Non-interventional Study Report

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 report
includes redaction of personal identifiable information only.

PASS information

Title	Vaginal estradiol tablets (Vagifem®) and endometrial cancer risk in the treatment of postmenopausal vaginal atrophy: A register-based cohort study in postmenopausal women.
Version identifier of the final study report	1.0
Report date	14 December 2022
EU PAS Register number	EUPAS44951
EU PAS Register link	https://www.encepp.eu/encepp/viewResource.htm?id=45818
Active substance	Estradiol, ATC code: G03CA03
Medicinal product	Vagifem® 10 mcg, Vagifem® 25 mcg
Medicinal product	NDA 020908
Procedure number	NA
Joint PASS	No
Research question and objectives	To evaluate whether exposure to Vagifem® increases the rate of endometrial cancer in postmenopausal women. The primary objective was 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. The secondary objective was 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.
Countries of study	Denmark and the United States of America.
Authors	[redacted]
UTN	U1111-1270-0966

ClinicalTrials.gov identifier	NCT05243823
IND number	038483
Generic name	Estradiol
Indication	Atrophic vaginitis due to menopause
Physician	[redacted]
Study sites	This was a database study, and the following databases were used: • Danish population-based registries • US Claims data (Truven)
Study initiated	First data extraction date:15 January 2022
Study completed	Last data extraction date: 31 July 2022

Marketing authorisation holder

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

This study was conducted in accordance with the Guidelines for Good Pharmacoepidemiology Practices (2016).[1]

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1 Abstract

Please refer to separate document.

2 List of abbreviations and definitions of terms

ATC	Anatomical Therapeutic Chemical (Classification System)
ATE	average treatment effect
BMI	body mass index
CI	confidence interval
CPR	central person register
DDD	daily define dose
E2	Estradiol
FDA	Food and Drug Administration
GSM	genitourinary syndrome of menopause
GPP	good pharmacoepidemiological practice
HR	hazard ratio
HRT	hormone replacement therapy
ICD	International Classification of Diseases
IR	incidence rate
ITT	intention to treat
IUD	intrauterine device
LDVE	low dose vaginal estrogens
LTMLE	longitudinal targeted minimum loss estimation
MAH	marketing authorisation holder
mcg	microgram
NIS	non-interventional study
PCOS	polycystic ovarian syndrome
PY	person years
SAP	statistical analysis plan
SFU	start of follow-up
UTN	Universal Trial Number

3 Investigators

[redacted]

4 Other responsible parties

The present study collected third party data from nationwide Danish patient registries. The data management and analyses for the data from the Danish cohort were conducted at [redacted] and [redacted] Furthermore, the study included a cohort study conducted using the US-based database Truven Health Market Scan, including the Commercial Claims and Encounters Database and the Medicare Supplemental and Coordination of Benefits Database (hereafter referred to as Truven) where data management and analysis were conducted at Novo Nordisk A/S.

5 Milestones

The relevant milestones for the study are presented in Table 5-1.

Table 5-1 Milestones

Milestone	Planned date	Actual date
Start of data collection	15 January 2022	15 January 2022
End of data collection	31 July 2022	31 July 2022
Registration in the EU PAS Register	15 January 2022	15 January 2022
Final report of study results	14 December 2022	Please refer to PASS information table for the report date and other details.

Abbreviations: EU PAS = the EU electronic register of post-authorisation studies maintained by the EMA; US = United States.

6 Rationale and background

Vulvovaginal atrophy is part of the genitourinary syndrome of menopause (GSM) which may cause symptoms interfering with physical and sexual health in up to 45% of women. If untreated, it progresses over time. Local estrogen therapy is indicated for vulvovaginal atrophy. Local estrogen therapy restores the vaginal epithelium and normalizes the vaginal acidic pH, which then restores the normal flora, increases vaginal moisture and provides relief against vaginal dryness. Despite the established safety and efficacy of vaginal estrogen treatment, GSM represents an under recognized and undertreated condition.[2, 3] Current product labelling for low-dose vaginal estrogens do not distinguish between routes of administration and comprise several "black box" warnings in the US prescribing information. One of the "black box" warnings is an increased risk of endometrial cancer which has been established for oral hormone replacement therapy (HRT).[3-5]

Endometrial cancer is the most common cancer of the female reproductive system in the western world.[6] In a Danish study of a cohort of postmenopausal women, an incidence rate of 55 per 100,000 person-years was found. Standard treatment consists of total 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.[6] Multiple risk factors have been identified: overweight/obesity, long-lasting endogenous or exogenous hyperestrogenism (due to polycystic ovarian syndrome, tamoxifen therapy, anovulation, nulliparity), hypertension, and diabetes mellitus. The latter is the strongest risk factor and body mass index (BMI) > 30 kg/m^2 is associated with a 3-4-fold increase in the risk of developing endometrial cancer.[6, 7] There is a theoretical increased risk of endometrial cancer in women exposed to vaginal estrogens.

Systemic estrogen exposure with 10 mcg estradiol (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.[8, 9] Endometrial safety of 10 mcg E2 tablets has been evaluated in a pooled population from two 52-week studies in which the incidence of endometrial hyperplasia and carcinoma was assessed.[10] 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,[11] and study 2 was an open-label endometrial safety trial conducted in 336 postmenopausal women in Europe.[8] Treatment regimens in the trials were identical. 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 suggesting no increased risk for endometrial hyperplasia or carcinoma in postmenopausal women undergoing treatment with 10 mcg E2 vaginal tablets for one year.

Three research publications of registry studies in the period between 2016-2018 evaluated the association between endometrial cancer and vaginal estrogen therapy.[5, 12, 13] These studies have shown conflicting results; Mørch et al[5] concluded that vaginal estrogen therapy increased the risk of endometrial cancer in post-menopausal women with a relative risk (RR) of 1.96 (95% CI 1.77-2.17) compared to women who were never on hormone therapy, whereas, the other two studies concluded that vaginal estrogen therapy does not increase the risk of endometrial cancer.[12] They reported a RR of 1.47 (0.75-2.90)[13] and a hazard ratio (HR) of 1.62 (CI 95%: 0.88-2.97)[12, 13] for endometrial cancer in women exposed to vaginal estrogen therapy compared to non-treated women. All three studies showed an overall risk estimate across different types (e.g. tablets, creams, rings)

and dosages of vaginal estrogens. None of the studies published so far have focused on low-dose vaginal estrogen, where it is expected that there will be low to no systemic effect of the treatment. A recent review article by Constantine et al,[14] 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 does 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 26 March 1999 and sNDA 20908/S-013 for 10 mcg was approved on 25 November 2009. The sale of Vagifem® 25 mcg in the US was discontinued as of 30 July 2010 and in Denmark Vagifem® 25 mcg was discontinued on 03 February 2014 due to commercial reasons.

The FDA had 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. The current report is based on a non-interventional PASS performed to fulfil this commitment to the FDA. The study included data from a nationwide Danish cohort of postmenopausal women and these results were supported with the data from a US cohort of postmenopausal women. The Danish nation-wide cohort was established through linkage of Danish national patient registries. The US cohort was established based on data from Truven. The aim of this study was to assess if there was a long-term increased rate of endometrial cancer when postmenopausal women were exposed to Vagifem®.

The Vagifem® 10 mcg product is manufactured for different markets using the same formulation, facility, and process, therefore, data collected globally are 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.

To support this assumption, a literature search was performed to investigate whether Danish postmenopausal women treated with menopausal hormone therapy are similar, in terms of demographics and management of menopausal symptoms, to postmenopausal women from the US treated with menopausal hormone therapy, thereby allowing Danish data to be used for evaluating the theoretical risk of endometrial cancer in women exposed to vaginal estrogens. The overall conclusion of this literature search was that Danish and US postmenopausal women are generally similar in terms of their demographics, attitude towards menopause, menopausal hormone therapy prescription habits, medication, and management strategies for postmenopausal symptoms (Appendix 16.1.1, Menopause literature search and summary report).

Ethics

Approval of the final study protocol was received from FDA. It was 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 was not required in Denmark for purely registry-based studies.

The study was conducted in accordance with Guidelines for Good Pharmacoepidemiology Practice (GPP) and any applicable local regulations.

Informed consent

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

In the US, participants at the initiation of the health insurance plan gave a general consent for the usage of their data.

All the data of relevance to the objectives of the study were anonymized data and extracted for analysis. Special attention was 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.

7 Research question and objectives

As stated in the protocol and amendments (included in Appendix 16.1.1) the aim of this study is to evaluate whether exposure to Vagifem® increases the rate of endometrial cancer in postmenopausal women, more specifically, to assess the following objectives and endpoints:

Primary objective

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.

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.

Endpoints

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 follow-up) to exit (end of study period, date of emigration, or date of hysterectomy, or date of death).

8 Amendments and updates

Please refer to Table 8-1 for details on amendments or updates made to the study protocol.

Table 8-1 Amendments to protocol

Number	Date	Section of study protocol	Amendment or update	Reason
1	05 April 2022	Section 9.3.1	Outcome definition for the Danish cohort updated to include information on disease pathophysiology based on the codes confirmed as endometrial cancer from the ICDO-3 system	The Danish Cancer Register does not contain information on subcodes of C54 or C55 in the ICD-10 system after 2005.

9 Research methods

9.1 Study design

9.1.1 Type of study

This was a longitudinal, observational, non-interventional, post-authorisation safety study (PASS) to investigate whether Vagifem® (LDVE) increases the occurrence of endometrial cancer in postmenopausal women. The study mainly used data from two countries: Denmark and the US during the period of 2000-2020 and period of 2007-2021, respectively.

The study data were extracted from the health care registries of Denmark (nationwide data) and supported by data from US (Claims data from Truven health analytics) (see Appendix 16.1.1, Section 9.2).

The study design contains two separate cohort studies, one for each country. All women meeting the inclusion and exclusion criteria will be part of the analyses. The two cohort studies follow postmenopausal women over time from start of follow-up (SFU).

Intention-to-treat analysis

For the primary objective, the study design was a propensity score matched cohort design with time-conditional propensity scores. Each woman exposed to LDVE was matched 1:2 to unexposed women by time-conditional propensity score matching procedure. A new user entered the study at the initiation of any LDVE treatment. At that date she was matched to 2 women with same year of birth and closest propensity score among all eligible controls, namely women that were still at risk, who were not matched earlier and who have not started any LDVE treatment up to that date. This date was defined as the enrolment date, and it was fixed for both exposed woman and matched controls. For the secondary objective the study design was a propensity score matched cohort design, where women exposed to Vagifem® were matched 1:2 to women exposed to a systemic HRT product. At first date of prescription, each new user of a Vagifem® product was matched to HRT users that had started the hormonal replacement therapy within the same year as the exposed woman and at same age. The two selected matched controls were the ones with closest propensity score of initiating Vagifem® treatment. The date of the first prescription of a Vagifem® product was set as enrolment date for the exposed woman and the respective matched controls.

For all analyses the matching was done without replacement and if a woman was matched as a control, she was not included in the exposed group at her start of LDVE treatment. Moreover, start of follow-up (SFU) was set to 6 months i.e., 180 days after the enrolment date, and women who fulfilled an exclusion criterion up to the SFU were excluded, together with the corresponding matches.

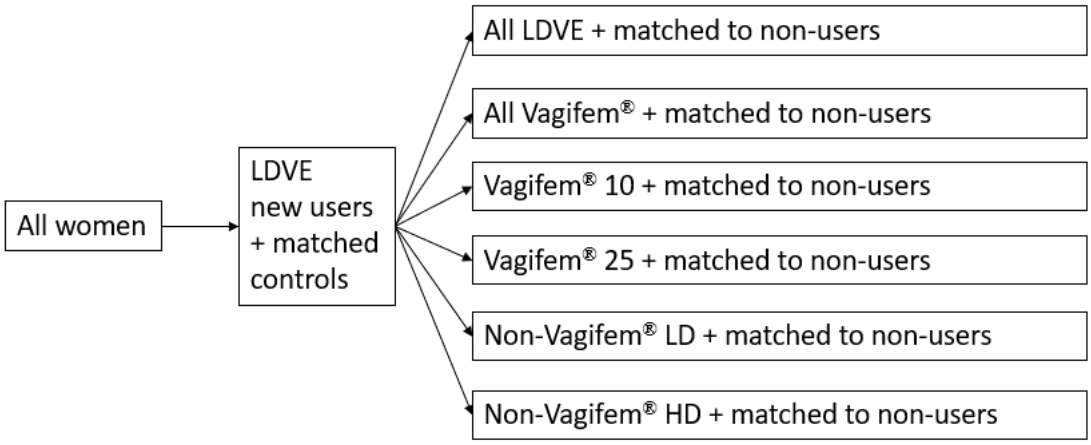
Dynamic exposures analyses based on causal inference

For the primary objective, the study design was a matched cohort design where new LDVE users were matched by birth year to non-users (Figure 9-1). At date of first prescription of LDVE treatment, users were matched to two women with same year of birth that were still at risk and had not yet started any LDVE treatment up to that date. This was defined as the enrolment date, and it is fixed for both exposed woman and matched controls. Similar matching procedure was used for the

secondary objective for the comparison of Vagifem® new-users and HRT users (Figure 9-2). Here, at the first date of prescription of a Vagifem® treatment, the new user was matched to two HRT users that had started the hormonal replacement therapy within the same year as the exposed woman and at same age. The date of the first prescription of a Vagifem® product was set as enrollment date for the exposed woman and the respective matched controls.

