Sodium-Glucose Co-Transporter 2 Inhibitors and Risk of Early Breast Cancer Among Women with Type 2 Diabetes

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ABSTRACT

Background: Sodium-glucose co-transporter 2 (SGLT-2) inhibitors are the newest class of antidiabetic drugs and have been shown to have cardiovascular benefits. However, numerical imbalances in breast cancer events were observed with certain SGLT-2 inhibitors (i.e., dapagliflozin) in premarketing trials, all occurring within one year of randomization. While this may have been a chance finding, it remains unclear whether use of SGLT-2 inhibitors, either as a class or by individual molecule, is associated with an early increased risk of breast cancer.

Objective: The objective of this thesis was to determine whether the use of SGLT-2 inhibitors is associated with an early increased risk of breast cancer among female patients with type 2 diabetes.

Methods: We used the United Kingdom Clinical Practice Research Datalink to identify a cohort of female patients newly treated with either a SGLT-2 inhibitor or a dipeptidyl peptidase 4 (DPP-4) inhibitor between January 1, 2013 and June 30, 2018, with follow-up until December 31, 2018. Cox proportional hazards models were used to estimate hazard ratios and corresponding 95% confidence intervals of breast cancer incidence, comparing new users of SGLT-2 inhibitors with new users of DPP-4 inhibitors using both intention-to-treat and as-treated approaches. The models were weighted using propensity score fine stratification.

Results: This thesis study included a total of 9,938 new users of SGLT-2 inhibitors and 36,631 new users of DPP-4 inhibitors. During a median follow-up of 2.6 years, there were 67 (2.8 per 1000 person-years) and 382 (3.7 per 1000 person-years) incident breast cancer events among SGLT-2 inhibitor and DPP-4 inhibitor users, respectively. Compared with use of DPP-4 inhibitors, use of SGLT-2 inhibitors was not associated with an overall increased risk of breast cancer in both the intention-to-treat and as-treated approaches (hazard ratio 1.00, 95% confidence interval: 0.77 to 1.30 and hazard ratio 0.92, 95% confidence interval: 0.67 to 1.26, respectively). In secondary

analyses, the hazard ratios for empagliflozin and canagliflozin were around the null value, with the exception of dapagliflozin (hazard ratio 1.16, 95% confidence interval 0.86 to 1.56) where the cumulative incidence curves diverged around 1.5 years after treatment initiation.

Conclusions: SGLT-2 inhibitors are effective antihyperglycemic drugs which offer cardiovascular benefits, but their safety with regards to cancer risk is still under investigation. The results of this study provide reassurance on the short-term safety of these drugs on breast cancer.

RÉSUMÉ

Contexte: Les inhibiteurs du cotransporteur sodium-glucose de type 2 (SGLT-2) sont une nouvelle classe de médicaments antidiabétiques et sont reconnus pour leurs avantages cardiovasculaires importants. Cependant, certains essais cliniques randomisés faits avant la commercialisation du premier inhibiteur de la SGLT-2, la dapagliflozine, ont rapporté un nombre plus élevé de cancers du sein dans les groupes exposés. Bien que cela ait pu être une découverte fortuite, on ne sait toujours pas si l'utilisation des inhibiteurs du SGLT-2, en tant que classe ou par molécule individuelle, est associée à un risque accru précoce de cancer du sein.

Objectif: L'objectif de cette thèse était de déterminer si l'utilisation des inhibiteurs du SGLT-2 est associée à une augmentation précoce du risque de cancer du sein chez les patientes atteintes de diabète de type 2.

Méthodes: Nous avons utilisé le Clinical Practice Research Datalink du Royaume-Uni pour identifier une cohorte de patientes nouvellement traitées avec un inhibiteur du SGLT-2 ou un inhibiteur de la dipeptidyl peptidase 4 (DPP-4) entre le 1er janvier 2013 et le 30 juin 2018, avec suivi jusqu'au 31 décembre 2018. Les modèles de risques proportionnels de Cox ont été utilisés pour estimer les rapports de risque de l'incidence du cancer du sein et les intervalles de confiance à 95% correspondants, en comparant les nouvelles utilisatrices d'inhibiteurs du SGLT-2 avec les nouvelles utilisatrices d'inhibiteurs de la DPP-4. Nous avons utilisé deux approches pour l'analyse primaire : *intention-to-treat* et *as-treated*. Les modèles ont été pondérés en utilisant la méthode *Propensity score fine stratification.*

Résultats: Au total, 9,938 nouvelles utilisatrices d'inhibiteurs du SGLT-2 et 36,631 nouvelles utilisatrices d'inhibiteurs de la DPP-4 ont été incluses dans l'étude. Au cours d'un suivi médian de 2,6 ans, il y a eu 67 (2,8 pour 1000 personnes-années) et 382 (3,7 pour 1000 personnes-années)

incidents de cancer du sein chez les utilisatrices des inhibiteurs du SGLT-2 et des inhibiteurs de la DPP-4, respectivement. Comparé aux inhibiteurs de la DPP-4, les inhibiteurs du SGLT-2 n'étaient pas associée à une augmentation du risque de cancer du sein dans les analyses *intention-to-treat* et *as-treated* (hazard ratio 1,00, intervalle de confiance à 95%: 0,77 à 1,30 et rapport de risque 0,92, intervalle de confiance à 95%: 0,67 à 1,26, respectivement). Dans les analyses secondaires, les rapports de risque pour l'empagliflozine et la canagliflozine se situaient autour de la valeur nulle, à l'exception de la dapagliflozine (hazard ratio 1,16, intervalle de confiance à 95% 0,86 à 1,56) où les courbes d'incidence cumulée divergeaient environ 1,5 an après le début du traitement.

Conclusions: Les inhibiteurs du SGLT-2 sont des médicaments antihyperglycémiants efficaces qui offrent des avantages cardiovasculaires, mais leurs effets cancérigènes ne sont pas encore tout-à-fait compris. Les résultats de cette étude sont rassurants par rapport au risque du cancer du sein avec ces médicaments.

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CONTRIBUTION OF AUTHORS

Melanie Suissa developed the research question with guidance from her supervisor Dr. Laurent Azoulay. Melanie Suissa drafted the thesis and corresponding manuscript. Melanie Suissa, Hui Yin and Dr. Laurent Azoulay contributed to the statistical analyses. Dr. Oriana Yu (diabetologist) and Dr. Stephanie Wong (breast cancer specialist) contributed to the methods and interpretation of results and provided expertise on the biological plausibility. All contributing authors revised the manuscript and provided input during manuscript revision for intellectual content. Dr. Azoulay acquired the data, supervised the study and is the guarantor.

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ABBREVIATIONS

AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
BMI	Body mass index
CI	Confidence interval
CPRD	Clinical Practice Research Datalink
DPP-4	Dipeptidyl peptidase 4
EMA	European Medicines Agency
FDA	Food and Drug Administration
FPG	Fasting plasma glucose
GLP-1	Glucagon-like peptide-1
HR	Hazard ratio
OGTT	Oral glucose tolerance test
RCT	Randomized controlled trial
SD	Standard deviation
SGLT-2	Sodium-glucose co-transporter 2
TZD	Thiazolidinedione
UK	United Kingdom
US	Unites States

CHAPTER 1: INTRODUCTION

Type 2 diabetes mellitus represents an important global health and economic burden. This disease is highly prevalent worldwide; its incidence has almost doubled in the last decade, affecting nearly 10% of adults in 2019.[1] Type 2 diabetes, if not treated, leads to long-term microvascular and macrovascular complications, particularly kidney failure and cardiovascular disease.[2] In addition, type 2 diabetes has been shown to be associated with an increased risk of several malignancies, such as liver, pancreas, endometrium, colorectal, bladder, and breast cancer.[3-10]

The pharmacological management of type 2 diabetes has considerably changed over the years. For centuries, the prognosis of type 2 diabetes was poor given the lack of effective treatments. The discovery of insulin in the 1920s was a medical breakthrough, and had an important impact on the prognosis of patients with type 2 diabetes.[11] This was followed by the approval of metformin (currently the recommended first-line pharmacotherapy)[12 13] in the 1970s and of sulfonylureas in the 1980s, which were the most common therapies for type 2 diabetes until about two decades ago. Since then, the pharmacological landscape of type 2 diabetes has improved further with the introduction of several effective second- to third- line treatments, such as dipeptidyl peptidase 4 (DPP-4) inhibitors, glucagon-like peptide-1 (GLP-1) receptor agonists, and sodium-glucose co-transporter 2 protein (SGLT-2) inhibitors.[12 14]

SGLT-2 inhibitors represent the newest class of antidiabetic drugs. Dapagliflozin was the first drug of this class that was approved in November 2012 in Europe, January 2014 in the United States, and December 2014 in Canada.[15 16] This was followed by canagliflozin in March 2013 and empagliflozin in May 2014.[17] SGLT-2 inhibitors effectively lower blood glucose levels by increasing the excretion of glucose in the urine.[2] Importantly, these drugs have been shown to reduce the risk of heart failure and cardiovascular mortality when compared to placebo.[18-20]

However, this drug class has been associated with some safety concerns, including hypotension, diabetic ketoacidosis, fractures, lower extremity amputation, genital infections, and certain malignancies, such bladder and breast cancer.[12]

With respect to breast cancer, the United States (US) Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have noted imbalances in breast cancer events reported in patients randomized to dapagliflozin in premarketing trials. Interestingly, all of these breast cancer events occurred within one year of randomization.[21 22] While the timing of these events is incompatible with long latency of breast cancer, these findings, in addition to an imbalance in bladder cancer events, led to the delayed approval of dapagliflozin by the US FDA in 2011.[23] In contrast, no imbalances were observed in subsequent large cardiovascular outcome trials of dapagliflozin and other SGLT-2 inhibitors.[18-20] It remains unclear whether the discrepant findings between the premarketing and postmarking trial was due to chance, potential tumor promoter effects, or possible over-detection of breast cancer.

To date, no observational studies have been conducted to assess the association between SGLT-2 inhibitors and early breast cancer. Given the increasing use of these drugs in patients with type 2 diabetes,[24] this thesis aimed at investigating this safety question in order to provide physicians, patients, and regulatory agencies with the necessary information when assessing the risks and benefits of SGLT-2 inhibitors.

CHAPTER 2: LITERATURE REVIEW

The following chapter consists of three sections. The first section provides an overview of type 2 diabetes, including its epidemiology, pathophysiology, clinical management, and its association with overall cancer incidence. The second section provides a brief overview of breast cancer and describes its association with type 2 diabetes. Finally, the third section describes what is known on the association between SGLT-2 inhibitors and breast cancer in the scientific literature.

