each question of the Checklist, the investigator should indicate whether or not it has been addressed in the study protocol. If the answer is "Yes", the section number of the protocol where this issue has been discussed should be specified. It is possible that some questions do not apply to a particular study (for example, in the case of an innovative study design). In this case, the answer 'N/A' (Not Applicable) can be checked and the "Comments" field included for each section should be used to explain why. The "Comments" field can also be used to elaborate on a "No" answer.

This Checklist should be included as an Annex by marketing authorisation holders when submitting the protocol of a non-interventional post-authorisation safety study (PASS) to a regulatory authority (see the Guidance on the format and content of the protocol of non-interventional post-authorisation safety studies). The Checklist is a supporting document and does not replace the format of the protocol for PASS presented in the Guidance and Module VIII of the Good pharmacovigilance practices (GVP).

Study title:

EU PAS Register® number: **Study reference number (if applicable):**

Section 1: Milestones	**Yes**	**No**	**N/A**	**Section Number**
1.1 Does the protocol specify timelines for				
1.1.1 Start of data collection[1]	☒	☐	☐	6

[1] Date from which information on the first study is first recorded in the study dataset or, in the case of secondary use of data, the date from which data extraction starts.

Section 1: Milestones	**Yes**	**No**	**N/A**	**Section Number**
1.1.2 End of data collection[2]	☒	☐	☐	6
1.1.3 Progress report(s)	☐	☐	☒	
1.1.4 Interim report(s)	☐	☐	☒	
1.1.5 Registration in the EU PAS Register®	☒	☐	☐	6
1.1.6 Final report of study results.	☒	☐	☐	6

Comments:

Section 2: Research question	**Yes**	**No**	**N/A**	**Section Number**
2.1 Does the formulation of the research question and objectives clearly explain:	☐	☐	☐	
2.1.1 Why the study is conducted? (e.g. to address an important public health concern, a risk identified in the risk management plan, an emerging safety issue)	☒	☐	☐	7
2.1.2 The objective(s) of the study?	☒	☐	☐	8
2.1.3 The target population? (i.e. population or subgroup to whom the study results are intended to be generalised)	☒	☐	☐	8
2.1.4 Which hypothesis(-es) is (are) to be tested?	☒	☐	☐	8
2.1.5 If applicable, that there is no *a priori* hypothesis?	☐	☐	☒	

Comments:

Section 3: Study design	**Yes**	**No**	**N/A**	**Section Number**
3.1 Is the study design described? (e.g. cohort, case-control, cross-sectional, other design)	☒	☐	☐	9.1
3.2 Does the protocol specify whether the study is based on primary, secondary or combined data collection?	☒	☐	☐	9.1
3.3 Does the protocol specify measures of occurrence? (e.g., rate, risk, prevalence)	☒	☐	☐	9.7.2
3.4 Does the protocol specify measure(s) of association? (e.g. risk, odds ratio, excess risk, rate ratio, hazard ratio, risk/rate difference, number needed to harm (NNH))	☒	☐	☐	9.7.2

[2] Date from which the analytical dataset is completely available.

Section 3: Study design		**Yes**	**No**	**N/A**	**Section Number**
3.5	Does the protocol describe the approach for the collection and reporting of adverse events/adverse reactions? (e.g. adverse events that will not be collected in case of primary data collection)	☐	☐	☒	

Comments:

Section 4: Source and study populations		**Yes**	**No**	**N/A**	**Section Number**
4.1	Is the source population described?	☒	☐	☐	9.2.1
4.2	Is the planned study population defined in terms of:				
	4.2.1 Study time period	☒	☐	☐	9.2.1
	4.2.2 Age and sex	☒	☐	☐	9.2.2
	4.2.3 Country of origin	☒	☐	☐	9.2.1
	4.2.4 Disease/indication	☒	☐	☐	8
	4.2.5 Duration of follow-up	☒	☐	☐	9.2.1
4.3	Does the protocol define how the study population will be sampled from the source population? (e.g. event or inclusion/exclusion criteria)	☒	☐	☐	9.2.2-3

Comments:

Section 5: Exposure definition and measurement		**Yes**	**No**	**N/A**	**Section Number**
5.1	Does the protocol describe how the study exposure is defined and measured? (e.g. operational details for defining and categorising exposure, measurement of dose and duration of drug exposure)	☒	☐	☐	9.3.2
5.2	Does the protocol address the validity of the exposure measurement? (e.g. precision, accuracy, use of validation sub-study)	☒	☐	☐	9.8.1-2
5.3	Is exposure categorised according to time windows?	☒	☐	☐	9.7.3
5.4	Is intensity of exposure addressed? (e.g. dose, duration)	☒	☐	☐	9.7.3
5.5	Is exposure categorised based on biological mechanism of action and taking into account the pharmacokinetics and pharmacodynamics of the drug?	☐	☐	☒	

Section 5: Exposure definition and measurement	**Yes**	**No**	**N/A**	**Section Number**
5.6 Is (are) (an) appropriate comparator(s) identified?	☒	☐	☐	8

Comments:

Section 6: Outcome definition and measurement	**Yes**	**No**	**N/A**	**Section Number**
6.1 Does the protocol specify the primary and secondary (if applicable) outcome(s) to be investigated?	☒	☐	☐	9.3.1
6.2 Does the protocol describe how the outcomes are defined and measured?	☒	☐	☐	9.3.1
6.3 Does the protocol address the validity of outcome measurement? (e.g. precision, accuracy, sensitivity, specificity, positive predictive value, use of validation sub-study)	☒	☐	☐	9.8.1-2, 9.7.4
6.4 Does the protocol describe specific outcomes relevant for Health Technology Assessment? (e.g. HRQoL, QALYs, DALYS, health care services utilisation, burden of disease or treatment, compliance, disease management)	☐	☐	☒	