Matching was done without replacement and if a woman was matched as a control, she was not included in the exposed group. New users were followed from enrollment date until occurrence of endometrial cancer, emigration, hysterectomy, or death, whichever came first.

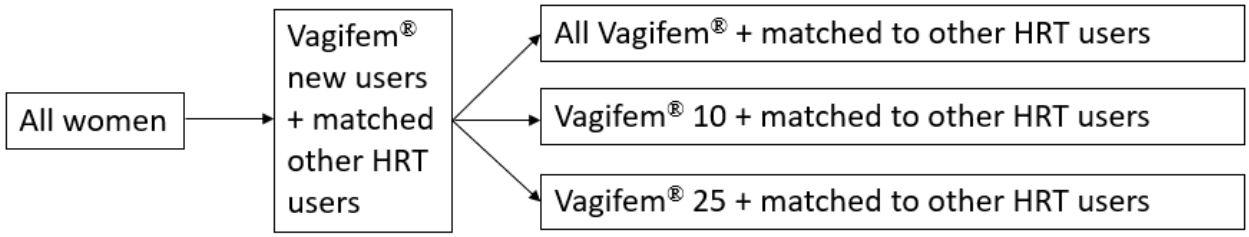
Figure 9-1 Representation of matching cohorts for the primary objective of both the ITT and the dynamic exposure model



Abbreviations: LD = low dose; HD = high dose.

Cross-reference: Section 9.3.1

Figure 9-2 Representation of matching cohorts for secondary objective



Abbreviations: HRT= hormone replacement therapy

Cross-reference: Section 9.3.1

Please refer to Section 7 for study objectives and endpoints.

Rationale for study design

A retrospective cohort study design, comparing new users of Vagifem® with a comparator group not using hormone replacement therapy, was chosen as the primary analysis. This design makes it possible to follow up postmenopausal women for first occurrence of endometrial cancer over time.

The study utilized data from Danish registries and US claims data. The Danish national health registries are of high quality with a high level of completeness and validity. Validation of Danish registries includes both manual and automatic checks to ensure the high degree of quality.[15-17] The US claims data (Truven) contain de-identified records for more than 186 million patients, reflecting real-world treatment patterns and enabling longitudinal follow up, as well as the inclusion of cross-sectional details in the analyses[18].

9.2 Setting

This non-interventional study was based on health care registries from Denmark (nationwide data) which has been supported by data from US (Claims data from Truven).

The study period i.e., the available data were collected between 01 January 2000 and 31 December 2020 for Danish population and 01 January 2007 and 30 September 2021 for US population. The data from the registry and the claims database were first and last extracted on 15 January 2022 and 31 July 2022, respectively. For further information on study population refer to Section 9.3.

9.3 Patients

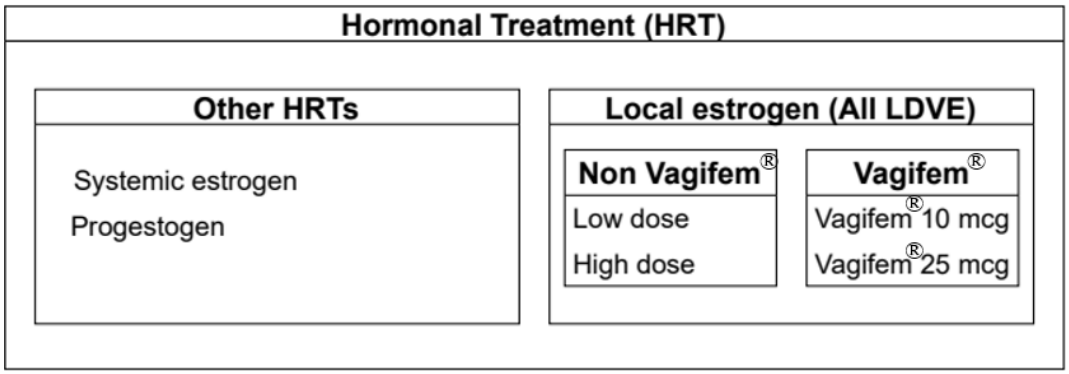
9.3.1 Study population

The study included population from a Danish and a US cohort. The study population for both countries consisted of new users of LDVE (split into Vagifem® and other LDVE products) in the study period, and a matched comparator group consisting of women using no hormone replacement therapy. Subgroup analyses were conducted by LDVE treatment definitions. Users of LDVE were split into Vagifem® users (Vagifem® 10 mcg, Vagifem® 25 mcg) and non-Vagifem® users (low-dose and high dose) (Figure 9-3). In particular, the low dose group was defined as estradiol treatment with maximal dose 10 mcg or conjugated estrogen with maximal dose 0.30 mg; and the high dose group was defined as estradiol treatment with maximal dose 25 mcg and minimal dose more than 10 mcg or conjugated estrogen with maximal dose 0.45 mg and minimal dose more than 0.30 mg. In the Danish cohort, there were no new users of high-dose non-Vagifem® LDVE that met inclusion/exclusion criteria, whereas in the US cohort both low and high dose LDVE analyses were implemented.

Subgroup analyses were conducted by selecting the matched groups by first treatment type for the exposed women in the matched population. For example, when considering only Vagifem® new-users, the exposed women that had Vagifem® as first LDVE treatment were selected with their respective matched groups from the matched population.

New users in both the cohorts were followed from the SFU (180 days after enrolment date), until occurrence of endometrial cancer, exposure to any non-LDVE hormone treatment, exposure to any non-Vagifem® LDVE (women in Vagifem® group only), emigration, hysterectomy, or death, whichever came first. For women in the US, data on death and emigration were not available.

Figure 9-3 Representation of treatment sub-groups for hormonal therapy



9.3.2 Inclusion criteria

To be eligible for the study, subjects were required to meet the following inclusion criteria:

- Female
- Age 50-75 years at entry

9.3.3 Exclusion criteria

- Endometrial cancer prior to entry
- Any use of vaginal estrogen products prior to entry
- Hysterectomy prior to entry

9.3.4 Rationale for selection criteria

The age range of 50-75 years was selected to capture the age group of postmenopausal females in whom HRT is prescribed. Females with a history of no hysterectomy were selected since females without a uterus cannot develop endometrial cancer.

9.3.5 Removal of patients from therapy or assessment

This was a non-interventional study based on registry and claims data, so patients were not able to be removed from therapy or assessment, since the registry and claims data included all patients who were already being treated with Vagifem® or another LDVE. Data collected were transferred, pseudonymised/anonymised to Novo Nordisk for analysis.

9.3.6 Sources of patients

The Danish nationwide cohort was established through linkage of Danish national patient registries. The US cohort was established based on data from the US claims database, Truven.

9.3.7 Methods of selection of patients

All women who fulfilled the inclusion criteria (see Section 9.3.2) and did not meet any of the exclusion criteria (see 9.3.3) were included in the current study.

9.4 Variables

See section 9.5 for a description of the data sources. The following variables were included in the analysis: Outcome, exposure, and adjustment variables. More information on these variables is included below.

9.4.1 Outcome

9.4.1.1 Danish cohort

The outcome was an incident endometrial cancer diagnosis in the National patient register and Danish Cancer Register as defined by the following diagnostic codes:

All C54 and C55 codes from the ICD-10 system, combined with information on the specific disease pathophysiology including only the codes confirmed as endometrial cancer from the ICDO-3 system (i.e., Type I and Type II cancers).

9.4.1.2 US Cohort

The following codes were used to identify endometrial cancer cases:

- ICD-10 C54.1 Malignant neoplasm of endometrium, or
- ICD-9 182.0 Malignant neoplasm of corpus uteri, except isthmus

9.4.2 Exposure

9.4.2.1 Danish cohort

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

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

See Appendix 16.1.1, Section 9.3.2 for further details on exposure.

9.4.2.2 US cohort

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

Exposure to treatment was 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[19]. The lag time was subject to sensitivity analyses for both the cohorts. Considerations regarding lag-time and exposure definitions are revisited in the sensitivity and supplementary analyses section of the study protocol (see Appendix 16.1.1, Sections 9.7.3 and 9.7.4).

9.4.3 Adjustment variables

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

The list below provides an overview of the selected adjustment variables for both the cohorts.

Demographic and sociodemographic variables

- Age
- Education (Danish cohort only) – Basic school, Vocational Training, Bachelor, Higher education. Education was fixed at the age of 50 to make education comparable between groups.
- Country of origin (Danish cohort only)– Denmark versus other country
- Income (Danish cohort only) – Average income over past 5 years, for individuals with no apparent income a low income was assumed. The income used was individual income based on family income and standardised to the same year. Income was included as tertiles. Income was fixed at the age of 50 to make groups comparable.

Comorbidities

- Polycystic ovarian syndrome
- Endometriosis
- Hypertension
- Diabetes
- Ischemic heart disease – myocardial infarction or angina
- Family history of cancer (Danish cohort only) – registered cancer in siblings and parents. Parental relations were only established for people born after approximately 1954 and family history of cancer was categorized as not present for people without registration of parents.
- Treatment for or diagnosis of infertility if available (ICD10 N97 or ICD9 628, or procedure code KLBF (Danish cohort only))

Medication use (including proxies for comorbidities):

- Drug index to define hypertension medication. Most hypertension is treated in general practice without registration of a diagnosis. A validated algorithm using prescription of 2 different classes of antihypertensive drugs (pharmacy claim of 2 different antihypertensive drugs in each of two consecutive 3 month periods, the drugs being defined as antiadrenergic (C02A, C02B, C02C), diuretic (C02DA, C03A, C03B, C03D, C03E, C03X, C02DA, C07B, C07C, C07D, C08G, C09BA, C09DA, C09XA52, C02L) vasodilator (C02DB, C02DD, C02DG), beta-blocker

(C07A, C07B, C07C,C 07D, C07F), calcium antagonist (C07FB, C08, C08G, C09BB, C09DB) or renin angiotensin inhibitor (C09AA, C09BA, C09BB, C09CA, C09DA, C09DB, C09XA02, C09XA52)
- Antidiabetic medication (ATC, A10) – any antidiabetic medication within the last 6 months
- Hormonal contraceptives including hormone IUD (Danish cohort only) (ATC G03AA, G03AB, G03AC, G03HB01[except vnr 014662,119758,154252,426114], G02BA03, G02BB01)
- Prior use of systemic estrogens or other estrogen- or progesterone-containing products

Other variables [(Danish cohort only) (when available)]

Danish cohort measured in women who had either given birth in Denmark or who had not given birth, but who have had continuous residence in Denmark since the age of 20.
- Parity, measured prior to enrolment date, categorized into following groups: 0 childbirths, 1-2 childbirths, more than 2 childbirths
- Body mass index (kg/m^2), measured in parous women, categorized: underweight (<18.5), normal (18.5 to <25), overweight (25 to <30), obese (≥ 30)
- Smoking (yes/no)

9.5 Data sources and measurement

9.5.1 Danish cohort

The national prescription, cancer and patient registers in Denmark were used in this study. Denmark was 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, 20] The unique person identifier was introduced more than 50 years ago in the Denmark[21]. It is assigned to all residents and hence 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[22].

For the current project all necessary data were made available in the research environment of Statistics Denmark where multiple registries were combined using the CPR number. The data were 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.

Please refer to Table 9-1 for the list of Danish registries used in the analysis.