2.1 TYPE 2 DIABETES

2.1.1 *EPIDEMIOLOGY AND RISK FACTORS OF TYPE 2 DIABETES*

Type 2 diabetes mellitus is prevalent worldwide, and its incidence has been increasing over the years. The International Diabetes Federation estimated in 2019 that 9.3% of adults aged 20 to 79 are affected diabetes, representing 463 million people worldwide; this is a drastic increase compared to 285 million in 2009.[25] Particularly, about nine out of 10 cases of diabetes are type 2 diabetes.[26 27] In 2017, type 2 diabetes was estimated to affect 7.3% and 6.6% of the Canadian and Quebec populations, respectively.[28]

The etiology of type 2 diabetes is multifactorial, resulting from an interaction between genetic and environmental factors.[27 29] On top of genetic predisposition, major risk factors include obesity, sedentary lifestyle, smoking, and nutrition.[27 30] Indeed, the majority (at least 80%) of people with type 2 diabetes are obese.[30] Type 2 diabetes is also well-known for its long-term macrovascular (coronary heart disease, peripheral vascular disease and cerebrovascular disease)

and microvascular (nephropathy, retinopathy and neuropathy) complications.[27] Type 2 diabetes and its complications lead to a reduced life span and quality of life in many patients.[27]

2.1.2 *PATHOPHYSIOLOGY OF TYPE 2 DIABETES*

Type 2 diabetes is a multifactorial disorder which affects the metabolism of insulin in the body. Insulin resistance and impaired insulin secretion play an important role in the pathogenesis of this disease.[29-31] Insulin resistance occurs when the action of insulin on target tissues such as fat, liver and particularly muscles, is reduced.[30] In early phases of the disease, beta cells in the pancreas are able to compensate for insulin resistance by producing more insulin, leading to a hyperinsulinemic state.[30] However, with time, the beta cells in some individuals are no longer able to maintain a hyperinsulinemic state, which leads to impaired insulin secretion and eventually results in sustained hyperglycemia.[30] Insulin resistance in the liver results in reduced inhibition of gluconeogenesis, which also contributes to hyperglycemia.[30]

There is a strong genetic component in the pathogenesis of type 2 diabetes. In fact, identical twins have a concordance for type 2 diabetes between 70 and 90%.[30] Individuals with two parents with diabetes have a nearly 40% risk of developing type 2 diabetes.[30] Interestingly, insulin resistance is often present in non-diabetic individuals who have family members affected by type 2 diabetes.[30] Thus, it is well-established that genetic predisposition plays an important role in the development of diabetes. However, progression to type 2 diabetes in predisposed individuals is determined by the interaction between genetic and environmental factors (such as obesity and lack of physical activity).[30]

Type 2 diabetes is diagnosed when an elevated random plasma glucose accompanies classic diabetes symptoms (polyuria, polydipsia, hyperglycemic crisis).[32] Generally, symptoms

only appear in later stages of type 2 diabetes, in which case a diagnosis can be made from two abnormal glycemic test results.[32] Prediabetes refers to individuals whose blood glucose levels are above normal values but do not meet the criteria for diabetes.[32] Glycemic criteria for diabetes diagnosis include a fasting plasma glucose ≥7.0 mmol/L, a 2-hour plasma glucose after a 75 gram oral glucose tolerance test ≥11.1 mmol/L and a hemoglobin A1C ≥ 6.5%.[32] The hemoglobin A1C test provides an "estimated average glucose" of the past three months, and a normal value is < 5.7%.[33 34] This test has become increasingly popular due to its convenience, since it does not require fasting, and stability.[32-34] Hemoglobin A1C is also used to monitor diabetes and to establish a target for patients (target A1C is generally ≤7.0% as this has been shown to reduce long-term complications of diabetes).[12]

2.1.3 *Clinical management of type 2 diabetes*

The clinical management of type 2 diabetes is evolving rapidly, with a wide selection of treatment options. Management of type 2 diabetes is patient-centered and requires a multifaceted treatment plan which includes patient education, lifestyle modifications and pharmacological treatment.[12 13] The aim of type 2 diabetes treatment is to prevent or delay complications and improve the quality of life of patients.[13]

2.1.3.1 Lifestyle measures

The first-line treatment for type 2 diabetes consists of lifestyle modifications including diet and physical activity.[12 13] These measures have been shown to be effective in preventing or delaying the onset of disease in patients presenting with prediabetes.[33] In patients with new-onset type 2 diabetes, healthy behaviours and weight loss have been shown to lead to

remission of disease in some patients (21.2% remission rate) and withdrawal of antidiabetic drugs.[35] Patients already diagnosed with diabetes can also benefit from weight loss as it can improve glycemic levels.[33] Physical activity, with or without significant weight loss, is also beneficial as it increases insulin sensitivity.[33] Other lifestyle modifications that should be considered include moderate alcohol consumption and smoking cessation.[13 33] Thus, healthy behaviours and weight loss should be encouraged in all patients presenting with prediabetes or diabetes by ensuring regular counselling and patient education.[33] Healthy behaviour interventions are considered successful if A1C levels are reduced to target within 3 months.[12] When lifestyle modifications have been attempted and proved ineffective, pharmacologic treatment is generally required, as detailed in the following section.

2.1.3.2 Pharmacological treatments

There are several classes of antidiabetic drugs on the market, which include biguanides, sulfonylureas, alpha-glucosidase inhibitors, thiazolidinediones (TZDs), incretin-based drugs, and finally, SGLT-2 inhibitors. Details of each class can be found in **Table 1**. Pharmacological treatment of patients with type 2 diabetes requires constant monitoring and adjustments, with establishment of hemoglobin A1C targets. The following section provides information on the history, mechanism of action, clinical indications, and adverse effects of each of these drug classes.

2.1.3.2.1 Biguanides

Biguanides, including metformin, phenformin and buformin, are a class of oral antidiabetic drugs initially introduced in France in the 1950s.[36 37] By the late 1970s, phenformin and buformin were withdrawn from the Canadian market because they were associated with a high risk of lactate

acidosis with a mortality rate of 40 to 50%;[37] thus, metformin, which was approved in Canada in 1972, is the only biguanide currently available on the market. It is because of this safety concern that metformin entered the US market in 1995.[36] In 1998, the efficacy and safety of metformin was established by the large UK Prospective Diabetes Study (UKPDS)-34, which demonstrated that metformin reduces the risk of diabetes-related outcomes including diabetes-related death and all-cause mortality in diet-treated overweight patients with type 2 diabetes.[38] Metformin has become widely accepted due to its high efficacy in lowering glycemic levels and its suitable safety profile.[13] This drug is recommended by clinical practice guidelines of Diabetes Canada, the American Diabetes Association and the European Association for the Study of Diabetes, as the first line medication for the treatment of type 2 diabetes in addition to lifestyle measures, alone or in combination with other antidiabetic drugs.[12 13] Lactate acidosis with metformin use is rare and has generally been reported in patients with severe illness or kidney injury.[13] Thus, metformin is strictly contraindicated in the setting of renal impairment and should not be used if glomerular filtration rate is <60mL/min.[30]

Metformin reduces blood glucose levels by decreasing hepatic glucose production and decreasing insulin resistance.[30 39] Its mechanism of action involves the activation of AMP-dependent protein kinase in the liver thus inhibiting gluconeogenesis, and activation of the insulin receptor expression which increases insulin sensitivity.[30 40] Metformin has no effect on insulin secretion and therefore does not cause hypoglycemia or weight gain when used in monotherapy.[39 40] This drug is also thought to have positive effects on the lipid profile related to cardiovascular disease.[30 40] Indeed, metformin has been suggested to reduce the risk of cardiovascular disease,[38] although a recent meta-analysis reported the contrary.[13] Nonetheless, metformin may reduce cardiovascular mortality compared to sulfonylureas.[41] Metformin is generally weight neutral but

may be associated with modest weight loss in obese individuals through incretin-like actions.[30] This drug may also be associated with gastrointestinal side effects such as nausea, diarrhea and abdominal discomfort, although these are usually transient and can be avoided by slow titration.[30 42]

2.1.3.2.2 Sulfonylureas

The hypoglycemic effects of sulfonylureas were discovered as early as 1937.[36] In 1956, the first sulfonylurea, tolbutamide, was approved in Europe, followed by other first-generation sulfonylureas.[36 39] Later in the 1980s, second-generation sulfonylureas such as glyburide and glipizide were introduced in the US, followed by a third-generation sulfonylurea, glimepiride, in 1995.[36] Sulfonylureas, which are administered orally,[30] are known as secretagogues since they stimulate pancreatic beta-cell insulin secretion by blocking potassium ATP-dependent channels, which leads to cellular depolarization thus stimulating insulin release.[30 36 39] These drugs are most effective in patients with functional beta-cells, thus among patients with relatively new-onset type 2 diabetes (<5 years) who have some endogenous insulin production.[30 39] Sulfonylureas have been shown to effectively lower A1C levels[43] and reduce microvascular complications.[44 45] However, this drug class has been associated with severe hypoglycemia and weight gain,[13 46] as well as with a possible increased risk of cardiovascular events and mortality.[47]

2.1.3.2.3 Meglitinides

Meglitinides are non-sulfonylurea insulin secretagogues of short duration.[36 30]The first meglitinide, repaglinide, was approved in 1997.[36] Meglitinides stimulate pancreatic insulin secretion through a similar mechanism as that of sulfonylureas, though they form a weaker bond

with the receptor making them short-acting and they require higher glucose levels to effectively stimulate insulin secretion.[36] Meglitinides are associated with a risk of hypoglycemia.[30] While these drugs are infrequently used for the treatment of type 2 diabetes,[13] they may be useful in patients who experience delayed postprandial hypoglycemia while on sulfonylureas.[33]

2.1.3.2.4 Alpha glucosidase inhibitors

Alpha glucosidase inhibitors are a class of antidiabetic drugs that are not commonly used for the treatment of type 2 diabetes.[13] The first of this class of drugs, acarbose, was approved by the US FDA in 1995 followed by miglitol in 1996.[36] These drugs act by inhibiting the alpha-glucosidase enzyme in the membrane of the small intestine, which delays glucose absorption thus lowering postprandial hyperglycemia.[30 36] These drugs have a very modest effect on hemoglobin A1C levels (lowers by 0.5%) and are associated with multiple gastro-intestinal side effects.[36]

2.1.3.2.5 Thiazolidinediones

TZDs are a class of oral antidiabetic drugs discovered in Japan in the 1980s.[36] The first molecule of this class, troglitazone, was approved in 1997 but removed from the market three years later due to its association with liver damage.[36] Rosiglitazone and pioglitazone were approved in 1999 and became increasingly popular in the treatment of type 2 diabetes over the next 10 years.[36] These drugs decrease insulin resistance by binding to the peroxisome proliferator-activated receptors (PPARs) nuclear receptor and stimulating glucose uptake by muscles, liver and mainly fat cells.[30] As agonists of the PPAR receptor, TZDs promote adipocyte differentiation, body fat redistributions from central to peripheral areas and fatty acid storage, and reduce the accumulation of fat in the liver.[30] These drugs are highly effective at lowering glucose levels and reducing

hemoglobin A1C levels.[28 29] However, the safety of TZDs has become a contentious issue over the years. Rosiglitazone has been shown to be associated with adverse cardiovascular events,[48 49] which led to the restriction of its use by the US FDA and Health Canada.[36 50] Pioglitazone has been shown to reduce cardiovascular outcomes[51 52] and steatohepatitis,[53] but was found to be associated with bladder cancer.[54 55] There are also concerns with these drugs regarding heart failure,[56] weight gain[51 52] and bone fracture.[57 58] Thus, the use of TZDs has fallen out of favor in recent years.