Comments:

Section 7: Bias	**Yes**	**No**	**N/A**	**Section Number**
7.1 Does the protocol address ways to measure confounding? (e.g. confounding by indication)	☒	☐	☐	9.3.3
7.2 Does the protocol address selection bias? (e.g. healthy user/adherer bias)	☒	☐	☐	9.9
7.3 Does the protocol address information bias? (e.g. misclassification of exposure and outcomes, time-related bias)	☒	☐	☐	9.9

Comments:

Section 8: Effect measure modification	**Yes**	**No**	**N/A**	**Section Number**
8.1 Does the protocol address effect modifiers? (e.g. collection of data on known effect modifiers, sub-group analyses, anticipated direction of effect)	☒	☐	☐	9.3

Comments:

Section 9: Data sources	**Yes**	**No**	**N/A**	**Section Number**
9.1 Does the protocol describe the data source(s) used in the study for the ascertainment of:				
9.1.1 Exposure? (e.g. pharmacy dispensing, general practice prescribing, claims data, self-report, face-to-face interview)	☒	☐	☐	9.4
9.1.2 Outcomes? (e.g. clinical records, laboratory markers or values, claims data, self-report, patient interview including scales and questionnaires, vital statistics)	☒	☐	☐	9.4
9.1.3 Covariates and other characteristics?	☒	☐	☐	9.4
9.2 Does the protocol describe the information available from the data source(s) on:				
9.2.1 Exposure? (e.g. date of dispensing, drug quantity, dose, number of days of supply prescription, daily dosage, prescriber)	☒	☐	☐	9.4
9.2.2 Outcomes? (e.g. date of occurrence, multiple event, severity measures related to event)	☒	☐	☐	9.4
9.2.3 Covariates and other characteristics? (e.g. age, sex, clinical and drug use history, co-morbidity, co-medications, lifestyle)	☒	☐	☐	9.4
9.3 Is a coding system described for:				
9.3.1 Exposure? (e.g. WHO Drug Dictionary, Anatomical Therapeutic Chemical (ATC) Classification System)	☒	☐	☐	9.4
9.3.2 Outcomes? (e.g. International Classification of Diseases (ICD), Medical Dictionary for Regulatory Activities (MedDRA))	☒	☐	☐	9.4
9.3.3 Covariates and other characteristics?	☒	☐	☐	9.3.3
9.4 Is a linkage method between data sources described? (e.g. based on a unique identifier or other)	☒	☐	☐	9.4

Comments:

Section 10: Analysis plan	**Yes**	**No**	**N/A**	**Section Number**
10.1 Are the statistical methods and the reason for their choice described?	☒	☐	☐	9.7.2
10.2 Is study size and/or statistical precision estimated?	☒	☐	☐	9.5
10.3 Are descriptive analyses included?	☒	☐	☐	9.7.2
10.4 Are stratified analyses included?	☒	☐	☐	9.7.2

Section 10: Analysis plan	**Yes**	**No**	**N/A**	**Section Number**
10.5 Does the plan describe methods for analytic control of confounding?	☒	☐	☐	9.3.3
10.6 Does the plan describe methods for analytic control of outcome misclassification?	☒	☐	☐	9.7.4
10.7 Does the plan describe methods for handling missing data?	☒	☐	☐	9.7.5
10.8 Are relevant sensitivity analyses described?	☒	☐	☐	9.7.4

Comments:

Section 11: Data management and quality control	**Yes**	**No**	**N/A**	**Section Number**
11.1 Does the protocol provide information on data storage? (e.g. software and IT environment, database maintenance and anti-fraud protection, archiving)	☒	☐	☐	9.8
11.2 Are methods of quality assurance described?	☒	☐	☐	9.8
11.3 Is there a system in place for independent review of study results?	☐	☒	☐	

Comments:

Section 12: Limitations	**Yes**	**No**	**N/A**	**Section Number**
12.1 Does the protocol discuss the impact on the study results of:				
12.1.1 Selection bias?	☒	☐	☐	9.9
12.1.2 Information bias?	☒	☐	☐	9.9
12.1.3 Residual/unmeasured confounding? (e.g. anticipated direction and magnitude of such biases, validation sub-study, use of validation and external data, analytical methods).	☒	☐	☐	9.9
12.2 Does the protocol discuss study feasibility? (e.g. study size, anticipated exposure uptake, duration of follow-up in a cohort study, patient recruitment, precision of the estimates)	☒	☐	☐	9.5, Appendix A, Appendix B

Comments:

Section 13: Ethical/data protection issues	**Yes**	**No**	**N/A**	**Section Number**
13.1 Have requirements of Ethics Committee/ Institutional Review Board been described?	☒	☐	☐	10.2
13.2 Has any outcome of an ethical review procedure been addressed?	☐	☒	☐	
13.3 Have data protection requirements been described?	☒	☐	☐	10.2

Comments:

Section 14: Amendments and deviations	**Yes**	**No**	**N/A**	**Section Number**
14.1 Does the protocol include a section to document amendments and deviations?	☒	☐	☐	5

Comments:

Section 15: Plans for communication of study results	**Yes**	**No**	**N/A**	**Section Number**
15.1 Are plans described for communicating study results (e.g. to regulatory authorities)?	☒	☐	☐	12
15.2 Are plans described for disseminating study results externally, including publication?	☒	☐	☐	12

Comments:

Name of the main author of the protocol: [redacted]

Date: dd/Month/year [redacted]

Signature: _____

List of figures and tables

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