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 National 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[a]	• Parity • BMI • Smoking

Abbreviation: BMI = body mass index

[a]BMI and smoking were included in the National Birth register in 2004

9.5.1.1 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[16]. Of relevance to the present study, the national prescription registries in 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.

9.5.1.2 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)[17].

9.5.1.3 The National Health Registry

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)[23]

9.5.1.4 Statistics Denmark

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

9.5.2 US Cohort

The Truven database is split into two parts, the Commercial Claims and Encounters Database and the Medicare Supplemental and Coordination of Benefits Database. The maximal age of a patient in the first database is 65 years. Older patients can be found in the Medicare database, records for these patients start at 65 years, and it is possible to link between the two databases.

9.6 Bias

The Danish registries cover the full population in Denmark and there is therefore no selection bias in the source population. The Truven data covers a population with health insurance and might therefore not be entirely representative of the background of the US population.

For the Danish registries, it was possible to take into account a range of variables in the matching processes, including socioeconomic status. This information was not available in the US claims data, and the comparison could therefore not take the following differences into account: socioeconomic status between the Vagifem® or LDVE users, and the postmenopausal women not using HRT.

The Danish data allows for a longer follow-up of each individual woman, as full data are available for all residents in Denmark. In the US claims data, follow-up will end when the patient leaves the health insurance plan. If LDVE leads to a higher risk of endometrial cancer in the long term, this would more likely be detected in the Danish data, and not in the US data.

Validation of the endpoint endometrial cancer was not possible in the US claims data. Several sets of ICD codes were therefore used to explore the impact of these on the effect estimates.

Several sensitivity analyses were included in the study to limit potential bias in the interpretation of the results (see Appendix 16.1.2, Section 3.3.2.10).

9.7 Study size

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

In order to calculate the power for the detectable Hazard Ratio for endometrial cancer, we assumed a 2:1 ratio between the comparator group and the Vagifem® group. Further the statistical tests were performed as two-sided with a 5% significance level. For the Danish cohort, the calculations were based on assumptions for the study period 01 January 2000 to 31 December 2019, based on the feasibility counts (see Appendix 16.1.1, Section 9.5) for the period 1995-2017. With an endometrial cancer rate of 55 per 100,000 person years, and assuming that around 80,000 women were 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 was 1.2 in the Danish cohort with a power of 80%.

In the US cohort the calculations were based on data between 01 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%.

9.8 Data transformation

9.8.1 Danish cohort

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

The academic collaborators retrieved as much information as possible about how data was generated and interpreted. This information included level of coverage, a full dictionary for all variables, data on migration, data on co-prescribed medication, and co-morbidity.

9.8.2 US cohort

All extraction of raw data and data analysis were performed at Novo Nordisk A/S.

9.9 Statistical methods

9.9.1 Main summary measures

The baseline characteristics, demographics, comorbidities, medications used, and other variables were analysed for both the cohorts (see Appendix 16.1.1, Section 4.6).

9.9.2 Main statistical methods

9.9.2.1 Primary analysis

The primary analysis was an intention to treat analysis (ITT) with a lag time of 6 months contrasting new users of LDVE with propensity-matched non-users at the enrolment date. Patients were considered at risk after start of follow up regardless of treatment discontinuation. Covariate balance between exposed and controls was assessed to verify the propensity score matching model.

The incidence rate of endometrial cancer was calculated both for the exposure groups and the matched comparator group within the maximal follow-up period. This was presented with a 95% confidence interval calculated by a Poisson regression model with log-follow-up time as offset. The follow-up-time was time from SFU to the first event, death, hysterectomy or censoring, whatever came first.

A Cox regression model was used to calculate the hazard ratio with 95% confidence interval. An adjusted model including all the additive effects of the covariates used in the propensity score matching was used. The hazard ratios of the adjusted model were considered the primary estimate of the relative rate between the exposure groups and the control group.

The analyses were conducted for the primary objective where new LDVE users, split into Vagifem® and other LDVE users, were compared with non-users.

Equivalently, incidence rates and hazard ratios for endometrial cancer were calculated in the secondary analysis for the population of Vagifem® new-users and propensity score matched users of systemic cyclic HRT and oral, transdermal and opposed injectable systemic HRT products.

9.9.2.2 Dynamic exposure analyses based on causal inference

The additional statistical analyses used methods from causal inference[24-27] to estimate the estimands of target trials based on the observational data. Here, the study population included new users of LDVE treatment and matched controls randomly sampled among the eligible controls. In the Danish cohort death and hysterectomy were treated as competing events. For women in US, data on death and emigration were not retrieved, thus no competing event was defined.

The estimands for the time-dynamic exposure analyses, including the accumulated duration analyses, were estimated by longitudinal targeted minimum loss estimation[28] (LTMLE) (Described in Appendix 16.1.2, Section 7.2) The 5-year risk of endometrial cancer was obtained for the treatment strategies of always treated and never treated (during the 5 years of follow-up). A time grid of 6 months long time interval was considered. Patients were defined as treated at 'k' months if they had been under treatment at least one day in the previous 6 months. The LTMLE used a time sequence of propensity score models and models for the censoring used baseline covariates and time-dependent covariates (as for the primary analysis). Here, time-dependent covariates were included considering their value at previous step. As an example, models for the event at 12 months included baseline covariates and time-dependent covariates at 6 months. Standard error was calculated by solving the efficient influence function.

The 5-year risk of endometrial cancer was estimated if women were treated during the 5 years (always treated / exposed women) and it was compared with the 5-years risk of endometrial cancer if women were not treated during the 5 years (never treated / matched controls). 5 years risk in each group, the absolute risk difference (average treatment effect, ATE) and the risk ratio with 95% confidence interval were calculated.

9.9.2.3 Supplementary analyses

Supplementary analyses were conducted as described in Appendix 16.1.2, Section 3.3.2.

9.9.3 Missing values

Danish cohort

For the women that had not given birth or had given birth before 2004, some of the known risk factors for endometrial cancer, including parity, BMI and smoking, were not available in the National Birth Registry.

For the Danish cohort, sensitivity analyses were performed without including information on family history of cancer, as this information was not available for all patients.

US cohort

In the US database, missing data was expected for history of hysterectomy and prior hormonal product use due to short follow up. See Appendix 16.1.2, Section 3.3.2.10 for further details.

9.9.4 Sensitivity analyses

By use of the Danish data several sensitivity analyses were performed to check the influence from the analytical/design choices on the study findings. The following analyses were done:

- 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 were not captured in the National Birth Registry. However, it was expected that this information was estimated by multiple imputations based on the information available on these confounders among the younger women in the cohort. Analysis using BMI was not implemented due to lack of data.
- Including additional data from the cancer register with information on histology and restricting to Type I malignancy only. Type I tumors were considered hormone sensitive tumors. For this analysis, the tumors were 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 included 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 included 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 tumors we also used cases with ICD10 code C55 if they had a histology defined as Type I according to the ICD-O-3 coding system.

In both data sources the following sensitivity analyses were 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

9.9.5 Amendments to the statistical analysis plan

Version Number	Date	Amendment or update	Reason
2	02 February 2022	Sections 3.3, 4, 7, and 8 were updated to include additional details on the method of conducting the statistical analyses.	FDA information request dated 20 December 2021
		Section 8 was updated to reflect changes in ICD codes.	A change in the Danish Cancer Register, which subsequently combined the National Patient Register with the Pathology Register. As a result, the ICD codes used were different across the Registers.
3	06 April 2022	Sections 3, 4, and 7 were updated to include additional technical details about the planned statistical analyses. The main updates were for the primary analysis, which is now based on propensity score matching, with a final update to the outcome definition.	FDA information request dated 09 March 2022

9.10 Quality control

The SAP and records of statistical programming performed to generate the results were stored by the academic collaborators for the Danish cohort and by Novo Nordisk A/S for the US cohort. The academic collaborators filed the necessary documents that allowed a replication of all data extractions and analyses performed during the study.

9.10.1 Danish registries

Data quality control was 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[16, 17, 22]. Data extraction was performed and transferred to a server at Statistics Denmark, where it was made available for analysis. Such centralized analysis was expected to provide the highest level of quality and accountability. Data was structured according to a common data model that was developed for the study. The academic collaborators ensured that the standard operating procedures applicable for this type of study in their research unit were followed.

9.10.2 US database

The Truven database contained 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 provided a wide breadth of information for detailed research quality[28].

9.10.3 Protocol deviations

A few sensitivity analyses using parameters such as BMI, smokers, parity, family history of cancer were not possible to perform due to substantial amount of missing data.

For the Danish cohort, information on vulvovaginal atrophy and data on non-Vagifem® high dose were limited. A code defining endpoint i.e., all C54 codes and 182 codes, except 182.1, 182.8, C54.2, and C54.3 was not used for ITT analysis. The LTMLE analysis was not conducted using different formulations.

For the US cohort, no active ingredient or exposure formulations were presented for ITT analysis. LTMLE analysis and sensitivity studies were only presented for all LDVE and not for specific Vagifem® groups. LTMLE analysis was not performed for various accumulated doses.

10 Results

10.1 Participants

Danish cohort

During the study period (from 2000 to 2020) a total of 1,312,834 women were qualified for inclusion in this study.

In women belonging to the Danish Cohort, majority of the LDVE users had utilised Vagifem®. Additionally, in the Danish cohort there were more users of Vagifem® 25 mcg when compared to Vagifem®10 mcg (167,812 vs 110,350) (Table 10-1).

US cohort

The total number of eligible women between 2007-2019 were 51,538,311.

In women belonging to the US cohort, only a smaller subset of LDVE users relied on Vagifem® and there were more users of Vagifem® 10 mcg compared to Vagifem® 25 mcg (104,997 vs 24,245) (Table 10-1).

Table 10-1 New users of LDVE in Danish and US cohort

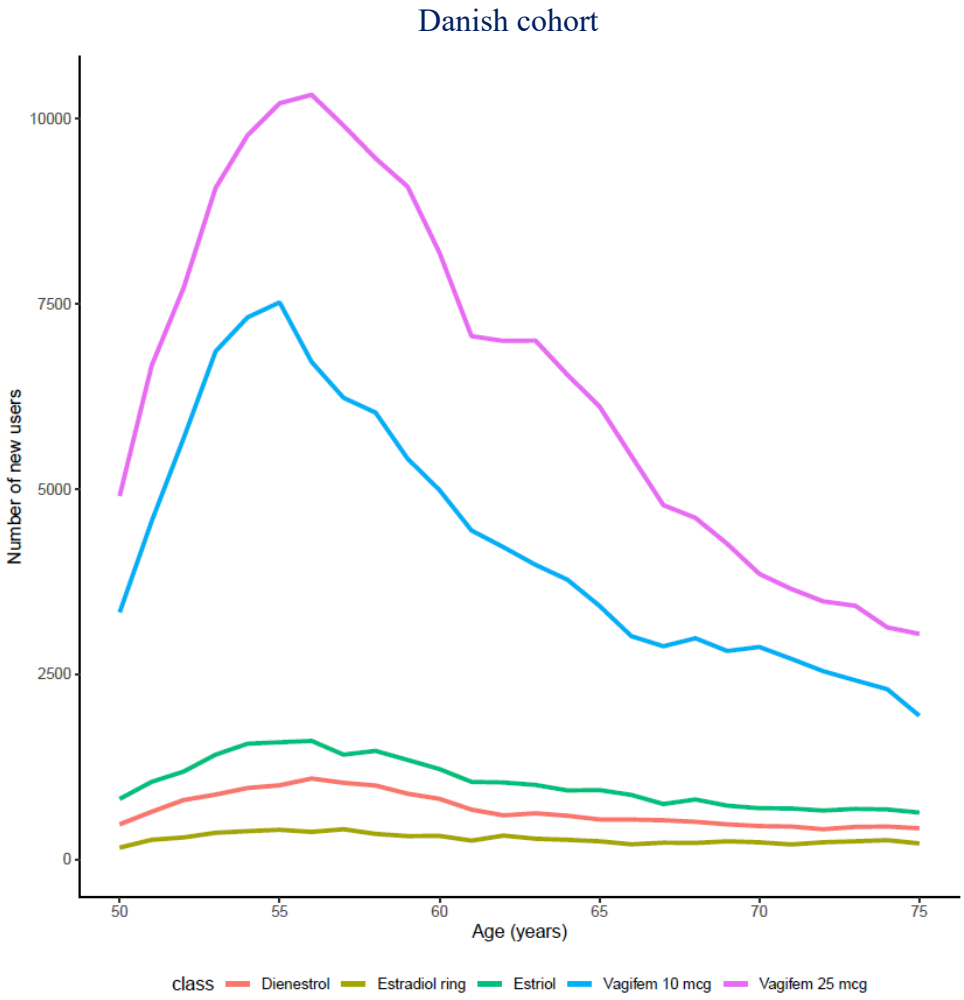
Population	New Users			
	All LDVE	**Vagifem® All**	**Vagifem® 25 mcg**	**Vagifem® 10 mcg**
Danish Cohort	328842	278162	167812	110350
US Cohort	961056	129237	24245	104997