2.1.3.2.6 Incretin-based drugs

Incretin-based drugs are a newer group of antidiabetic drugs introduced in 2005.[36] This group of drugs consists of two classes: GLP-1 receptor agonists, administered by subcutaneous injections, and DPP-4 inhibitors, administered orally.[13 30 36] GLP-1 is a hormone secreted by the small intestine within minutes after ingesting carbohydrate- or fat- containing foods.[36] The human GLP-1 hormone possesses natural antidiabetic properties; it stimulates glucose-dependent insulin secretion and insulin synthesis by pancreatic beta-cells, suppresses glucagon production and delays gastric emptying thereby suppressing appetite.[36 59] It also reduces beta-cell apoptosis and stimulates beta-cell differentiation.[59] However, this hormone is rapidly degraded and inactivated by the DPP-4 enzyme.[36 59 60]

GLP-1 receptor agonists, such as exenatide and liraglutide, are structurally similar to the endogenous GLP-1 hormone, but are more resistant to DPP-4 degradation and thus have a longer half-life.[36 59] These drugs are able to bind to GLP-1 receptors and replicate the exact antidiabetic properties of GLP-1 for longer periods.[59] DPP-4 inhibitors, including alogliptin, linagliptin, saxagliptin, sitagliptin and vildagliptin, are able inhibit the DPP-4 enzyme to reduce degradation and inactivation of GLP-1, which enables the antidiabetic action of GLP-1.[59] Incretin-based drugs,

when used in monotherapy, are not associated with a risk of hypoglycemia due to the highly glucose-dependant mechanism of GLP-1.[33 59] These drugs also do not cause weight gain; in fact, GLP-1 receptor agonists have been found to induce weight loss, while DPP-4 inhibitors have been found to be weight neutral.[13 36 59] Common adverse effects of incretin based drugs include nausea, vomiting and diarrhea, although these tend to resolve over time.[13] Finally, contrary to initial concerns, GLP-1 receptor agonists have not been found to be associated with an increased risk of acute pancreatitis.[61 62] However, an association between DPP-4 inhibitors and this adverse event remains controversial.[62 63]

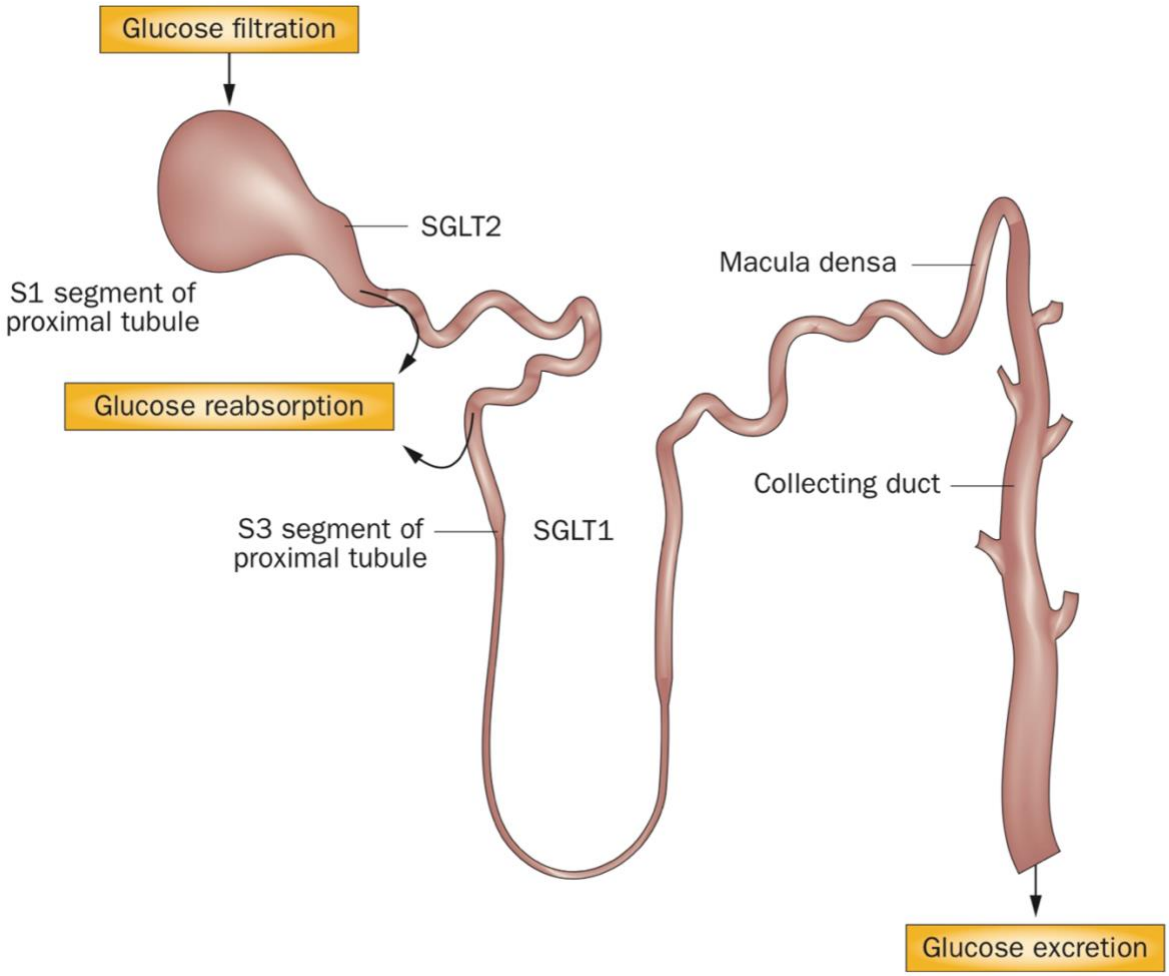
2.1.3.2.7 SGLT-2 inhibitors

SGLT-2 inhibitors are a new class of antihyperglycemic drugs used for the treatment of type 2 diabetes. Dapagliflozin was the first approved SGLT-2 inhibitor in Europe in November 2012, followed by canagliflozin approved in the US in March 2013, followed by empagliflozin in May 2014.[15 17] Current guidelines recommend SGLT-2 inhibitors as second line therapy for patients with type 2 diabetes, either in monotherapy or in combination with other types of antidiabetic drugs.[12 13 64]

The mechanism of action of SGLT-2 inhibitors is illustrated in **Figure 1**. The SGLT-2 proteins are responsible for the majority of glucose reabsorption by the kidneys, a key component in maintaining glucose hemostasis.[2] These proteins ensure the uptake of glucose across the apical membrane of proximal renal tubules, which is followed by the transportation of intracellular glucose across the basolateral membrane by basolateral glucose transporter 2 (GLUT2).[65] SGLT-2 inhibitors inhibit sodium-glucose co-transporter 2 proteins in the kidneys, thereby enabling the excretion of glucose in the urine (glycosuria) and the lowering of blood glucose levels.[2] These

drugs effectively reduce A1C levels by 0.5-0.8%, induce weight loss and lower blood pressure.[17 66] Importantly, they have been shown to significantly reduce the risk of cardiovascular disease related outcomes including death.[14] However, there are concerns that their use may increase the risk of several important adverse events, including diabetic ketoacidosis,[67] genital infections,[68] lower extremity amputations,[69] and certain malignancies such as bladder and breast cancer.[18 66 70] The latter will be discussed in detail in the subsequent section.

FIGURE 1. Mechanism of action of SGLT-2 inhibitors. Representation of distribution of sodium-glucose co-transporter 2 proteins along the nephron



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2.1.3.2.8 Insulin

The discovery of human insulin therapies in the 1920s was both a medical and scientific breakthrough.[11] However, conventional insulins had some limitations; their slow absorption and short duration of action lead to late postprandial hypoglycemia and nocturnal hypoglycemia.[72] It was not until the 1990s that insulin analogues, which better mimic the natural secretion of insulin, were developed to reduce the incidence of hypoglycemia.[11] Lispro was the first short-acting insulin analogue to be approved by the US FDA in 1993, followed by the first basal insulin analogue, glargine, approved in 2000.[11] Insulin therapy is indicated when non-insulin drugs fail to maintain glycemic control, in individuals with renal or hepatic disease preventing the use of other antidiabetic drugs, in patients with severe hyperglycemia, or during hospitalization or surgery.[30 39] Ultimately, many patients with type 2 diabetes require insulin therapy due to eventual insulin deficiency that develops in late stages of the disease.[30] The advantage of insulin over other antidiabetic drugs is that it reduces glucose in a dose-dependent manner making it easier to reach a glycemic target, though limited by an increased risk hypoglycemia.[13] Insulin therapy has also been shown to significantly reduce the risk of microvascular complications.[44] Other than hypoglycemia, disadvantages of insulin therapy include weight gain and the inconvenience of frequent injections, titrations and monitoring.[73]

Overall, there is a wide variety of pharmacological therapies available for the treatment of type 2 diabetes. Each class of drugs is associated with a number of advantages and disadvantages (see **Table 1** below for a summary). Thus, treatment regiments and glycemic targets are specific to each individual and require constant monitoring and adjustment.

TABLE 1. Types of antidiabetic drugs and their characteristics, % reduction in hemoglobin A1C, advantages and disadvantages

Class	Route	Mechanism of action	Hemoglobin A1C (%) reduction	Advantages	Disadvantages
Biguanides	Oral	Decrease insulin resistance	1-2	Weight neutral, do not cause hypoglycemia	Diarrhea, nausea, lactic acidosis
Sulfonylureas	Oral	Increase insulin secretion	1-2	Effective in lowering glycemia and A1C levels	Weight gain, hypoglycemia, CV mortality
Meglitinides	Oral	Increase insulin secretion	1-2	Similar to sulfonylureas	Hypoglycemia
Alpha-glucosidase inhibitors	Oral	Decrease glucose absorption	0.5	Modest A1C reduction	Multiple GI side effects
TZDs	Oral	Decrease insulin resistance	0.5-1.4	Effective	Weight gain, fractures, CV mortality
GLP-1 agonists	Injectable	Increase insulin secretion (glucose dependent)	0.5-1.0	Weight loss, do not cause hypoglycemia	GI side effects
DPP-4 inhibitors	Oral	Increase insulin secretion (glucose dependent)	0.5-0.8	Weight neutral, do not cause hypoglycemia	Pancreatitis controversial
SGLT-2 inhibitors	Oral	Increase urinary glucose excretion	0.5-0.8	Weight loss, significantly reduce CV mortality	DKA, genital infections, amputations, cancers
Insulin therapy	Injectable	Increase insulin levels (dose dependent)	No limit	Highly effective	Weight gain, hypoglycemia

Abbreviations: CV, cardiovascular; DKA, diabetes ketoacidosis; DPP-4, dipeptidyl peptidase-4; GI, gastrointestinal; GLP-1, glucagon-like peptide-1; SGLT-2, sodium-glucose co-transporter 2

2.1.4 Association between type 2 diabetes and cancer incidence

Diabetes and cancer are both prevalent diseases and represent growing burdens worldwide. For over half a century, physicians have reported patients presenting with both type 2 diabetes and cancer concurrently.[5] In the 1960s, a formal association between type 2 diabetes and cancer incidence was first reported in population-based studies.[5] Over the years, several observational studies have reported associations between type 2 diabetes and several malignancies, including liver, pancreas, endometrium, colon, rectum, bladder and breast cancer.[3-10] Conversely, the risk of prostate cancer has been found to be reduced in patients with type 2 diabetes.[74 75]

The relationship between diabetes and cancer is not completely understood, although it is likely related to insulin. Insulin is a mild growth factor which promotes cellular differentiation, slows apoptosis and mediates cellular response to other more important growth factors (such as insulin-like growth factors).[4 76] In the presence of insulin resistance, high levels of insulin can significantly increase the mitogenic response to important growth factors and promote tumor proliferation.[76] In addition, some studies have indicated that the content of insulin receptors is increased in some tumors (breast and colorectal cancers).[77 78] The following section will focus on breast cancer, which is the outcome of interest of this thesis.