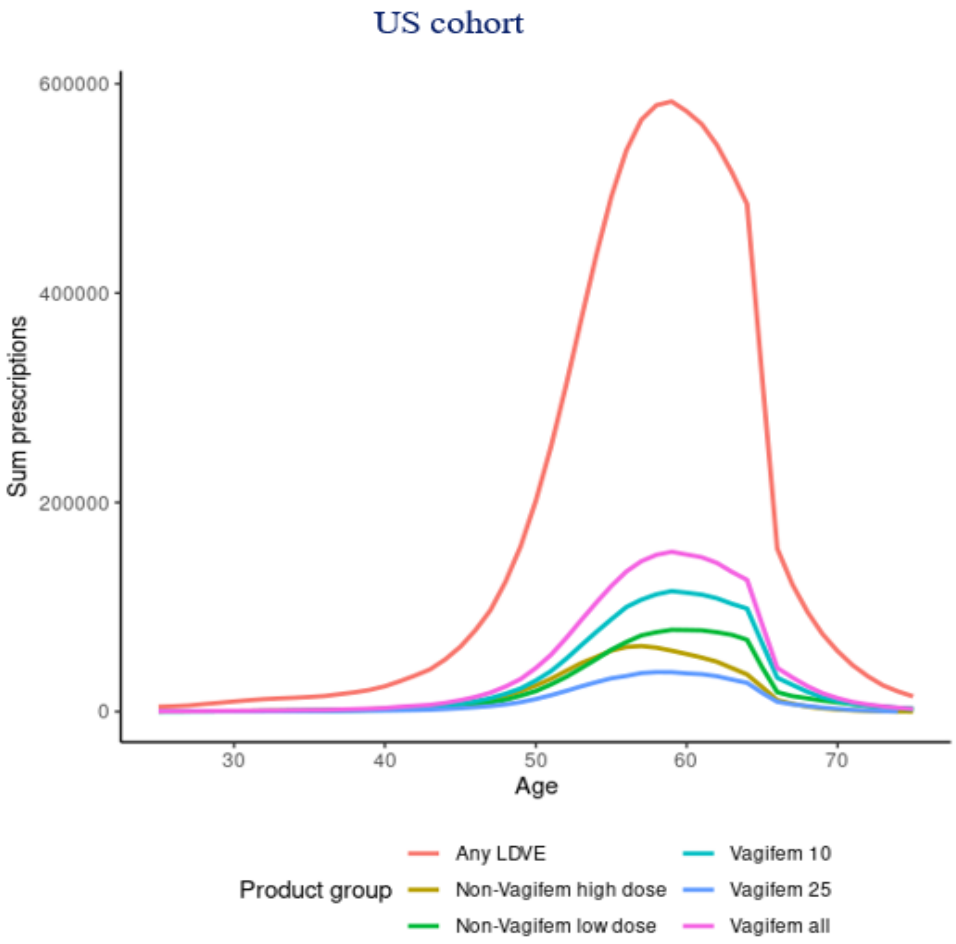
Abbreviations: LDVE = low dose vaginal estrogens; US = United States

10.2 Descriptive data

In the Danish cohort, a higher number of Vagifem® users were found to be in the 50−60 years age range. In the US population, the fraction of claims and number of prescriptions for Vagifem® were found to be high in women aged 55−65 years (Figure 10-1).

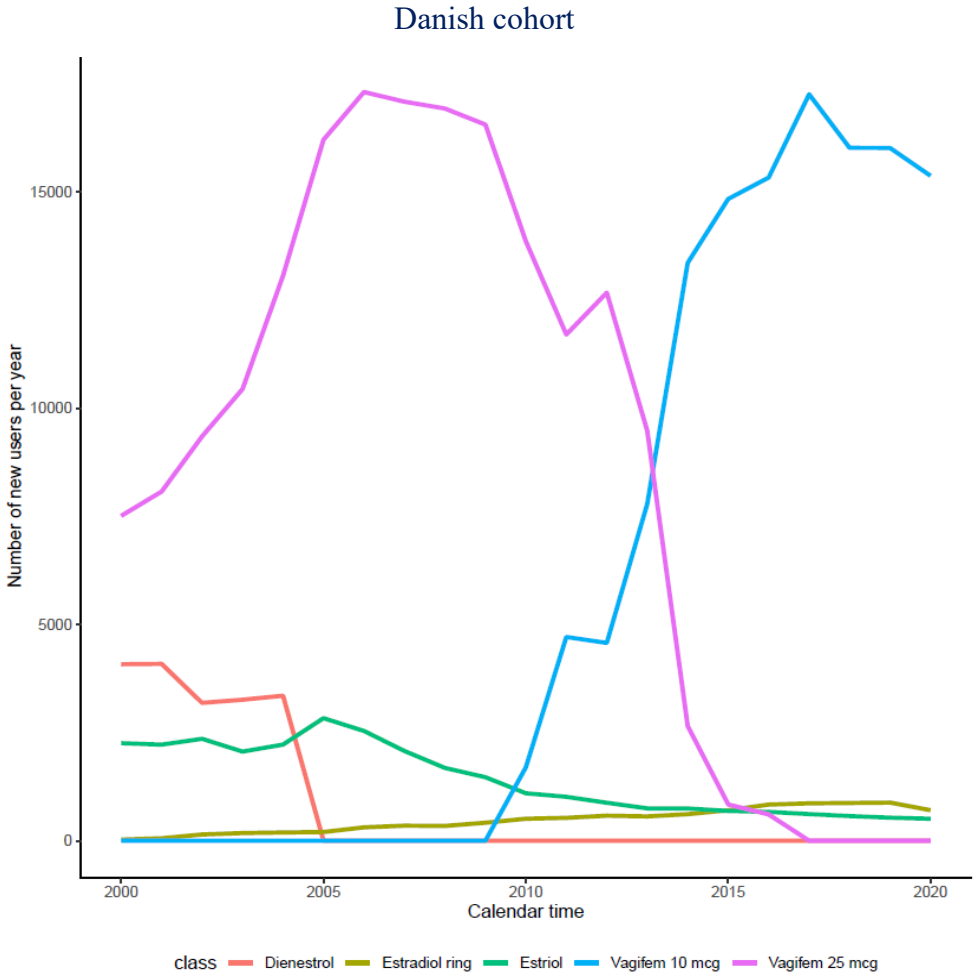
Figure 10-1 LDVE usage by age for Danish and US cohorts

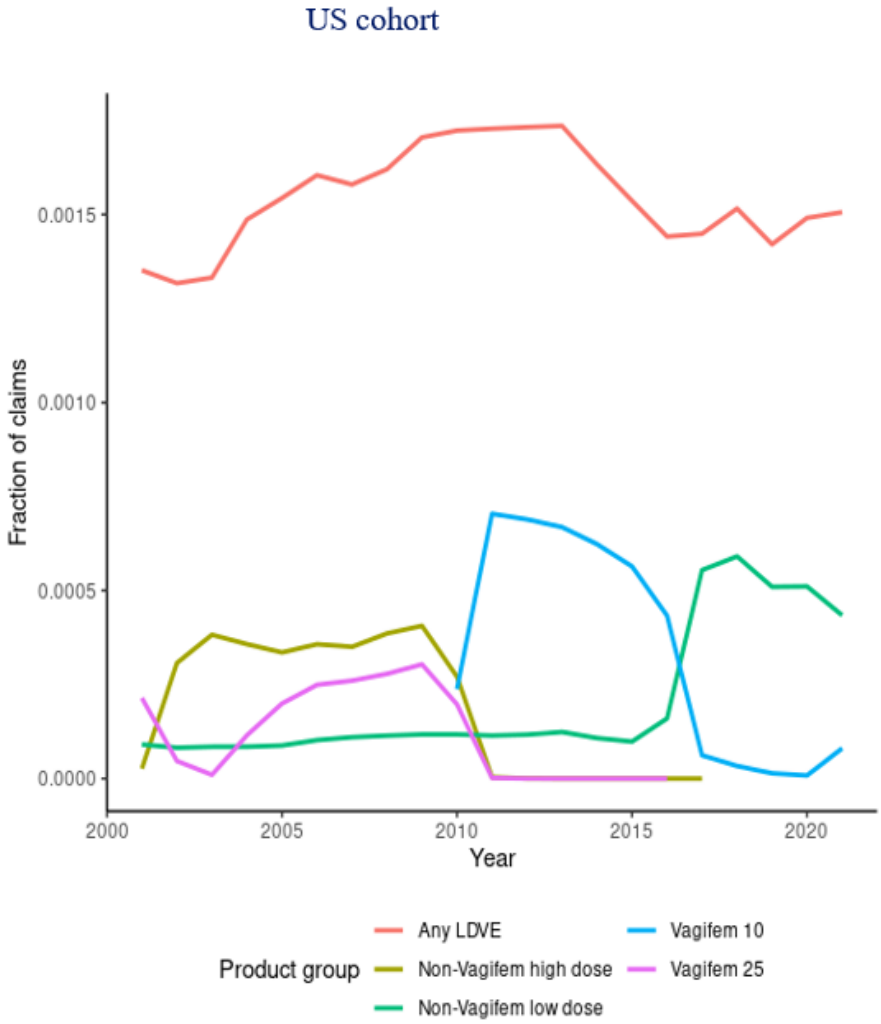



US cohort
600000
400000
200000
0
Sum prescriptions
30
40
50
60
70
Age
Product group
Any LDVE
Non-Vagifem high dose
Non-Vagifem low dose
Vagifem 10
Vagifem 25
Vagifem all

In the Danish cohort, the usage of Vagifem® 10 mcg was seen to increase after 2010 with peak usage between the years 2015 to 2020, whereas a decline in the number of Vagifem® 25 mcg users was observed from 2010 to 2015 and beyond. In the US cohort, the claims and sum of prescriptions were high after 2010 and declined after 2015 (Figure 10-2). One of the possible reasons for this decline could be the introduction of generics to the market.

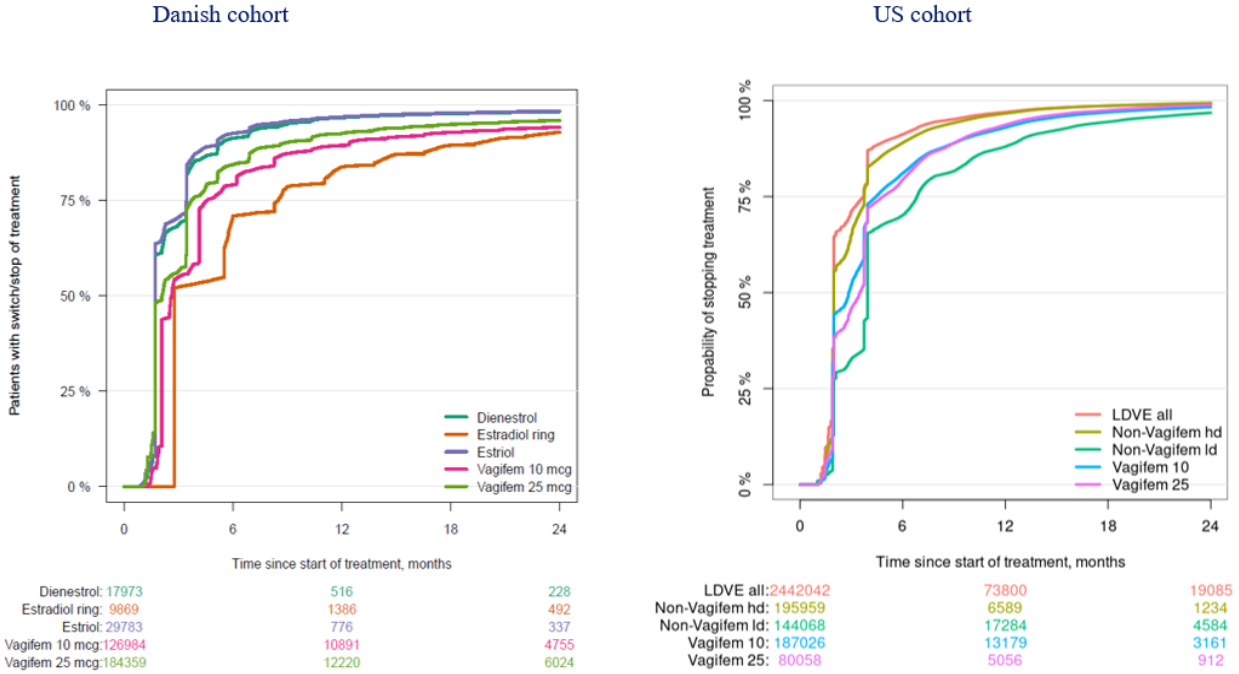
Figure 10-2 LDVE usage by year for Danish and US cohorts




US cohort
Fraction of claims
0.0015
0.0010
0.0005
0.0000
2000
2005
2010
2015
2020
Year
Product group
Any LDVE
Non-Vagifem high dose
Non-Vagifem low dose
Vagifem 10
Vagifem 25

Data from the Danish registry showed that more than 75% of the new users switched or stopped LDVE therapy after 6 months of initiation. In the US cohort, the number of new users who claimed LDVE products declined to 10% before first year of therapy (Figure 10-3). In both the Danish and US cohorts all participants were followed-up as mentioned in Section 9.3.1.

Figure 10-3 Non-adherence of LDVE treatment in both cohorts



10.3 Outcome data

Results are presented in section 10.4.

10.4 Main results

In both the Danish and US cohorts, new users of LDVE/Vagifem® (exposed) in the primary analysis were matched based on the year of birth to non-users of any LDVE (comparator) at the enrolment date i.e., the date of first prescription. In the secondary analysis, new users of LDVE/Vagifem® (exposed) were matched to systemic hormonal therapy users (comparator). The hazard ratio for exposed group compared to matched controls, together with the crude incidence rate for treated groups was calculated.

10.4.1 Primary Analysis

10.4.1.1 Intention to treat analysis

Danish Cohort

Based on the propensity score model, the matched cohort for the primary analysis was obtained in which a total of 328,842 women had started treatment during follow-up, but only 253,184 were matched to two controls. The ITT analysis was conducted on a sample of 624,498 women (208,166 in the exposed group and 416,332 in the control group). The start of follow-up was set 180 days

after the enrolment date. A total of 53,347 women were excluded because of the 180 days of lag-time.

The primary analysis was performed by fitting a Cox regression model to the matched cohort. The balance of characteristics at start of follow-up for new-users of any LDVE and matched population by time-dependent propensity score. is represented in the Table 10-2 below. However, information on family history of cancer and polycystic ovarian syndrome were excluded because of non-convergency.

Table 10-2 Balance in covariates after matching for Danish cohort

Variable	Level	Matched controls (n=416332) (%)	Exposed women (n=208166) (%)
Age categories	[50,55]	121789 (29.3)	67671 (32.5)
	[55,60]	128339 (30.8)	60039 (28.8)
	[60,65]	74068 (17.8)	34028 (16.3)
	[65,70]	48411 (11.6)	23931 (11.5)
	[70,75]	43725 (10.5)	22497 (10.8)
Dementia	0	415813 (99.9)	207844 (99.8)
	1	519 (0.1)	322 (0.2)
Diabetes	0	402828 (96.8)	203158 (97.6)
	1	13504 (3.2)	5008 (2.4)
Endometriosis	0	414548 (99.6)	206584 (99.2)
	1	1784 (0.4)	1582 (0.8)
Heart Failure	0	412317 (99.0)	206537 (99.2)
	1	4015 (1.0)	1629 (0.8)
Hypertension	0	336194 (80.8)	170173 (81.7)
	1	80138 (19.2)	37993 (18.3)
Infertility	0	414250 (99.5)	206712 (99.3)
	1	2082 (0.5)	1454 (0.7)
Ischemic Heart Disease	0	400892 (96.3)	201003 (96.6)
	1	15440 (3.7)	7163 (3.4)
Myocardial Infarction	0	410240 (98.5)	205575 (98.8)
	1	6092 (1.5)	2591 (1.2)
Polycystic Ovarian Syndrome	0	416252 (100.0)	208,129 (100.0)
	1	80 (0.0)	37 (0.0)
Renal Disease	0	414211 (99.5)	207,220 (99.5)

Variable	Level	Matched controls (n=416332) (%)	Exposed women (n=208166) (%)
	1	2121 (0.5)	946 (0.5)
Systemic Estrogen	0	359333 (86.3)	172457 (82.8)
	1	56999 (13.7)	35709 (17.2)
Progesterone	0	282289 (67.8)	138997 (66.8)
	1	134043 (32.2)	69169 (33.2)
Hormonal Contraceptive	0	290311 (69.7)	149616 (71.9)
	1	126021 (30.3)	58550 (28.1)
Education	Bachelor	85347 (20.5)	44064 (21.2)
	Basic	136286 (32.7)	71252 (34.2)
	Higher education	19332 (4.6)	10203 (4.9)
	Upper secondary	12861 (3.1)	6026 (2.9)
	Vocational training	162506 (39.0)	76621 (36.8)
Income	low	86102 (20.7)	48162 (23.1)
	medium	144283 (34.7)	66698 (32.0)
	high	185947 (44.7)	93306 (44.8)
Origin	Danish	388927 (93.4)	196469 (94.4)
	Foreigner	27405 (6.6)	11697 (5.6)
BMI	Underweight	52 (1.4)	42 (1.9)
	Normal weight	2335 (63.0)	1493 (69.2)
	Pre-obesity	844 (22.8)	437 (20.3)
	Obesity	476 (12.8)	186 (8.6)
	missing	412625	206008
Smoker	mean (sd)	0.2 (0.4)	0.2 (0.4)
	missing	395601	196914
Family histroy of cancer	0	416239 (100.0)	208124 (100.0)
	1	93 (0.0)	42 (0.0)
Vulvovaginal atrophy	0	416323 (100.0)	208124 (100.0)
	1	9 (0.0)	42 (0.0)

Note: 0 = No; 1=Yes.

Abbreviation: BMI = body mass index

Primary objective

A Poisson regression model was used to estimate incidence rate of endometrial cancer comparing new-users and matched controls. A Cox regression model controlled for all variables used in the propensity score model was fitted to estimate the hazard ratio. However, information on family history of cancer and polycystic ovarian syndrome were excluded from the cox regression due to non-convergence. Women were censored on first occurrence of either death, hysterectomy, start on a non-LDVE treatment, emigration or when no further data were available.

Hazard ratio for new users of LDVE and different product groups of LDVE compared to non-users and incidence rate per 10000 person years of endometrial cancer are presented in Table 10-3. Users of Vagifem® 10 mcg and non-Vagifem® low dose products had lower IRs of 4.731 [95% CI, 3.865; 5.790] and 3.129 [95% CI, 1.492; 6.563] respectively, compared to other LDVE products. IRs were not controlled for covariates. Vagifem® represented in the analysis refers to sum of Vagifem® 10 mcg and Vagifem® 25 mcg. For the Danish cohort there are no new users of non-Vagifem® high dose product.

Table 10-3 Hazard Ratio of endometrial cancer for LDVE new users compared to non-users and Incidence Rate of endometrial cancer for LDVE new users (Danish cohort)

Product name	Number of women exposed	Hazard Ratio	HR 95% CI	Incidence Rate (per 10000 PY)	IR 95%CI (per 10000 PY)
All LDVE	208166	1.105	[1.029;1.186]	6.648	[6.235;7.088]
Vagifem®	171970	1.135	[1.044;1.233]	6.539	[6.072;7.042]
Vagifem® 10	62706	1.026	[0.801;1.315]	4.731	[3.865;5.790]
Vagifem® 25	109264	1.151	[1.054;1.258]	6.951	[6.419;7.527]
Non-Vagifem®	32643	0.98	[0.838;1.145]	6.878	[5.928;7.979]
Non-Vagifem® low dose	4254	0.611	[0.297;1.258]	3.129	[1.492;6.563]

Abbreviations: HR = hazard ratio; CI = confidence interval; PY = patient years; IR= Incidence Rate

US Cohort

The number of women who started on treatment were 961,056, but only 875,099 were left after matching (16 women got matched to less than two controls, and 85,941 women starting treatment was selected as controls before their treatment started). The ITT analysis was conducted on a sample of 1,221,840 women (407,280 in the exposed group and 814,560 in the control group). A total of 467,831 case-control sets were excluded during 180 days of lag-time.

The balance of characteristics at start of follow-up for new-users of any LDVE and matched population by time-dependent propensity score is represented in the Table 10-4 below. The US cohort was matched using fewer covariates than the Danish cohort.

Table 10-4 Balance in covariates after matching for US cohort

Variable	Level	Matched controls (n=814560) (%)	Exposed women (n=407280) (%)
Age at enrolment	mean (SD)	58.6 (5.5)	58.6 (5.5)
Age categories	[50,55]	266520 (32.7)	133260 (32.7)
	[55,60]	282318 (34.7)	141159 (34.7)
	[60,65]	171756 (21.1)	85878 (21.1)
	[65,70]	58520 (7.2)	29260 (7.2)
	[70,75]	35446 (4.4)	17723 (4.4)
Follow-up time	mean (SD)	928.8 (844.9)	960.4 (862)
Time before enrolment	mean (SD)	1372.2 (1145.3)	1238.4 (1097.1)
Progesterone	0	784228 (96.3)	392120 (96.3)
	1	30332 (3.7)	15160 (3.7)
Other estrogen	0	782557 (96.1)	391278 (96.1)
	1	32003 (3.9)	16002 (3.9)
Ischemic heart disease	0	747602 (91.8)	373771 (91.8)
	1	66958 (8.2)	33509 (8.2)
Diabetes	0	122211 (15.0)	61108 (15.0)
	1	692349 (85.0)	346172 (85.0)
Hypertension	0	472535 (58.0)	236259 (58.0)
	1	342025 (42.0)	171021 (42.0)
Endometriosis	0	804448 (98.8)	402222 (98.8)
	1	10112 (1.2)	5058 (1.2)
Polycystic Ovarian Syndrome	0	813009 (99.8)	406499 (99.8)
	1	1551 (0.2)	781 (0.2)
Infertility	0	812410 (99.7)	406189 (99.7)
	1	2150 (0.3)	1091 (0.3)

Note: 0 = No; 1=Yes

Abbreviation: SD = standard deviation

Primary objective

The hazard ratio for various LDVE new users compared to non-users and incidence rate per 1000 person years of endometrial cancer are represented in Table 10-5. It was found that the HR and IR of endometrial cancer for all product groups were below one in the US cohort.

Table 10-5 Hazard Ratio of endometrial cancer for LDVE new users compared to non-users and Incidence Rate of endometrial cancer for LDVE new users (US cohort)

Product name	Number of women exposed	Hazard Ratio	HR 95% CI	Incidence Rate (per 1000 PY)	IR 95%CI (per 1000 PY)
Any LDVE	407280	0.714	[0.661; 0.772]	0.820	[0.767; 0.876]
Vagifem®	55436	0.676	[0.547; 0.835]	0.782	[0.652; 0.938]
Vagifem® 10	44161	0.616	[0.481; 0.789]	0.728	[0.587; 0.903]
Vagifem® 25	11277	0.894	[0.592; 1.350]	0.962	[0.684; 1.350]
Non-Vagifem®	352913	0.718	[0.661; 0.781]	0.824	[0.768; 0.885]
Non-Vagifem® low dose	22419	0.443	[0.290; 0.678]	0.499	[0.340; 0.732]
Non-Vagifem® high dose	34418	0.692	[0.538, 0.891	0.839	[0.676, 1.04]

Abbreviations: LDVE = low dose vaginal estrogens; HR = hazard ratio; CI = confidence interval; PY = patient years; IR= Incidence Rate

10.4.1.2 Longitudinal Targeted Minimum Loss Estimation (LTMLE) analysis:

Danish Cohort

The difference in 5-year absolute risk of endometrial cancer was obtained by LTMLE. A time grid of 6 months long time interval was considered and the average treatment effect comparing the interventions always treated and never treated during the 5 years of follow-up was calculated. Death and hysterectomy were treated as competing events. Patients were censored if they started a non-LDVE treatment, emigrated or when no further data was available. Time dependent covariates were used for censoring in this method. The start of follow up for LTMLE analysis was the date of enrolment unlike 180 days in the primary analysis.