2.2 BREAST CANCER

2.2.1 EPIDEMIOLOGY AND RISK FACTORS

Breast cancer is the most common female malignancy and represents an important health burden globally, accounting for one million new cancer cases each year.[79] Breast cancer is the leading cause of death among women aged 40 to 50 years.[79] It is the most prominent cause of cancer-related mortality among women of all ages, accounting for about 411,000 deaths each year.[79 80] In Canada, it is predicted that 27,400 women will be diagnosed with breast cancer in 2020 and 5,100 will die from the disease.[81] Finally, an estimated 1 in 8 Canadian women will develop breast cancer over their lifetime.[80 81]

Estrogen-dependent breast cancer is the most common type of this malignancy, and thus its incidence is directly associated with lifetime estrogen exposure.[82] Accordingly, it has been shown that age at menarche, age at first pregnancy, and age at menopause are the most influential breast cancer risk factors.[82] Indeed, women who commence menarche at age 12 have almost double the breast cancer risk than women who have their menarche at age 16.[82] Similarly, while early menopause reduces the risk of breast cancer, women who experience menopause at later ages have greater breast cancer risk.[82] Nulliparity and late age at first full-term pregnancy also increase the lifetime risk of developing breast cancer.[82] Finally, exposure to exogenous hormones such as oral contraceptives and hormone replacement therapy can lead to a small increase breast cancer risk.[79 82]

Age and geographic location are also important risk factors of breast cancer. Indeed, the incidence of breast cancer increases with age, doubling every 10 years until menopause.[79] Additionally, the risk of breast cancer is between five to 10 times greater in women from developed countries such as North America or Western Europe compared to women from Asia.[79 82] This

difference is most likely due to environmental factors since migrants from Asia have been found to develop identical breast cancer rates as the country they migrated to within one or two generations.[79 82] Other established risk factors include family history of breast cancer in first degree relative, previous benign atypical hyperplasia of the breast, previous history of breast cancer, excessive alcohol consumption and exposure to ionising radiation before age 30.[79 82]

2.2.2 Association between type 2 diabetes and breast cancer

The association between type 2 diabetes and breast cancer is well-established. Several observational studies and meta-analyses have found that type 2 diabetes is associated with an increased risk of developing breast cancer.[5 83 84] A meta-analysis combining 40 observational studies reported a 27% increased risk (summary relative risk: 1.27, 95% confidence interval (CI): 1.16 to 1.39) of breast cancer in women with type 2 diabetes.[83] In studies that adjusted for body mass index, there was a more modest increased risk of 16% (summary relative risk: 1.16, 95% CI: 1.08 to 1.24).[83]

The mechanism explaining this association is not completely understood, although it is believed to be related to the hyperinsulinemic state in individuals with type 2 diabetes.[83] First, insulin has been found to have a direct mitogenic effect on breast tissue.[84] Second, insulin has a lowering effect on sex hormone binding globulin levels resulting in higher levels of circulating estrogen and other hormones, which could be involved in the development of hormone-dependent breast cancers.[83] Similarly, obesity, which is associated with type 2 diabetes, causes an increase in circulating endogenous estrogen.[83] Finally, hyperinsulinemia also has joint effects with insulin-like growth factor 1 which may play a role in breast carcinogenesis.[83]

2.3 SGLT2 INHIBITORS AND BREAST CANCER

To date, there is limited data on the long-term safety of SGLT2 inhibitors, particularly with respect to their association with cancer incidence. While the available evidence has been documented in several randomized controlled trials (RCTs), observational studies investigating the carcinogenicity of this drug class are current lacking. This section will review what is known on the association between SGLT-2 inhibitors and breast cancer in the scientific literature.

2.3.1 Safety reviews: United States Food and Drug Administration and European Medicines Agency

The US FDA and the EMA are regulatory agencies who review data from premarketing RCTs to asses drug safety before market release. Dapagliflozin was the first molecule of the SGLT-2 inhibitor class to be reviewed by the FDA and EMA, followed by canagliflozin and empagliflozin. While canagliflozin and empagliflozin were not found to have any effect of breast cancer in premarketing trials,[85 86] a signal was observed with dapagliflozin. Overall, there were 21 phase 2b and 3 premarketing trials that assessed dapagliflozin safety. The US FDA and EMA reviews of these trials noted some differences between exposed and control groups with regards to malignancies. In addition to a discrepancy in bladder cancer events, they noted imbalances in breast cancer outcomes between the randomized groups.[70 87-89]

Overall, there was a total of 12/2693 (0.45%) versus 3/1439 (0.21%) breast cancer events among patients randomized to dapagliflozin versus placebo, generating incidence rates of 40.0 versus 19.0 per 10,000 person-years, respectively (rate ratio 2.47, 95% confidence interval (CI) 0.64 to 14.10).[21 70 88] It should be noted that the initial estimated rate ratio in the 2011 US FDA Report was almost double that of the updates 2013 report (4.41, 95% CI 0.57 to 200.86); the rate

was reduced by the time the drug application was re-submitted in 2013 as a result of additional cases in the control arm.[70 88] However, these RCTs were all of short durations, with follow-up periods ranging between 24 and 208 weeks.[70] Interestingly, 10 out of the 12 breast cancer events occurring in the dapagliflozin group were diagnosed within one year of randomization, two of which occurred within 30 days of drug initiation, which is not consistent with a temporal association.[70 88] In addition, nine of the 12 cases were hormone receptor positive and patients were generally obese, Caucasian women, over the age of 50. Also, only three of the cases has routine screening as there was no mammographic screening before study entry.[70]

2.3.2 POST-MARKETING RANDOMIZED CONTROLLED TRIALS

Post-marketing RCTs are conducted after a drug has been released on the market and generally assess drug efficacy and safety. Since the approval of dapagliflozin and other SGLT-2 inhibitors thereafter, three post-marketing trials have been conducted to evaluate cardiovascular outcomes in type 2 diabetes with the use of these drugs. These include Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients (EMPA-REG OUTCOME), Canagliflozin Cardiovascular Assessment Study (CANVAS), and Multicenter Trial to Evaluate the Effects of Dapagliflozin on the Incidence of Cardiovascular Events (DECLARE-TIMI58).[18-20]

The EMPA-REG OUTCOME study was a large trial designed to assess cardiovascular safety among patients with type 2 diabetes using empagliflozin.[18] This randomized, double-blind, placebo-controlled trial assessed once daily empagliflozin (either 10 mg or 25 mg) compared with placebo in a total 7020 patients from 42 countries. Patients were randomized according to a 1:1:1 ratio to receive either 10 mg or 25 mg empagliflozin or placebo and were followed for a median

time of 3.1 years. The primary endpoint was a composite of cardiovascular death, nonfatal myocardial infarction and nonfatal stroke, which occurred in 490/4687 (10.5%) empagliflozin users and in 282/2333 (12.1%) placebo users. The hazard ratio (HR: 0.86, 95.02% CI: 0.74 to 0.99) revealed a 14% risk reduction in the primary endpoint in empagliflozin users. In this large RCT, no imbalances in breast cancer events were observed with empagliflozin versus placebo (7/4687 vs 3/2333, respectively).[18 90]

The CANVAS trial assessed cardiovascular outcomes among patients with type 2 diabetes using canagliflozin.[19] This study was a randomized, single-blind, placebo-controlled trial of a total of 10,142 participants from 30 countries. Patients were randomized in a 1:1:1 ratio to receive either 300 mg canagliflozin, 100 mg canagliflozin or placebo, and were followed for a median time of 126.1 weeks. The primary endpoint (composite of cardiovascular death, nonfatal myocardial infarction and nonfatal stroke) occurred at a significantly lower rate among the canagliflozin group than the placebo group (26.9 versus 31.5 per 1000 patient-years, respectively), with an observed hazard ratio (HR: 0.86, 95% CI: 0.75 to 0.97) showing a risk reduction of 14%. In assessing other safety outcomes, the rates of cancers (renal cell, bladder) were similar in the exposed and unexposed groups. Specifically, there were no important imbalances of breast cancer events with canagliflozin versus placebo (3.1 versus 2.6 events per 1000 patient-years, respectively).

The DECLARE-TIMI 58 study was a randomized, double-blind, multinational, placebo-controlled, phase 3 trial designed to assess cardiovascular safety among patients with type 2 diabetes using dapagliflozin.[20] This trial evaluated a total of 17,160 patients which were assigned in a 1:1 ratio to receive either 10 mg of dapagliflozin daily or placebo. The median follow-up time was 4.2 years. The primary endpoint was MACE (defined as cardiovascular death, myocardial infarction or ischemic stroke), for which dapagliflozin met the criterion for noninferiority. The

MACE endpoint occurred in 756/8582 (8.8%) dapagliflozin users and in 803/8578 (9.4%) placebo users (HR: 0.93, 95% CI: 0.84 to 1.03). Evaluation of other safety outcomes revealed no imbalances of breast cancer events with dapagliflozin versus placebo (36/8574 vs 35/8569 events, respectively).[20]

2.3.3 BIOLOGICAL PLAUSIBILITY

The discrepant findings between premarketing and post-marketing trials on breast cancer events may have been due to chance. Indeed, there is no clear biological mechanism to explain a potential association with increased breast cancer incidence. There are two hypotheses that can explain the discrepant findings. First, SGLT-2 inhibitors are known to induce weight loss, which could facilitate the detection of pre-existing breast lumps, leading to an early diagnosis of breast cancer relatively soon after treatment initiation.[12] Indeed, lower body mass index may be associated with greater tumour palpability.[91] This mechanism was recently observed with GLP-1 receptor agonists, where rapid and significant weight loss was associated with an increased detection of breast cancer.[92] This could explain the occurrence of the majority of cases within one year of drug initiation, including events diagnosed within one month of randomization. A second hypothesis involves a tumour promoting effect, in which carcinogenesis would occur by an accelerated process. However, SGLT-2 proteins are not expressed in mammary tissue and animal studies have failed to demonstrate that SGLT-2 inhibitors have any neoplastic activity.[93] Likewise, a recent experimental study even showed that the SGLT-2 inhibitor, ipragliflozin, induces apoptosis in breast cancer cells via membrane hyperpolarization and mitochondrial dysfunction.[94] It remains unclear whether these *in vitro* findings translate to a decreased risk of breast cancer in humans.[93]

2.3.4 KNOWLEDGE GAPS

In summary, the available evidence on the association between SGLT-2 inhibitors and breast cancer is limited. Signals of a possible association between SGLT-2 inhibitors and breast cancer were first observed in premarketing RCTs of dapagliflozin, all occurring within one year of randomization. However, no imbalances in breast cancer events were observed in subsequent large cardiovascular outcome trials, although these studies were not designed to assess breast cancer as a safety outcome.