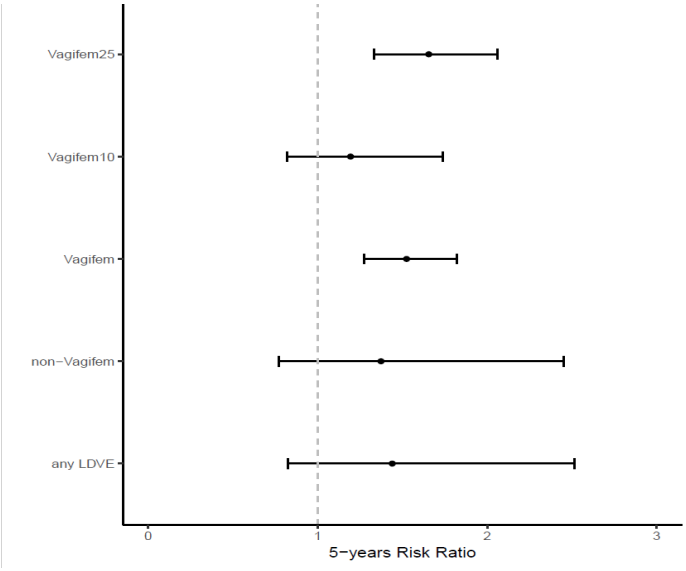
The 5 year risk of endometrial cancer in each treatment group (exposed women and matched controls), the absolute risk difference (average treatment effect, ATE), and the risk ratio with the respective confidence interval are presented in Table 10-6 and Figure 10-4. The relative risk of endometrial cancer with Vagifem® 10 mcg when compared to non-users was found to be lower (1.1939 [95% CI, 0.8193; 1.7398]) than other products.

Table 10-6 LTMLE analysis of LDVE new users compared to non-users (Danish cohort)

Product Name	Risk for exposed	Risk for controls	ATE	Relative Risk
All LDVE	0.0040 [0.0035;0.0046]	0.0028 [0.0013;0.0043]	0.0012 [-0.0004;0.0028]	1.4398 [0.8247;2.5136]
Vagifem®	0.0040 [0.0033;0.0046]	0.0026 [0.0024;0.0028]	0.0014 [0.0007;0.0020]	1.5245 [1.2750;1.8228]
Vagifem® 10	0.0027 [0.0018;0.0035]	0.0022 [0.0018;0.0026]	0.0004 [-0.0005;0.0014]	1.1939 [0.8193;1.7398]
Vagifem® 25	0.0047 [0.0038;0.0055]	0.0028 [0.0025;0.0031]	0.0018 [0.0009;0.0028]	1.6553 [1.3309;2.0588]
Non-Vagifem®	0.0036 [0.0017;0.0054]	0.0026 [0.0020;0.0032]	0.0010 [-0.0010;0.0029]	1.3735 [0.7700;2.4499]

Abbreviations: LDVE = low dose vaginal estrogens; ATE = average treatment effect

Figure 10-4 LTMLE analysis representing Relative Risk



US Cohort

The design of the analysis aimed at determining the 5-year absolute risk of endometrial cancer for women on 5 years of treatment. However, in the US cohort there were fewer women followed up for that long. At 5 years less than 2% of women were still on treatment. Thus, in order to arrive at relevant and robust measures, the 5-year risk is reported for women with 3 years or more on treatment. However, it is to be noted that the outcome of this analysis might not be fully reliable owing to the short duration of treatment. The results observed were inconsistent and with much larger confidence intervals compared to other analyses (Table 10-7).

Table 10-7 LTMLE Analysis of LDVE new users compared to non-users (US cohort)

Product Name	Risk for exposed	Risk for controls	ATE	Relative Risk
All LDVE	0.0060 [0.0018, 0.0101]	0.0069 [0.0065, 0.0073]	-0.0009 [-0.0051, 0.0033]	0.8689 [0.4341, 1.7392]
Vagifem® 10	0.0088 [0.0000, 0.0199]	0.0071 [0.0059, 0.0084]	0.0017 [-0.0094, 0.0128]	1.2372 [0.3495, 4.3792]
Vagifem® 25	0.0068 [0.0000, 0.0248]	0.0083 [0.0054, 0.0112]	-0.0015 [-0.0198, 0.0168]	0.8205 [0.0562, 11.9806]
Non-Vagifem®	0.0057 [0.0000, 0.0174]	0.0069 [0.0065, 0.0073]	-0.0012 [-0.0129, 0.0105]	0.8218 [0.1039, 6.4998]

Abbreviations: LDVE = low dose vaginal estrogens; ATE = average treatment effect.

10.4.2 Secondary Analysis

10.4.2.1 ITT analysis for Secondary endpoint

For Danish cohort, the hazard ratio and incidence rate per 10000 person years of endometrial cancer for Vagifem® 10 mcg and 25 mcg new-users compared to users of other HRTs are represented in Table 10-8. This analysis shows that Vagifem® 10 mcg had lower HR and IR of endometrial cancer than Vagifem® 25 mcg when compared with systemic HRT users.

Table 10-8 Hazard ratio and incidence rate of endometrial cancer for Vagifem® products compared to systemic HRT users (Danish cohort)

Product name	Number of women exposed	Hazard Ratio	HR 95% CI	Incidence Rate (per 10000 PY)	IR 95%CI (per 10000 PY)
Vagifem® 10	3163	0.506	[0.138;1.849]	2.924	[0.943;9.065]
Vagifem® 25	8736	0.907	[0.670;1.227]	5.722	[4.213;7.771]

Abbreviations: HR = hazard ratio; CI = confidence interval; PY = patient years; IR= Incidence Rate

For the US cohort, hazard ratio and incidence rate per 1000 person years of endometrial cancer for all Vagifem® new users compared to users of other HRTs are shown in Table 10-9. The HR and IR were below one for Vagifem® 10 mcg. Similar to the observations in primary analysis, the HR and IR of endometrial cancer were lower for Vagifem 10 mcg than Vagifem 25 mcg when compared with systemic HRT users.

Table 10-9 Hazard ratio and incidence rate of endometrial cancer for Vagifem® products compared to HRT users (US cohort)

Product name	Number of women exposed	Hazards Ratio	HR 95% CI	Incidence Rate (per 1000 PY)	IR 95%CI (per 1000 PY)
Vagifem®	86913	0.841	[0.715;0.989]	0.817	[0.714;0.935]
Vagifem® 10	68057	0.808	[0.666;0.980]	0.767	[0.652;0.901]
Vagifem® 25	18861	0.926	[0.685;1.25]	0.964	[0.755;1.23]

Abbreviations: HR = hazard ratio; CI = confidence interval; PY = patient years; IR= Incidence Rate

10.4.2.2 LTMLE analysis for secondary endpoint

For the US cohort, data on LTMLE analysis supporting the secondary endpoint were not available. In the Danish cohort, Vagifem® 10 mcg and 25 mcg users were compared to systemic HRT users by LTMLE. The relative risks of endometrial cancer with Vagifem® 10 mcg and 25 mcg were 0.4793 [0.2973; 0.7727] and 1.3335 [0.9403; 1.8911], respectively (Table 10-10).

Table 10-10 LTMLE analysis of Vagifem®10/25 mcg users compared to systemic HRT

Product Name	Risk for exposed	Risk for controls	ATE	Relative Risk
Vagifem® 10 mcg	0.0029 [0.0018;0.0040]	0.0061 [0.0043;0.0079]	-0.0032 [-0.0053; -0.0011]	0.4793 [0.2973;0.7727]
Vagifem® 25 mcg	0.0050 [0.0037;0.0064]	0.0038 [0.0029;0.0046]	0.0013 [-0.0003;0.0029]	1.3335 [0.9403;1.8911]

Abbreviations: LDVE = low dose vaginal estrogens; ATE = average treatment effect

10.4.3 Summary of main results

The study included data from a nationwide Danish cohort of postmenopausal women and a US cohort of postmenopausal women to evaluate the risk of endometrial cancer in participants using low dose vaginal estrogens.

A total of 328842 and 961056 LDVE new users were considered for Danish and US cohorts, respectively. The proportion of Vagifem® 25 mcg users were higher in the Danish population whereas Vagifem® 10 mcg was used by majority in the US population. In both the cohorts, usage of Vagifem® 10 mcg increased and 25 mcg declined around the year 2010. The number of women continuing LDVE products decreased after first 6 months of therapy in both the populations.

The women were followed up after 180 days of enrolment. Time dependent propensity scores were used to match the new users with controls. In the Danish cohort, the hazard ratio for women using low dose vaginal estrogens when compared to women using no hormone replacement therapy was 1.105 [95% CI, 1.029; 1.186] and incidence rate (per 10000 person years (PY)) of endometrial cancer for LDVE new users was 6.648 [95% CI, 6.235; 7.088]. Vagifem® 10 mcg showed a lower hazard ratio and incidence rate (HR 1.026 [95% CI, 0.801; 1.315] and IR 4.731 [95% CI, 3.865; 5.790] per 10000 PY) for endometrial cancer than Vagifem® 25 mcg (HR 1.151 [95% CI, 1.054; 1.258] and IR 6.951 [95% CI, 6.419; 7.527] per 10000 PY) (Table 10-3).

In the US cohort, the hazard ratio for women using low dose vaginal estrogens when compared to women using no hormone replacement therapy was 0.714 [95% CI, 0.661; 0.772] and incidence rate (per 1000 PY) of endometrial cancer for LDVE new users was 0.820 [95% CI, 0.767; 0.876]. For Vagifem® 10 mcg, the hazard ratio and incidence rate were lower (HR 0.616 [95% CI, 0.481; 0.789] and IR 0.728 [95% CI, 0.587; 0.903] per 1000 PY) than Vagifem® 25 mcg (HR 0.894 [95% CI, 0.592; 1.350] and IR 0.962 [95% CI, 0.684; 1.350] per 1000 PY) (Table 10-5).

For the Danish cohort, the hazard ratio for Vagifem® 10 mcg compared to systemic HRT users and incidence rate per 10000 PY of endometrial cancer were 0.506 [95% CI, 0.138; 1.849] and 2.924 [95% CI, 0.943; 9.065] whereas the HR and IR for Vagifem® 25 mcg were 0.907 [95% CI, 0.670; 1.227] and 5.722 [95% CI, 4.213; 7.771] (Table 10-8).

For the US cohort, the hazard ratio for Vagifem® 10 mcg compared to systemic HRT users and incidence rate per 1000 PY of endometrial cancer were 0.808 [95% CI, 0.666;0.980] and 0.767 [95% CI, 0.652; 0.901] whereas the HR and IR for Vagifem® 25 mcg were 0.926 [95% CI, 0.685; 1.25] and 0.964 [95% CI, 0.755; 1.23] (Table 10-9). The incidence rates for all Vagifem® products were lower than systemic hormone replacement therapy.

The LTMLE analysis in the Danish cohort using new users of LDVE compared to non-users identified the difference in 5-years absolute risk of endometrial cancer, where the relative risk was (1.4398 [0.8247; 2.5136]) for all LDVE, and similar risk was identified in different product groups with Vagifem®10 mcg having lower relative risk compared to Vagifem® 25 mcg. In addition, the findings indicate that there is no significantly elevated risk with Vagifem® 10 mcg when compared to non-users. (Table 10-6). In the US cohort, the 5-year risk for women was reported with 3 years or more duration on treatment because there were only about 2% women who were on continuous treatment for 5 years, the relative risk observed for all LDVE was 0.8689 [0.4341; 1.7392] (Table 10-7).

The pattern of results representing the risk of endometrial cancer among various sensitivity parameters using intention to treat analysis and LTMLE analysis were found to be similar (Detailed description is provided in Section 10.5).

10.5 Other analyses

The supplementary and sensitivity analyses were conducted as described in Appendix 16.1.1, Section 9.7.3 and Section 9.7.4.

10.5.1 Danish Cohort

10.5.1.1 ITT for other analyses

Sensitivity and supplementary analyses were performed for all product groups in the Danish cohort. Analysis using vulvovaginal atrophy could not be completed as the number of women with positive diagnosis were minimal in the Danish cohort. The results for ITT analysis showed similar pattern across various sensitivity parameters representing lower risk of endometrial cancer for all LDVE (Table 10-11).

Table 10-11 Summary of other analyses for Danish cohort

Other analyses	Product Name	Number of women exposed	HR	HR 95% CI	Incidence Rate (per 1000 PY)	IR 95%CI (per 1000 PY)
Code C54	Any LDVE	203175	1.039	[0.967;1.117]	6.601	[6.182;7.049]
	Vagifem®	166274	1.065	[0.978;1.159]	6.580	[6.099;7.099]
	Vagifem® 10 mcg	62048	0.948	[0.745;1.206]	5.022	[4.116;6.127]
	Vagifem® 25 mcg	104226	1.084	[0.990;1.187]	6.947	[6.399;7.541]
	Non-Vagifem®	33312	0.95	[0.813;1.109]	6.730	[5.793;7.818]
	Low dose non-Vagifem®	4843	1.113	[0.562;2.205]	4.622	[2.559;8.345]
Codes C54.1, C54.2, C54.3, C54.8 and C54.9 definition	Any LDVE	203271	1.068	[0.993;1.148]	6.662	[6.240;7.112]
	Vagifem®	166464	1.091	[1.002;1.188]	6.630	[6.148;7.151]
	Vagifem® 10 mcg	61924	1.012	[0.796;1.286]	5.179	[4.258;6.301]
	Vagifem® 25 mcg	104503	1.105	[1.009;1.210]	6.971	[6.423;7.566]
	Non-Vagifem®	33290	0.961	[0.821;1.125]	6.672	[5.738;7.757]
	Low dose non-Vagifem®	4827	0.671	[0.367;1.228]	5.493	[3.190;9.460]
3 months lag time	Any LDVE	255118	1.091	[1.029;1.156]	7.200	[6.824;7.597]
	Vagifem®	211651	1.126	[1.054;1.203]	7.164	[6.747;7.607]
	Vagifem® 10 mcg	67474	1.089	[0.867;1.368]	5.178	[4.306;6.226]
	Vagifem® 25 mcg	118213	1.206	[1.109;1.312]	7.217	[6.691;7.786]
	Non-Vagifem®	35509	1.046	[0.902;1.214]	7.166	[6.226;8.249]
	Low dose non-Vagifem®	4570	0.621	[0.294;1.310]	2.922	[1.393;6.129]
12 months lag time	Any LDVE	191237	1.045	[0.969;1.128]	5.634	[4.872;6.515]
	Vagifem®	156090	1.07	[0.978;1.170]	5.369	[4.524;6.371]
	Vagifem® 10 mcg	55685	0.897	[0.679;1.184]	4.168	[2.659;6.535]
	Vagifem® 25 mcg	118213	1.095	[0.996;1.205]	5.644	[4.690;6.793]

Other analyses	Product Name	Number of women exposed	HR	HR 95% CI	Incidence Rate (per 1000 PY)	IR 95%CI (per 1000 PY)
	Non-Vagifem®	29867	0.952	[0.807;1.123]	5.991	[4.302;8.344]
	Low dose non-Vagifem®	3879	0.7	[0.329;1.487]	1.963	[0.276;13.936]
Age 55-75 years	Any LDVE	140597	1.148	[1.060;1.243]	7.694	[7.167;8.260]
	Vagifem®	114131	1.17	[1.064;1.285]	7.538	[6.940;8.189]
	Vagifem® 10 mcg	36787	0.971	[0.727;1.297]	5.721	[4.519;7.244]
	Vagifem® 25 mcg	77344	1.197	[1.083;1.322]	7.890	[7.222;8.618]
	Low dose non-Vagifem®	23945	1.066	[0.897;1.267]	7.917	[6.747;9.291]
Type-I malignancy	Any LDVE	229000	1.159	[1.078;1.246]	6.360	[5.971;6.775]
	Vagifem®	188796	1.243	[1.142;1.352]	6.459	[6.011;6.941]
	Vagifem® 10 mcg	69763	1.328	[1.049;1.680]	5.261	[4.382;6.315]
	Vagifem® 25 mcg	119033	1.232	[1.126;1.349]	6.741	[6.233;7.290]
	Non-Vagifem®	36209	0.941	[0.799;1.108]	6.023	[5.171;7.016]
	Low dose non-Vagifem®	5312	0.731	[0.364;1.466]	3.392	[1.765;6.520]
Censoring for any non-endometrial cancer	Any LDVE	209165	1.081	[1.004;1.162]	6.621	[6.201;7.070]
	Vagifem®	172279	1.125	[1.033;1.225]	6.664	[6.183;7.184]
	Vagifem® 10 mcg	62979	1.236	[0.960;1.591]	5.022	[4.116;6.128]
	Vagifem® 25 mcg	109300	1.11	[1.014;1.216]	7.047	[6.498;7.641]
	Non-Vagifem®	33210	0.954	[0.813;1.121]	6.402	[5.475;7.486]
	Low dose non-Vagifem®	5312	0.644	[0.327;1.271]	4.145	[2.230;7.704]
Active ingredient	Dienestrol	11150	0.892	[0.698;1.141]	6.935	[5.449;8.828]
	Estradiol	179777	1.131	[1.044;1.225]	6.532	[6.086;7.010]
	Estriol	17239	1.095	[0.887;1.353]	7.456	[6.135;9.062]
Treatment formulation	Ring	4254	1.111	[1.034;1.193]	6.705	[6.287;7.150]
	Tablet	203912	0.611	[0.297;1.258]	3.129	[1.492;6.563]

Abbreviations: LDVE = low dose vaginal estrogens

10.5.1.2 LTMLE for sensitivity and supplementary analyses

Each parameter pertaining to sensitivity analyses were considered and LTMLE was performed for all LDVE compared to non-users. Similar to the presentation in primary analysis, Vagifem® 10 mcg showed lower risk of endometrial cancer than Vagifem® 25 mcg (Table 10-12).

Table 10-12 Summary of LTMLE analysis of 5-years risk in each of the exposed group, together with the average treatment effect for Danish cohort

Other analyses	Product Name	Risk for exposed	Risk for controls	ATE	Relative Risk
Age 55-75 years	Any LDVE	0.0040 [0.0035;0.0046]	0.0028 [0.0013;0.0043]	0.0012 [-0.0004;0.0028]	1.4398 [0.8247;2.5136]
	Vagifem®	0.0048 [0.0040;0.0056]	0.0031 [0.0028;0.0034]	0.0017 [0.0008;0.0025]	1.5339 [1.2732;1.8478]
	Vagifem® 10 mcg	0.0033 [0.0021;0.0044]	0.0028 [0.0023;0.0034]	0.0004 [-0.0008;0.0017]	1.1503 [0.7741;1.7094]
	Vagifem® 25 mcg	0.0057 [0.0046;0.0067]	0.0033 [0.0029;0.0036]	0.0024 [0.0012;0.0035]	1.7323 [1.3848;2.1670]
	Non-Vagifem®	0.0035 [0.0022;0.0049]	0.0030 [0.0023;0.0037]	0.0005 [-0.0010;0.0021]	1.1710 [0.7409;1.8507]
180 days lag time	Any LDVE	0.0035 [0.0027;0.0043]	0.0027 [0.0026;0.0028]	0.0008 [0.0000;0.0016]	1.3019 [1.0360;1.6360]
	Vagifem®	0.0034 [0.0026;0.0043]	0.0027 [0.0025;0.0029]	0.0007 [-0.0001;0.0016]	1.2763 [0.9843;1.6549]
	Vagifem® 10 mcg	0.0024 [0.0013;0.0036]	0.0026 [0.0024;0.0029]	-0.0002 [-0.0014;0.0010]	0.9202 [0.5638;1.5021]
	Vagifem® 25 mcg	0.0039 [0.0028;0.0050]	0.0027 [0.0025;0.0029]	0.0012 [0.0000;0.0023]	1.4289 [1.0550;1.9354]
	Non-Vagifem®	0.0025 [0.0014;0.0037]	0.0027 [0.0025;0.0029]	-0.0002 [-0.0013;0.0010]	0.9413 [0.5937;1.4924]
3 months lag time	Any LDVE	0.0036 [0.0029;0.0044]	0.0027 [0.0025;0.0030]	0.0009 [0.0002;0.0017]	1.3364 [1.0778;1.6572]
	Vagifem®	0.0037 [0.0029;0.0045]	0.0027 [0.0025;0.0029]	0.0010 [0.0001;0.0018]	1.3556 [1.0709;1.7161]
	Vagifem® 10 mcg	0.0026 [0.0014;0.0037]	0.0027 [0.0025;0.0029]	-0.0001 [-0.0013;0.0011]	0.9538 [0.6027;1.5093]
	Vagifem® 25 mcg	0.0042 [0.0031;0.0053]	0.0027 [0.0025;0.0030]	0.0014 [0.0003;0.0025]	1.5183 [1.1560;1.9940]
	Non-Vagifem®	0.0029 [0.0017;0.0041]	0.0027 [0.0025;0.0029]	0.0002 [-0.0010;0.0014]	1.0603 [0.6968;1.6135]
12 months lag time	Any LDVE	0.0033 [0.0025;0.0042]	0.0027 [0.0025;0.0029]	0.0006 [-0.0003;0.0015]	1.2218 [0.9351;1.5964]
	Vagifem®	0.0033 [0.0024;0.0043]	0.0027 [0.0025;0.0029]	0.0006 [-0.0004;0.0016]	1.2249 [0.9105;1.6479]
	Vagifem® 10 mcg	0.0024 [0.0011;0.0036]	0.0027 [0.0025;0.0029]	-0.0003 [-0.0016;0.0009]	0.8751 [0.5096;1.5025]
	Vagifem® 25 mcg	0.0036 [0.0024;0.0048]	0.0027 [0.0025;0.0030]	0.0009 [-0.0003;0.0021]	1.3306 [0.9434;1.8767]
	Non-Vagifem®	0.0022 [0.0010;0.0034]	0.0027 [0.0025;0.0029]	-0.0005 [-0.0017;0.0007]	0.8120 [0.4728;1.3945]
C54 codes in ICD-10	Vagifem®	0.0043 [0.0036;0.0049]	0.0026 [0.0024;0.0028]	0.0017 [0.0010;0.0023]	1.6466 [1.3844;1.9584]
	Vagifem® 10 mcg	0.0031 [0.0022;0.0041]	0.0025 [0.0021;0.0029]	0.0007 [-0.0004;0.0017]	1.2703 [0.9025;1.7880]
	Vagifem® 25 mcg	0.0048 [0.0039;0.0057]	0.0026 [0.0024;0.0029]	0.0022 [0.0012;0.0031]	1.8148 [1.4634;2.2507]
	Non-Vagifem®	0.0039 [0.0018;0.0060]	0.0028 [0.0020;0.0035]	0.0011 [-0.0011;0.0034]	1.4097 [0.7670;2.5910]
Sub-groups of C54 codes in ICD-10	Vagifem®	0.0041 [0.0035;0.0047]	0.0025 [0.0023;0.0027]	0.0016 [0.0009;0.0022]	1.6286 [1.3648;1.9434]
	Vagifem® 10 mcg	0.0029 [0.0019;0.0038]	0.0020 [0.0016;0.0024]	0.0008 [-0.0002;0.0018]	1.4129 [0.9656;2.0674]
	Vagifem® 25 mcg	0.0048 [0.0039;0.0057]	0.0027 [0.0024;0.0030]	0.0021 [0.0011;0.0030]	1.7538 [1.4108;2.1801]