To date, no observational studies have been conducted to evaluate the association between SGLT-2 inhibitors and the risk of breast cancer in the real-world setting. Given their increasing use in patients with type 2 diabetes,[24] and concerns regarding their potential carcinogenicity,[21 70 88] there is a need to assess whether this increasingly popular class of drugs is associated with an increased risk of breast cancer. To fill this gap in knowledge, this thesis assessed whether SGLT-2 inhibitors are associated with an early increased risk of breast cancer. The following chapters describe the objectives, methodology, and findings of this research work.

CHAPTER 3: OBJECTIVES AND HYPOTHESES

3.1 PRIMARY OBJECTIVE

The primary objective of this thesis is to determine whether the use of SGLT-2 inhibitors, when compared with use of DPP-4 inhibitors, is associated with an increased risk of early breast cancer among women with type 2 diabetes.

3.1.1 SECONDARY OBJECTIVES

This thesis has four secondary objectives:

1) To determine whether the association varies according to type of SGLT-2 inhibitor (dapagliflozin, empagliflozin, canagliflozin);
2) To determine whether there is a duration-response relationship in terms of cumulative duration of use and time since initiation;
3) To determine whether there is effect measure modification by obesity (<30 kg/m2, ≥30 kg/m2)
4) To determine whether there is effect measure modification by the mammography screening age limit in the UK breast cancer screening programme (below and above 70 years of age)

3.2 HYPOTHESIS

The primary hypothesis is that there is no association between use of SGLT-2 inhibitor and breast cancer, when compared with DPP-4 inhibitors among women with type 2 diabetes.

3.2.1 SECONDARY HYPOTHESES

1) The association does not vary according to type of SGLT-2 inhibitor.
2) There is no duration-response relationship in terms of cumulative duration of use and time since initiation.
3) There is no effect measure modification by obesity.
4) There is no effect measure modification by mammography screening age limit.

CHAPTER 4: METHODOLOGY

The methodology used in this thesis is described in the manuscript found in Chapter 5. However, this chapter will provide greater details on certain aspects that were not covered in the manuscript because of space constraints. Specifically, it will provide additional information on the data source, descriptions of the study cohort, the follow-up period, and covariates included in the propensity score model, along with an illustrative figure and table.

4.1 DATA SOURCE

In the UK, over 98% of the population is registered with a general practitioner (GP), and visits are free-of-charge as per the National Health Service (NHS).[95] GPs are known as the gatekeepers of the healthcare system in the UK, as they are at the very center of care for all patients. They are the first point of contact for all non-emergency medical visits which are then either managed in primary care or referred to secondary care as necessary.[95] When patients are referred to secondary care, specialists communicate diagnoses and treatment plans with the GP.[95] Each patient has a distinctive NHS number and patient data are routinely recorded by healthcare professionals.[95]

The Clinical Practice Research Datalink (CPRD) is one of the largest ongoing primary care databases worldwide.[96] Patients included in the CPRD have been shown to be representative of the general UK population in terms of age, sex and ethnicity.[95] The CPRD has been generating primary care data for over 30 years under the GOLD dataset. In 2017, CPRD Aurum was launched which captures data on over 19 million patients, representing 13% of the population of England.[97] Together, CPRD GOLD and Aurum include approximately 1800 primary care practices and over 50 million patients.[96] The CPRD collects electronic health records on a monthly basis from

participating GP practices, and all patients registered with participating practices are included in the dataset unless they individually opt out.[95] The data recorded includes diagnoses, symptoms, prescriptions, referrals and tests.[97] The CPRD database also records information on demographics, lifestyle measures (e.g., smoking, alcohol consumption), anthropometric measures (e.g., body mass index), and laboratory tests (e.g., HbA1c), providing a wide variety of covariates that can be used in observational studies.[95] Prescriptions are recorded using the British National Formulary product dictionary.[95 98] Therefore, the CPRD has access to a wide variety of patient data and has thus become the largest longitudinal primary care database in the world.

The CPRD records diagnoses and procedures using the Read code classification system. Read codes are a hierarchical clinical classification system consisting of over 96,000 codes.[95] The classification system consists of five character alphanumeric codes, with each level of the code having the possibility to be lower or upper case.[98] With 58 available characters, there is a maximum possibility of over 650 million available codes.[98] The Read code classification system is designed to be logical, hierarchical, computerised and dynamic and is cross-referenced to standard classifications.[98] Specifically, some classifications included and cross-referenced in the Read code system are the International Classification of Diseases, Ninth Revision(ICD 9), the Office of Population, Censuses and Surveys' Classification of Surgical Operations and Procedures (OPCS), the physicians' current procedural terminology (CPT-4), the British National Formulary and the OPCS classification of occupations.[98]

Diagnoses recorded in the CPRD have been shown to be of high validity and quality.[99 100] Indeed, in a meta-analysis of 212 publications reporting on a total of 357 validation analyses, 89% of cases with a computerised diagnosis were confirmed with medical records.[99] Importantly,

diagnoses of breast cancer have been shown to be well-recorded in the CPRD compared to the UK National Cancer Data Repository, with a positive predictive value of 90%.[101 102] Additionally, diabetes recording in the database has a high sensitivity exceeding 90%.[103] Finally, it was estimated that over 95% of prescriptions written by GPs are recorded in the CPRD.[103]

Due to its high validity, the CPRD has been used in several observational studies to investigate the effect of antidiabetic drugs on certain malignancies.[55 104-106] The CPRD has also been used to assess breast cancer as a safety outcome with the use of different drugs.[106-108] Thus, the use of CPRD as a data source was believed to be an appropriate choice to investigate our study question.

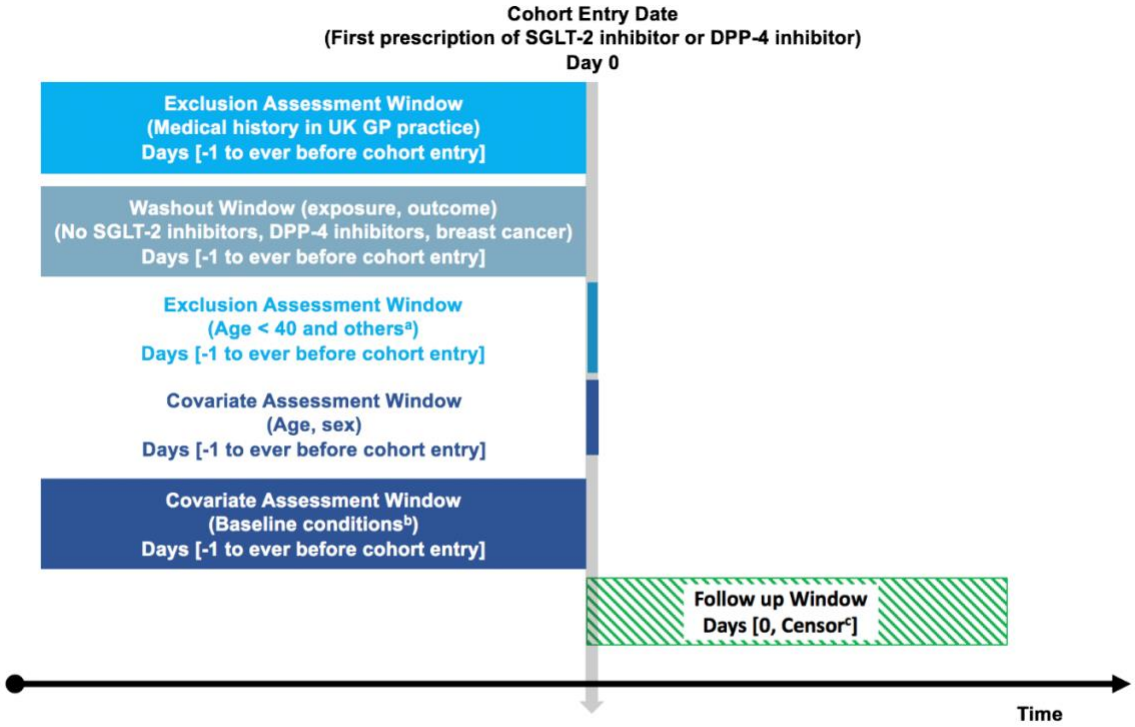
The study protocol was approved by the Independent Scientific Advisory Committee of the CPRD (protocol no. 19_272) and by the Research Ethics Research Board of the Jewish General Hospital, Montreal, Canada.

4.2 STUDY COHORT

The study cohort included all female patients newly treated with either an SGLT-2 inhibitor (dapagliflozin, canagliflozin, empagliflozin) or a DPP-4 inhibitor (sitagliptin, saxagliptin, linagliptin, alogliptin) between January 1, 2013 (the year the first SGLT-2 inhibitor, dapagliflozin, entered the UK market) and June 30, 2018. Cohort entry was defined as the date of the first prescription of either drug class during the study period. A graphical illustration of the cohort construction is presented in **Figure 2**. This representation shows that all exclusion assessment, washout window assessment and covariate assessment were measured before cohort entry. All patients were required to have at least one year of medical history in the CPRD before cohort entry, which is illustrated in the exclusion assessment window. To ensure a cohort of new users of SGLT-

2 inhibitors and DPP-4 inhibitors, patients who were previously prescribed these drugs were excluded, using a one-year washout window. In addition, patients with a previous diagnosis of breast cancer (Read codes provided in **Table 2**) any time prior to cohort entry were excluded. Additional exclusion criteria included age below 40 (as type 2 diabetes is mainly diagnosed after this age) and prior end-stage renal disease or dialysis (as these are contraindications to receiving SGLT-2 inhibitors).

FIGURE 2. Study cohort of female patients newly treated with SGLT-2 inhibitors and DPP-4 inhibitors



Abbreviations; UK: United Kingdom; GP: general physician; SGLT-2: sodium-glucose co-transporter 2; DPP-4: dipeptidyl-peptidase 4.

a. Other exclusion criteria included prior end-stage renal disease or dialysis
b. Baseline conditions are listed in the description of the propensity score model
c. Earliest of: incident diagnosis of breast cancer, death from any cause, or end of study period (December 31, 2018)

4.3 FOLLOW-UP PERIOD AND OUTCOME DEFINITION

As described in the manuscript, SGLT-2 inhibitor and DPP-4 inhibitor users were followed using two different approaches. In the primary approach, we used an intention-to-treat exposure definition in which patients were followed from cohort entry until an incident diagnosis of breast cancer, death from any cause, or end of study period (December 31, 2018), whichever occurred first. This approach assumed an irreversible effect of the exposure on the outcome, in which the risk of developing breast cancer remained even after treatment discontinuation. In a secondary approach, we used an as-treated exposure definition in which patients were followed while continuously exposed from cohort entry until treatment discontinuation, an incident diagnosis of breast cancer, death from any cause, or end of study period (December 31, 2018), whichever occurred first. Patients were considered continuously exposed if one prescription overlapped the date of the next prescription, using a one-year grace period in the event of non-overlapping prescriptions. The one-year grace period was chosen to account for possible diagnostic delays associated with a diagnosis of breast cancer. This approach assumed a reversible effect of the exposure on the outcome (i.e., the risk of developing breast cancer decreases after treatment discontinuation).

We identified incident breast cancer cases based on Read codes, which are used to record diagnoses in the CPRD (found in **Table 2)**. Diagnoses of breast cancer have been shown to be well-recorded in the CPRD with a positive predictive value of 90%, when compared with the UK National Cancer Data Repository.[101 102] In addition, the completeness of case recordings for breast cancer has been shown to be around 98%.[101]

TABLE 2. Breast cancer Read codes

Read term	Med Code	Read Code
[X]Other carcinoma in situ of breast	53803	ByuFG00
Benign neoplasm of breast	5411	B77..00
Benign neoplasm of female breast	19138	B770.00
Benign neoplasm of breast NOS	28870	B77z.00
Carcinoma in situ of skin of breast	8647	B825000
Carcinoma in situ of breast and genitourinary system	45681	B83..00
Carcinoma in situ of breast	7833	B830.00
Lobular carcinoma in situ of breast	10387	B830000
Intraductal carcinoma in situ of breast	18694	B830100
Neoplasm of uncertain behaviour of breast	41232	B933.00
Neoplasm of unspecified nature of breast	45906	BA03.00
Unspecified lump in breast		N63

4.4 PROPENSITY SCORE FINE STRATIFICATION

We used the propensity score fine stratification model to control for confounding. This approach is well suited for exposures with a relatively low prevalence of use (such as newly marketed drugs like SGLT-2 inhibitors).[109] This approach consists of creating strata using the propensity score distribution of the exposed patients. This design ensures that all exposed patients are assigned to a given stratum, which is key to minimizing loss of information while maximizing covariate balance between the exposure groups.[109] For this thesis, we created 50 strata based on the propensity score distribution of the SGLT-2 inhibitor users. All patients in the SGLT-2 inhibitor group were assigned a weight of one, while the DPP-4 inhibitor users were reweighed according of number of SGLT-2 inhibitor users in their stratum. The weight is calculated as such: (number of SGLT-2 inhibitors in PS stratum i/total number of SGLT-2 inhibitor users) / (number of DPP-4 inhibitor users in stratum i/total number of DPP-4 inhibitor users).[109] Thus, this approach does not use propensity scores directly to generate the weights; instead the weights are calculated based on stratum membership.[109] Therefore, this procedure is less sensitive to positivity violations (i.e., extreme weights due to propensity scores close to 0 or 1), which is often an issue in situations where the exposure has a relatively low prevalence of use.[110] As the weighting is performed on the comparator group only (i.e., DPP-4 inhibitors), the estimand generated by this approach is the average treatment effect among the treated population (ATT).[109]

4.5 CONFOUNDERS

The propensity score model considered 36 potential confounders. The following confounders were measured at or before cohort entry: age, body mass index (<30 kg/m$_2$, ≥30

kg/m2, unknown), alcohol-related disorders (alcoholism, cirrhosis, alcoholic hepatitis, hepatic failure), and smoking status (ever, never, unknown).

We included diabetes-related variables as proxies of disease severity, such as duration of diabetes, hemoglobin A1c (last measure before cohort entry), microvascular complications (nephropathy, neuropathy, retinopathy) and macrovascular complications (peripheral vascular disease, myocardial infarction, ischemic stroke). We also adjusted for previous use of antidiabetic drugs used at any time before cohort entry, including metformin, sulfonylureas, thiazolidinedione, meglitinides, alpha-glucosidase inhibitors, glucagon-like peptide-1 receptor agonists and insulin use (these were entered as mutually non-exclusive variables in the propensity score model).

In addition, we considered the use hypertensive drugs, such as beta blockers, calcium channel blockers, angiotensin converting enzyme inhibitors, angiotensin receptor blockers and diuretics, as there is some evidence these drugs may be associated with breast cancer (these were measured at any time before cohort entry and as mutually non-exclusive variables).[111] We also considered variables potentially associated with the incidence of breast cancer, including oophorectomy, cancer (other than non-melanoma skin cancer), congestive heart failure, coronary artery disease, chronic kidney disease, as well as ever use of other commonly prescribed drugs such as nonsteroidal anti-inflammatory drugs, antiplatelets (aspirin, clopidogrel, ticagrelor, prasugrel), statins, hormone replacement therapy and oral anticoagulants (warfarin, heparin). Finally, we included mammographic screening history in the year before cohort entry as measure of screening behavior. **Table 3** provides a summary of these covariates along with their corresponding definition, diagnostic and product classification system, and covariate assessment period.

TABLE 3. Covariate definitions and assessment periods

Covariate	Definition	Code classification system	Covariate assessment period	Variable type
Demographic/lifestyle variables				
Age	Cohort entry year minus year of birth	-	Cohort entry	Continuous
Body mass index	Weight (kg)/height (m^2)	-	Latest recorded	Categorical
Smoking status	Never, ever, unknown	-	Last recorded	Categorical
Alcohol-related disorders	Present/absent	Read codes	Ever before cohort entry	Binary
Diabetes-related variables				
Duration of diabetes	Cohort entry – (first of either a diagnosis of type 2 diabetes, antidiabetic drug prescription, hemoglobin A1c > 6.5 %)	Read codes	Ever before cohort entry	Continuous
Haemoglobin A1c	≤ 7 %, 7.1-8.0%, > 8.0%	Read codes	Ever before cohort entry	Categorical
Nephropathy	Present/absent	Read codes	Ever before cohort entry	Binary
Neuropathy	Present/absent	Read codes	Ever before cohort entry	Binary
Retinopathy	Present/absent	Read codes	Ever before cohort entry	Binary
Peripheral vascular disease	Present/absent	Read codes	Ever before cohort entry	Binary
Myocardial infarction	Present/absent	Read codes	Ever before cohort entry	Binary
Stroke	Present/absent	Read codes	Ever before cohort entry	Binary
Antidiabetic drugs				
Metformin	Present/absent	Read codes	Ever before cohort entry	Binary
Sulfonylureas	Present/absent	Read codes	Ever before cohort entry	Binary
Thiazolidinediones	Present/absent	Read codes	Ever before cohort entry	Binary
Meglitinides	Present/absent	Read codes	Ever before cohort entry	Binary
Alpha-glucosidase inhibitors	Present/absent	Read codes	Ever before cohort entry	Binary
GLP-1 receptor agonists	Present/absent	Read codes	Ever before cohort entry	Binary

Covariate	Definition	Code classification system	Covariate assessment period	Variable type
Insulin	Present/absent	Read codes	Ever before cohort entry	Binary
Breast cancer-related variables				
Oophorectomy	Present/absent	Read codes	Ever before cohort entry	Binary
Cancer	Present/absent	Read codes	Ever before cohort entry	Binary
Congestive heart failure	Present/absent	Read codes	Ever before cohort entry	Binary
Coronary artery disease	Present/absent	Read codes	Ever before cohort entry	Binary
Chronic kidney disease	Present/absent	Read codes	Ever before cohort entry	Binary
Antihypertensive drugs				
Beta-blockers	Present/absent	Product codes	Ever before cohort entry	Binary
Calcium channel blockers	Present/absent	Product codes	Ever before cohort entry	Binary
Angiotensin converting enzyme	Present/absent	Product codes	Ever before cohort entry	Binary
Angiotensin receptor blockers	Present/absent	Product codes	Ever before cohort entry	Binary
Diuretics	Present/absent	Product codes	Ever before cohort entry	Binary
Other prescription drugs				
Non-steroidal anti-inflammatory	Present/absent	Product codes	Ever before cohort entry	Binary
Acetylsalicylic acid	Present/absent	Product codes	Ever before cohort entry	Binary
Other antiplatelets	Present/absent	Product codes	Ever before cohort entry	Binary
Statins	Present/absent	Product codes	Ever before cohort entry	Binary
Hormone replacement therapy	Present/absent	Product codes	Ever before cohort entry	Binary
Oral anticoagulants	Present/absent	Product codes	Ever before cohort entry	Binary
Mammography	Present/absent	Product codes	Year before cohort entry	Binary

CHAPTER 5: MANUSCRIPT: SGLT-2 INHIBITORS AND EARLY BREAST CANCER EVENTS: POPULATION-BASED COHORT STUDY

The following chapter presents the manuscript of the study on the association between SGLT-2 inhibitors and breast cancer among women with type 2 diabetes. First, the introduction provides background information on SGLT-2 inhibitors and the study rationale. Second, the methods section describes the cohort creation, follow-up and statistical analyses. Then, the results are presented, including descriptive characteristics of the cohort and results of the primary and secondary analyses. Finally, the discussion provides an interpretation of the findings, comparisons with previous literature and strengths and limitations of the study. This manuscript was submitted to Diabetes Care and is undergoing second review.

Original Research Article

Sodium-Glucose Co-Transporter 2 Inhibitors and Risk of Early Breast Cancer Among Women with Type 2 Diabetes

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5.1 ABSTRACT

Objective: To determine whether use of sodium-glucose cotransporter-2 (SGLT-2) inhibitors is associated with an early increased risk of breast cancer among female patients with type 2 diabetes, when compared with use of dipeptidyl peptidase 4 (DPP-4) inhibitors.

Research Design and Methods: Using the United Kingdom Clinical Practice Research Datalink, we conducted a population-based cohort study among 9,938 new users of SGLT-2 inhibitors users and 36,631 new users of DPP-4 inhibitors between 2013 and 2018. Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) of breast cancer incidence, comparing SGLT-2 inhibitors with DPP-4 inhibitors using both intention-to-treat and as-treated approaches. The models were weighted using propensity score fine stratification.

Results: During a median of 2.6 years, there were 67 (2.8/1000 person-years) and 382 (3.7/1000 person-years) breast cancer events among SGLT-2 inhibitor and DPP-4 inhibitor users, respectively. Compared with DPP-4 inhibitors, SGLT-2 inhibitors were not associated with an overall increased risk of breast cancer in both the intention-to-treat and as-treated approaches (HR 1.00, 95% CI: 0.77-1.30 and HR 0.92, 95% CI: 0.67-1.26, respectively). In secondary analyses, the HRs for empagliflozin and canagliflozin were around the null value, except for dapagliflozin (HR 1.16, 95% CI: 0.86-1.56) where the cumulative incidence curves diverged around 1.5 years after treatment initiation.