Other analyses	Product Name	Risk for exposed	Risk for controls	ATE	Relative Risk
	Non-Vagifem®	0.0039 [0.0018;0.0059]	0.0025 [0.0019;0.0031]	0.0014 [-0.0007;0.0035]	1.5755 [0.8829;2.8115]
Type-I malignancy	Vagifem®	0.0040 [0.0034;0.0047]	0.0023 [0.0021;0.0025]	0.0017 [0.0011;0.0024]	1.7519 [1.4627;2.0984]
	Vagifem® 10 mcg	0.0028 [0.0019;0.0037]	0.0024 [0.0019;0.0028]	0.0004 [-0.0005;0.0014]	1.1857 [0.8282;1.6975]
	Vagifem® 25 mcg	0.0046 [0.0037;0.0055]	0.0023 [0.0020;0.0026]	0.0023 [0.0014;0.0032]	1.9885 [1.5887;2.4888]
	Non-Vagifem®	0.0045 [0.0023;0.0067]	0.0027 [0.0020;0.0033]	0.0018 [-0.0005;0.0041]	1.6697 [0.9711;2.8708]
Censoring for any non-endometrial cancer	Vagifem®	0.0046 [0.0039;0.0053]	0.0025 [0.0022;0.0028]	0.0021 [0.0014;0.0028]	1.8444 [1.5379;2.2120]
	Vagifem® 10 mcg	0.0029 [0.0020;0.0038]	0.0026 [0.0021;0.0031]	0.0003 [-0.0007;0.0013]	1.1153 [0.7708;1.6137]
	Vagifem® 25 mcg	0.0054 [0.0044;0.0064]	0.0025 [0.0022;0.0028]	0.0029 [0.0018;0.0039]	2.1535 [1.7228;2.6918]
	Non-Vagifem®	0.0040 [0.0018;0.0062]	0.0031 [0.0024;0.0038]	0.0009 [-0.0014;0.0032]	1.2988 [0.7173;2.3519]
Maximum gap of 90 days on treatment	Vagifem®	0.0040 [0.0034;0.0047]	0.0026 [0.0024;0.0028]	0.0014 [0.0007;0.0021]	1.5504 [1.2835;1.8728]
	Vagifem® 10 mcg	0.0027 [0.0018;0.0035]	0.0022 [0.0018;0.0026]	0.0004 [-0.0005;0.0014]	1.1972 [0.8203;1.7472]
	Vagifem® 25 mcg	0.0047 [0.0038;0.0056]	0.0028 [0.0025;0.0031]	0.0019 [0.0010;0.0028]	1.6645 [1.3444;2.0608]
	Non-Vagifem®	0.0039 [0.0018;0.0061]	0.0026 [0.0020;0.0032]	0.0013 [-0.0009;0.0036]	1.5091 [0.8356;2.7254]
On treatment gap of 180 days	Vagifem®	0.0040 [0.0034;0.0047]	0.0026 [0.0024;0.0028]	0.0014 [0.0007;0.0021]	1.5504 [1.2835;1.8728]
	Vagifem® 10 mcg	0.0027 [0.0018;0.0035]	0.0022 [0.0018;0.0026]	0.0004 [-0.0005;0.0014]	1.1973 [0.8204;1.7474]
	Vagifem® 25 mcg	0.0047 [0.0038;0.0056]	0.0028 [0.0025;0.0031]	0.0019 [0.0010;0.0028]	1.6646 [1.3445;2.0608]
	Non-Vagifem®	0.0039 [0.0018;0.0061]	0.0026 [0.0020;0.0032]	0.0013 [-0.0009;0.0036]	1.5090 [0.8356;2.7252]
Active ingredient	Dienestrol	0.0024 [0.0013;0.0036]	0.0024 [0.0012;0.0037]	0.0000 [-0.0017;0.0017]	1.0073 [0.5065;2.0032]
	Estradiol	0.0043 [0.0037;0.0049]	0.0027 [0.0025;0.0030]	0.0016 [0.0010;0.0022]	1.5736 [1.3492;1.8354]
	Estriol	0.0050 [0.0025;0.0074]	0.0029 [0.0021;0.0037]	0.0021 [-0.0005;0.0046]	1.7166 [0.9774;3.0151]

Abbreviations: LDVE = low dose vaginal estrogens; ATE = average treatment effect

10.5.2 US Cohort

10.5.2.1 ITT for other analyses

In the US cohort, the sensitivity and supplementary analyses were performed only for 'all LDVE' category. For the definition of endometrial cancer, ICD 9 and 10 codes were used. Please refer to Table 10-13 for the summary of sensitivity analyses performed. Similar to the primary analysis, sensitivity and supplementary analyses for US cohort showed that the risk ratios were below one.

Table 10-13 Summary of other analyses for US cohort

Other analyses	Number of women exposed	Hazard Ratio	HR 95% CI	Incidence Rate (per 1000 PY)	IR 95%CI (per 1000 PY)
Codes 182.0, 182.1, 182.8 and C54.1, C54.2, C54.3, C54.8, C54.9	408377	0.713	[0.662, 0.769]	0.864	[0.810, 0.921]
Codes 182 and C54, excluding 182.1 and C54.2, C54.3	407794	0.697	[0.646, 0.752]	0.840	[0.787, 0.897]
Codes 182 and C54	407625	0.716	[0.663, 0.773]	0.846	[0.793, 0.903]
Codes 182 and C54, excluding 182.1, 182.8 and C54.2, C54.3	408002	0.708	[0.656, 0.765]	0.818	[0.766, 0.874]
Lag time 3 months	539109	0.742	[0.694, 0.793]	0.860	[0.813, 0.911]
Lag time 12 months	250091	0.683	[0.617, 0.755]	0.786	[0.721, 0.857]
Censoring non-endometrial cancer	69514	0.548	[0.408, 0.735]	0.421	[0.324, 0.547]
Age 55-75 years	303247	0.735	[0.674, 0.801]	0.936	[0.869, 1.01]
Vaginal atrophy (Yes)	123402	0.729	[0.630, 0.844]	0.820	[0.724, 0.929]
Vaginal atrophy (No)	298813	0.703	[0.643, 0.768]	0.821	[0.761, 0.885]

Abbreviations: HR = hazard ratio; CI = confidence interval; PY = patient years; IR= Incidence Rate

10.5.2.2 LTMLE for sensitivity and supplementary analyses

Each parameter pertaining to sensitivity analyses were considered and LTMLE was performed for all LDVE compared to non-users using 182.0 and C54.1 codes (Table 10-14).

Table 10-14 Summary of LTMLE analysis of 3-years risk in all LDVE group, together with the average treatment effect for US cohort

Other analyses	Risk for exposed	Risk for controls	ATE	Relative Risk
Age 55-75 years	0.0071 [0.0003, 0.0140]	0.0075 [0.0070, 0.0081]	-0.0004 [-0.0073, 0.0064]	0.9413 [0.3580, 2.4746]
180 days lag time	0.0060 [0.0022, 0.0098]	0.0068 [0.0064, 0.0072]	-0.0008 [-0.0047, 0.0030]	0.8803 [0.4639, 1.6708]
3 months lag time	0.0060 [0.0020, 0.0101]	0.0069 [0.0065, 0.0073]	-0.0008 [-0.0049, 0.0032]	0.8770 [0.4467, 1.7218]
12 months lag time	0.0060 [0.0022, 0.0098]	0.0067 [0.0063, 0.0071]	-0.0007 [-0.0045, 0.0031]	0.8900 [0.4724, 1.6770]
Censoring for any non-endometrial cancer	0.0211 [0.0000, 0.1143]	0.0179 [0.0166, 0.0192]	0.0033 [-0.0899, 0.0964]	1.1820 [0.0143, 97.4597]
Vaginal atrophy (Yes)	0.0059 [0.0000, 0.0147]	0.0070 [0.0062, 0.0079]	-0.0011 [-0.0099, 0.0077]	0.8411 [0.1902, 3.7206]
Vaginal atrophy (No)	0.0060 [0.0013, 0.0108]	0.0069 [0.0064, 0.0073]	-0.0008 [-0.0056, 0.0039]	0.8807 [0.4010, 1.9342]
On treatment gap of 90 days	0.0059 [0.0043, 0.0074]	0.0069 [0.0065, 0.0073]	-0.0010 [-0.0026, 0.0005]	0.8509 [0.6525, 1.1095]
On treatment gap of 180 days	0.0060 [0.0054, 0.0067]	0.0069 [0.0065, 0.0073]	-0.0009 [-0.0016, -0.0001]	0.8744 [0.7724, 0.9899]

Abbreviations: LDVE = low dose vaginal estrogens; ATE = average treatment effect

10.6 Safety evaluation

This was a database study and was designed to collect information only about endometrial cancer risk. The data used in this study were already available in existing databases (secondary data usage), and hence single case collection and reporting from any other safety endpoints than endometrial cancer was not required.

11 Discussion

11.1 Key results

The total number of eligible people in the Danish and US cohort were 1,312,834 and 51,538,311, respectively. The total number of LDVE users was overall higher in the US population compared to the Danish population, however, higher rates of Vagifem® 10 and 25 mcg usage were observed in the Danish population. A majority of Vagifem® users in the US population utilized 10 mcg and in the Danish cohort a higher number of women had used Vagifem® 25 mcg.

The hazard ratio for LDVE new users compared to non-users were 1.105 [1.029; 1.186] and 0.714 [0.661; 0.772] for the Danish and US cohorts respectively. The primary analysis using ITT and LTMLE in the Danish cohort depicted that there might be a slightly higher risk shown while using Vagifem® 25 mcg, which was not present in the low dose i.e., Vagifem® 10 mcg group. For the US cohort, all risk ratios representing LDVE products were below one, however the same tendency with a slightly higher risk was observed in the Vagifem® 25 mcg group.

Similarly, in both the cohorts for analyses defining secondary endpoint, the hazard ratio was below one for all the LDVE products when compared to systemic HRT; with Vagifem® 10 mcg representing lower risk than Vagifem® 25 mcg.

For both cohorts, all the sensitivity analyses represented a similar pattern of endometrial cancer risk across the treatment groups, with slightly lower incidence rates for Vagifem® 10 mcg.

11.2 Limitations

The following limitations were noted for this study:

- The LTMLE analysis was affected by a short duration of treatment for a majority of the population.
- The Truven claims data used for the US cohort might not represent the background of US population entirely, as it covers only population with health insurance.
- The Truven claims data lacked details of socioeconomic status, and the comparison could therefore not take into account the differences in socioeconomic status between the Vagifem® or LDVE users, and the postmenopausal women not using HRT.
- Validation of the endpoint endometrial cancer was not possible in the US claims data. Hence, several sets of ICD codes were used to explore the impact of these on the effect estimates.
- Longer term risk of endometrial cancer with LDVE use could not be detected in the US data as the follow up ended when the patient discontinued the health insurance plan. The average follow-up period was 3 years, wherein 5% patients continued therapy.
- In the US database, additional bias analyses were not performed for the history of hysterectomy and prior hormonal product use due to missing data.
- In the Danish cohort, there were no data available on new users of high-dose non-Vagifem® LDVE that the met inclusion/exclusion criteria.
- The influence of BMI could not be accounted for due to a substantial amount of missing data in the Danish cohort and lack of data in the US cohort.

11.3 Interpretation

The primary and secondary analyses for both the Danish and US cohorts emphasize that low dose Vagifem® (10 mcg) has a lower risk of endometrial cancer when compared to Vagifem® 25 mcg.

Similarly, a systematic review previously conducted by Constantine et al[14], concluded that the evidence from clinical reports do not support an increased risk of endometrial hyperplasia or endometrial cancer with low-dose unopposed vaginal estrogens. A study conducted by Mørch et al[5] concluded that long term estrogen therapy increased the risk of endometrial cancer, however the data on Vagifem® 10 mcg were not available during the study period.

Both the cohorts showed a similar pattern of discontinuation, with most of the population discontinuing or switching the therapy or insurance plan before 1 year of being on treatment, which might have influenced the detection of longer-term risk of endometrial cancer especially in the US cohort.

11.4 Generalisability

The Danish data are of high quality derived from accurate population-based registers and contains information on all prescriptions filled and redeemed by Danish residents. Data collected from Truven database for the US population were similar to the Danish data in terms of demographics, prescription habits, management strategies and attitudes towards menopause.

In this study, since both the population based Danish registries and US Claims data were used in analyzing the risk of endometrial cancer, the results could be generalized to other countries of similar socioeconomic development. For further details see (Appendix 16.1.1, Menopause literature search and summary report).

12 Other information

No additional information to the report.

13 Conclusion

Overall, the study showed that usage representing all LDVE users combined have hazard ratio close to one for the Danish cohort and less than one for the US cohort. Therefore, the risk of developing endometrial cancer with low-dose vaginal estrogens do not appear to be different from that of non-users. In addition, users of Vagifem® 10 mcg did not show an increased risk of endometrial cancers vs. non-users.

The study concludes that Vagifem® 10 mcg has a persistently lower risk of endometrial cancer compared to Vagifem® 25 mcg, hence the results from this PASS did not give rise to new safety concerns related to endometrial cancer risks for Vagifem® 10 mcg.

14 Tables, figures and listings

Not applicable.

15 References

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Annex 1. List of stand-alone documents

Number	Document reference number	Title
1	16.1.1	Protocol and protocol amendments Summary of menopausal report
2	16.1.2	Statistical Analysis Plan

Annex 2. List of documents, available on request

Following documents were used in the study and are available on request.

Number	Title
1	Result analysis report of Danish cohort
2	Result analysis report of US cohort