Conclusions: In this first population-based study, use of SGLT-2 inhibitors was not associated with an overall increased risk of early breast cancer, when compared with DPP-4 inhibitors. Additional studies are needed to investigate a possible association with dapagliflozin.

5.2 INTRODUCTION

Sodium-glucose cotransporter-2 (SGLT-2) inhibitors are a novel class of second-to third-line antidiabetic drugs used in the management of patients with type 2 diabetes (1-3). While these drugs have been shown to have beneficial cardiovascular effects in randomized controlled trials (4-7), there are concerns that their use may increase the risk of several important adverse events, including diabetic ketoacidosis (8), genital infections (9), lower extremity amputations (10), and certain malignancies such as bladder and breast cancer (7, 11, 12).

With respect to breast cancer, SGLT-2 inhibitors trials have generated conflicting evidence on a possible association with these drugs. Regulatory agencies, such as the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA), have noted imbalances in breast cancer events in patients randomized to dapagliflozin versus placebo in premarketing trials (incidence rates 40.0 versus 19.0 per 10,000 person-years, respectively; rate ratio 2.47, 95% confidence interval [CI] 0.64-14.10); the majority of these events occurred in the first year of treatment (12-14). In subsequent cardiovascular outcome trials of canagliflozin and dapagliflozin, there were no important imbalances in breast cancer events with these SGLT-2 inhibitors versus placebo (4, 6). However, it remains unclear whether the imbalances observed in the SGLT-2 inhibitor premarketing trials was due to chance, potential tumor promoter effects, or possible over-detection of breast cancer.

To date, no observational studies have been conducted to evaluate the association between SGLT-2 inhibitors and the risk of breast cancer in the real-world setting. Given their increasing use in patients with type 2 diabetes (15), and concerns regarding their potential carcinogenicity (12-14), we conducted a population-based cohort study to determine whether the use of SGLT-2

inhibitors is associated with an early increased risk of breast cancer in a large cohort of female patients with type 2 diabetes.

5.3 METHODS

Data Source

This study was conducted using the GOLD and Aurum datasets of the United Kingdom (UK) Clinical Practice Research Datalink (CPRD) (16). The CPRD is a large database of longitudinal primary care medical records shown to be representative of the UK population (17). The CPRD records diagnoses and procedures using the Read code classification system, as well as lifestyle measures (e.g., smoking, alcohol consumption), anthropometric measures (e.g., body mass index), and laboratory tests (e.g., glycated hemoglobin A1c [HbA1c]) (17). Prescriptions are recorded using the British National Formulary product dictionary (17). Diagnoses recorded in the CPRD have been shown to be of high validity and quality (18, 19). Importantly, diagnoses of breast cancer have been shown to be well-recorded in the CPRD compared to the UK National Cancer Data Repository, with a positive predictive value of 90% (20, 21).

The study protocol was approved by the Independent Scientific Advisory Committee of the CPRD (protocol no. 19_272) and by the Research Ethics Research Board of the Jewish General Hospital, Montreal, Canada.

Study Population

We identified all female patients newly treated with either an SGLT-2 inhibitor (dapagliflozin, canagliflozin, empagliflozin) or a DPP-4 inhibitor (sitagliptin, saxagliptin, linagliptin, alogliptin) between January 1, 2013 (the year the first SGLT-2 inhibitor, dapagliflozin,

entered the UK market) and June 30, 2018. Cohort entry was defined as the date of the first prescription of either drug class during the study period. All patients were required to be at least 40 years of age and have at least one year of medical history in the CPRD before cohort entry. As DPP-4 inhibitors have been available since 2007, we excluded patients who were previously prescribed these drugs at any time before cohort entry. We also excluded patients previously diagnosed with end-stage renal disease or undergoing dialysis (as these are contraindications to receiving SGLT-2 inhibitors), and those previously diagnosed with breast cancer at any time before cohort entry (Read codes provided in **Supplemental Table 1**).

Propensity Score Fine Stratification

We used propensity score fine stratification to control for confounding, a method that is well suited for newly-marketed drugs with relatively low exposure prevalence (22). Indeed, propensity score fine stratification has been demonstrated to produce estimates with greater precision compared to propensity score matching methods that discard unmatched exposed or unexposed patients when prevalence of exposure is low (22). Thus, we used multivariate logistic regression to calculate the predicted probability (propensity score) of receiving an SGLT-2 inhibitor versus a DPP-4 inhibitor, conditional of the covariates listed below. After trimming the non-overlapping distributions of the propensity score, we created 50 strata based on the distribution of the SGLT-2 inhibitor patients. SGLT-2 inhibitor users received a weight of one, while DPP-4 inhibitor users were weighted in proportion to the distribution of their assigned strata (22).

The propensity score model considered the following potential confounders measured at or before cohort entry: age, body mass index (<30 kg/m2, ≥30 kg/m2, unknown), alcohol-related

disorders (alcoholism, cirrhosis, alcoholic hepatitis, hepatic failure), and smoking status (ever, never, unknown). We also included diabetes-related variables as proxies of disease severity, such as duration of diabetes, hemoglobin A1c (last measure before cohort entry), microvascular complications (nephropathy, neuropathy, retinopathy), macrovascular complications (peripheral vascular disease, myocardial infarction, ischemic stroke), and previous used of antidiabetic drugs used at any time before cohort entry (metformin, sulfonylureas, thiazolidinedione, meglitinides, alpha-glucosidase inhibitors, glucagon-like peptide-1 receptor agonists and insulin use; all entered as non-mutually exclusive variables). We also considered variables potentially associated with breast cancer risk, including oophorectomy, cancer (other than non-melanoma skin cancer), congestive heart failure, coronary artery disease, chronic kidney disease, ever use of antihypertension drugs (23) (beta blockers, calcium channel blockers, angiotensin converting enzyme inhibitors, angiotensin receptor blockers and diuretics), as well as ever use of other commonly prescribed drugs such as nonsteroidal anti-inflammatory drugs, antiplatelets (aspirin, clopidogrel, ticagrelor, prasugrel), statins, hormone replacement therapy and oral anticoagulants (warfarin, heparin). Finally, we included mammographic screening history in the year before cohort entry.

Follow-Up Period

SGLT-2 inhibitor and DPP-4 inhibitor users were followed using two different approaches. In the primary approach, we used an intention-to-treat exposure definition in which patients were followed from cohort entry until an incident diagnosis of breast cancer, death from any cause, or end of study period (December 31, 2018), whichever occurred first. This approach assumed an irreversible effect of the exposure on the outcome, in which the risk of developing breast cancer

remained even after treatment discontinuation. In a secondary approach, we used an as-treated exposure definition in which patients were followed while continuously exposed from cohort entry until treatment discontinuation, an incident diagnosis of breast cancer, death from any cause, or end of study period (December 31, 2018), whichever occurred first. Patients were considered continuously exposed if one prescription overlapped the date of the next prescription, using a one-year grace period in the event of non-overlapping prescriptions. The one-year grace period was chosen to account for possible diagnostic delays associated with a diagnosis of breast cancer. This approach assumed a reversible effect of the exposure on the outcome (i.e., the risk of developing breast cancer decreases after treatment discontinuation).

Statistical Analysis

Crude incidence rates of breast cancer were calculated for each exposure group, with 95% CIs based on the Poisson distribution. We assessed covariate balance by calculating standardized differences before and after weighting, with standardized differences of more than 0.10 as indicative of imbalance. For both the intention-to-treat and as-treated approaches, we constructed weighted Kaplan-Meier curves for each exposure group and used weighted Cox proportional hazards models to estimate hazard ratios (HRs) and corresponding 95% CIs of breast cancer, comparing SGLT-2 inhibitor users with DPP-4 inhibitor users.

We performed three prespecified secondary analyses. First, we investigated whether the association varied according to the type of SGLT-2 inhibitor (dapagliflozin, empagliflozin, canagliflozin). Second, we assessed whether there was a duration-response relationship in terms of cumulative duration of use and time since initiation, both modelled as time-varying variables (≤1 year, 1.1-2 years, >2 years). Finally, we assessed whether there was effect measure

modification by obesity (<30 kg/m2, ≥30 kg/m2), as it is a well-known risk factor for breast cancer (24-27), and mammography screening age limit in the UK *Breast Cancer Screening Programme* (below and above 70 years of age) (28). Effect measure modification was assessed by including interaction terms between these variables and the exposure in the outcome model.

We also conducted sensitivity analyses to assess the robustness of our results. First, we lagged the exposure groups by one and two years to assess whether breast cancer events were occurring earlier (possible over-detection) or later (potential biological association). This was done by censoring breast cancer events that occurred in the first year (one-year lag) or first two years (two-year lag) after treatment initiation. Lastly, we repeated the analysis after accounting for death as a competing risk using inverse probability of censoring weighting (22, 29).

5.4 RESULTS

The study included a total of 9,938 new users of SGLT-2 inhibitors and 36,631 new users of DPP-4 inhibitors (**Figure 1**). After a median follow-up of 2.6 years, these exposure groups generated 67 and 382 breast cancer events, yielding crude incidence rates of 2.8 (95% CI 2.2-3.6) and 3.7 (95% CI 3.3-4.1) per 1000 person-years, respectively. Among SGLT-2 inhibitor users, dapagliflozin accounted for the majority of users (n=5429, 54.6%), followed by empagliflozin (n=2771, 27.9%), and canagliflozin (n=1726, 17.4%). The median duration of use of SGLT-2 inhibitors and DPP-4 inhibitors was 367 and 466 days, respectively.

Table 1 presents baseline characteristics of SGLT-2 inhibitor and DPP-4 inhibitor users, before and after weighting. Before weighting, SGLT-2 inhibitor users were younger, more likely to be obese, and to have elevated hemoglobin A1c levels, compared with DPP-4 inhibitor users. They were also more likely to have previously received metformin, GLP-1 receptor agonists and

insulin, as well as to have undergone mammography in the year before cohort entry. In contrast, SGLT-2 inhibitor users were less likely to have been diagnosed with stroke, cancer, coronary artery disease, and chronic kidney disease. After weighting, all baseline characteristics were well balanced with no standardized difference exceeding 0.02.

The results of the intention-to-treat and as-treated analyses are presented in **Table 2**. In the intention-to-treat analysis, use of SGLT-2 inhibitor was not associated with an overall increased risk of breast cancer, compared with DPP-4 inhibitor use (HR 1.00, 95% CI 0.76-1.30). The cumulative incidence curves of the SGLT-2 inhibitor and DPP-4 inhibitors did not significantly diverge during the follow-up period (**Figure 2**). Overall, similar findings were observed in the as-treated analysis (**Table 2** and **Supplemental Figure 1**).

In secondary analyses, the molecule-specific analysis generated HRs below the null value for canagliflozin (HR 0.62, 95% CI 0.29-1.31) and empagliflozin (HR 0.83, 95% CI 0.44-1.56), while the HR was above the null value for dapagliflozin (HR 1.16, 95% CI 0.86-1.56) (**Supplemental Table 2**). In a post-hoc analysis, the cumulative incidence curves diverged at around 1.5 years after treatment initiation for dapagliflozin, with a higher cumulative incidence of breast cancer in dapagliflozin users compared with DPP-4 inhibitor users (**Supplemental Figure 2**). Overall, there was no clear duration-response relationship with SGLT-2 inhibitor cumulative duration of use and time since treatment initiation (**Supplemental Table 3**). In contrast, the HR was elevated among non-obese women compared with obese women, although the CIs were wide and overlapping (HR 1.68, 95% CI 0.99-2.83 and HR 0.87, 95% CI 0.64-1.19, respectively) (**Supplemental Table 4**). Finally, stratifying by age generated HRs around the null for all categories, but with wide CIs (**Supplemental Table 5**).

Sensitivity Analyses

The results of the sensitivity analysis are presented in **Supplemental Tables 6-8**. The use of a one-year lag period led to a slight increase in the HR (HR 1.12, 95% CI 0.82-1.54), while a two-year lag did not materially change the point estimate (HR 0.96, 95% CI 0.62-1.50). Finally, there was no evidence of competing risk by death as assessed using inverse probability of censoring weighting.

5.5 DISCUSSION

In this first population-based cohort study, use of SGLT-2 inhibitors was not associated with an overall early increased risk of breast cancer, when compared with use of DPP-4 inhibitors among women with type 2 diabetes. Similar results were observed in the intention-to-treat and as-treated analyses. When stratifying by SGLT-2 inhibitor molecule, dapagliflozin was associated with a moderately elevated HR, but with a wide CI that included the null value. Overall, these results remained consistent in several sensitivity analyses.

Signals of a possible association between SGLT-2 inhibitors and breast cancer were first observed in premarketing trials of dapagliflozin. In 21 phase 2b and 3 studies, there were 12/2693 and 3/1439 breast cancer events in the dapagliflozin versus placebo groups, generating incidence rates of 40.0 versus 19.0 per 10,000 person-years, respectively (rate ratio 2.47, 95% CI 0.64-14.10) (12-14). It is important to note that these trials were all of short durations, with follow-up periods ranging from 24 to 208 weeks (12, 14). Interestingly, 10 out of the 12 breast cancer events occurring in the dapagliflozin group were diagnosed within one year of randomization (12, 14). Nonetheless, these imbalances, in addition to imbalances in bladder cancer events, led to a delay in the approval of dapagliflozin by the US FDA in 2011. Since the approval of dapagliflozin and

other SGLT-2 inhibitors thereafter, three post-marketing trials were conducted to evaluate the cardiovascular safety of these drugs. These include Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients (EMPA-REG OUTCOME), Canagliflozin Cardiovascular Assessment Study (CANVAS), and Multicenter Trial to Evaluate the Effects of Dapagliflozin on the Incidence of Cardiovascular Events (DECLARE-TIMI 58) (4, 6, 7). In the CANVAS trial, there was a small imbalance in breast cancer events with canagliflozin than with placebo (3.1 vs 2.6 events per 1000 patient-years, respectively) (4). In contrast, there was no imbalances of breast cancer events in the DECLARE-TIMI 58 trial of dapagliflozin compared with placebo (36/8574 vs 35/8569 events, respectively; HR 1.02, 95% CI 0.64-1.63) (6, 30). While these trials had longer durations of follow-up (median of 2.4 years for canagliflozin and 4.2 years for dapagliflozin), they were not designed or powered to assess breast cancer as a safety outcome. To date, safety information on malignancies occurring in the EMPA-REG OUTCOME has not been reported.

The discrepant findings between premarketing and post-marketing trials on breast cancer events may have been due to chance. Indeed, there is no clear biological mechanism to explain a potential association with increased breast cancer incidence. While an accelerated tumor promoting effect is theoretically possible, it is unlikely given that sodium-glucose cotransporter proteins are not expressed in mammary tissue and animal studies have failed to demonstrate that SGLT-2 inhibitors have any neoplastic activity (31). It is possible, however, that the breast cancer imbalances observed in the premarketing trials may be due to a transient over-detection of breast lumps leading to an increase in breast cancer diagnoses. Specifically, SGLT-2 inhibitors are known to induce weight loss across all body mass index categories (32), which could facilitate the detection of pre-existing breast lumps, leading to an early diagnosis of breast cancer relatively

soon after treatment initiation. This mechanism was recently observed with glucagon-like peptide-1 receptor agonists, where rapid and significant weight loss was associated with an increased detection of breast cancer (33). In our study, dapagliflozin was associated with a slightly elevated HR and with cumulative incidence curves diverging after 1.5 years of treatment initiation. However, all SGLT-2 inhibitors have been associated with weight loss (32), and thus it is unclear why this effect was observed for dapagliflozin specifically. While these findings need to be interpreted with caution, they nonetheless appear concordant with the signals observed in the dapagliflozin premarketing trials. Additional large and well-conducted studies will be needed to further investigate this possible association.

This study has several strengths. We assembled a large cohort of female patients newly treated with either SGLT-2 inhibitors or DPP-4 inhibitors using the CPRD, a population-based database shown to be representative and of high quality (17). The inclusion of new users reduced the possibility of prevalent user biases in our study (34). Finally, the use of an active comparator, DPP-4 inhibitors, used at a similar stage in the disease management likely reduced confounding by indication.

This study has some limitations. First, misclassification of exposure is possible, since the CPRD records written prescriptions and thus it is unknown whether the drugs were filled and used as intended. However, such misclassification is likely to be non-differential between the exposure groups. Furthermore, the CPRD records prescriptions written by general practitioners and not by specialists, although type 2 diabetes is almost entirely managed by general practitioners in the United Kingdom (35). Second, outcome misclassification is possible, but breast cancer has been shown to be well recorded in the CPRD database, when compared with the National Cancer Data Repository (20). Third, SGLT-2 inhibitors are a relatively new class of drugs, which limited the

follow-up period (median 2.6 years). However, the rationale for conducting this study was based on breast cancer imbalances observed in premarketing trials of short durations (ranging from 24 to 208 weeks). As such, this study was specifically designed to assess whether SGLT-2 inhibitors are associated with an early risk of breast cancer. Thus, while this study provides some reassurance on the relatively short-term effects of SGLT-2 inhibitors on breast cancer risk, future studies will need to be conducted to assess their long-term effects on the incidence of this malignancy. Fourth, given the observational nature of this study, residual confounding is possible. However, the use of an active comparator used at a similar stage of the disease and propensity score fine stratification likely minimized this potential bias (22). Finally, while the study was well-powered to assess the association with SGLT-2 inhibitors as a class, some secondary analyses led to point estimates with wide CIs; these should be interpreted with caution.

In summary, the results of this study indicate that, compared with DPP-4 inhibitors, SGLT-2 inhibitors are not associated with an early increased risk of breast cancer among women with type 2 diabetes. While these findings should provide some reassurance to physicians and patients using these drugs, additional research is needed to investigate whether use of certain SGLT-2 inhibitors, such as dapagliflozin, is associated with an increased risk of this malignancy.

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5.7 FIGURES AND TABLES

FIGURE LEGEND

Figure 3: Study flow diagram

Figure 4: Weighted cumulative incidence curves of breast cancer comparing use of SGLT-2 inhibitors with use of DPP-4 inhibitors

FIGURE 3. Study flow diagram

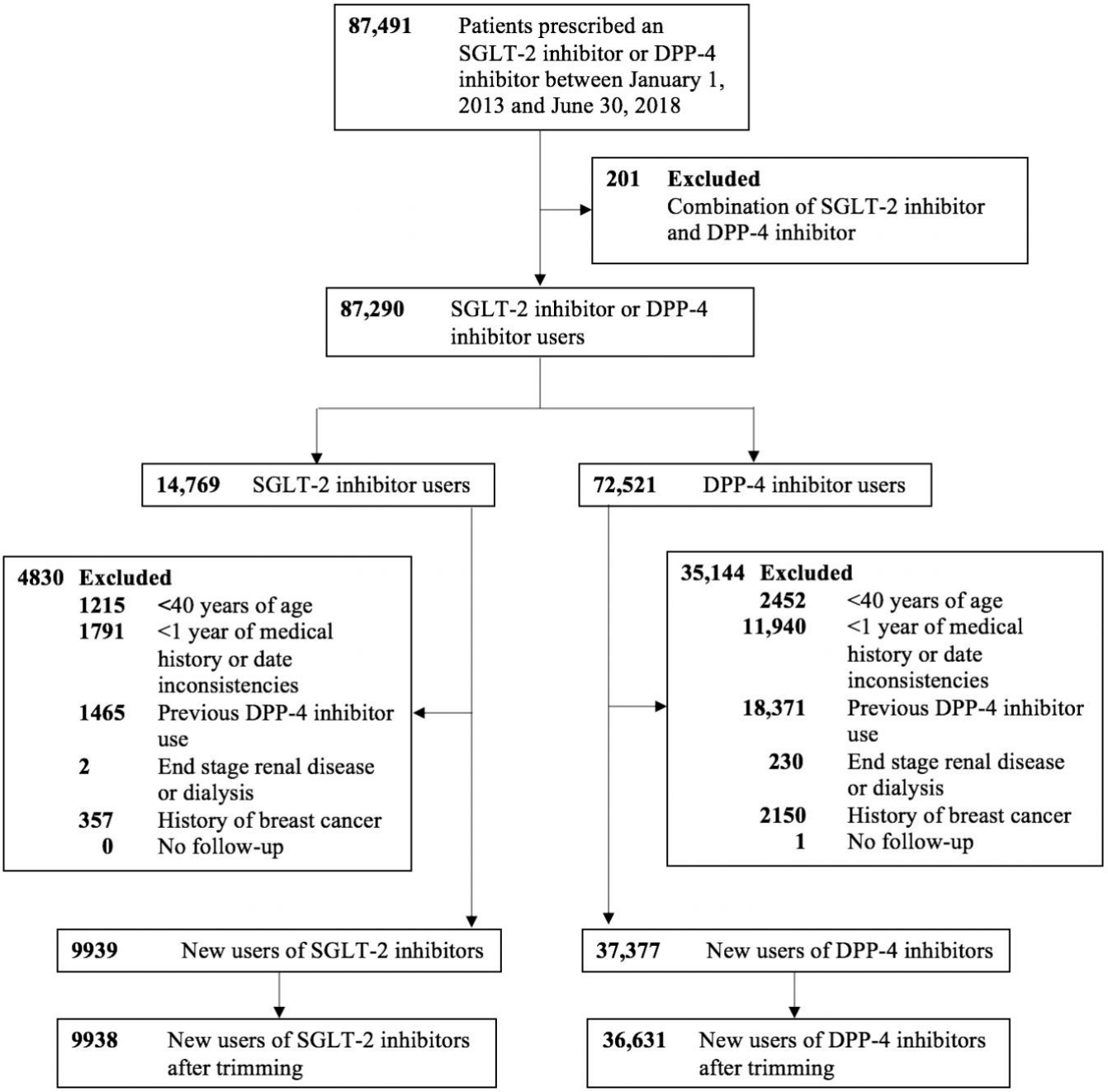


FIGURE 4. Weighted cumulative incidence curves of breast cancer comparing use of SGLT-2 inhibitors with use of DPP-4 inhibitors

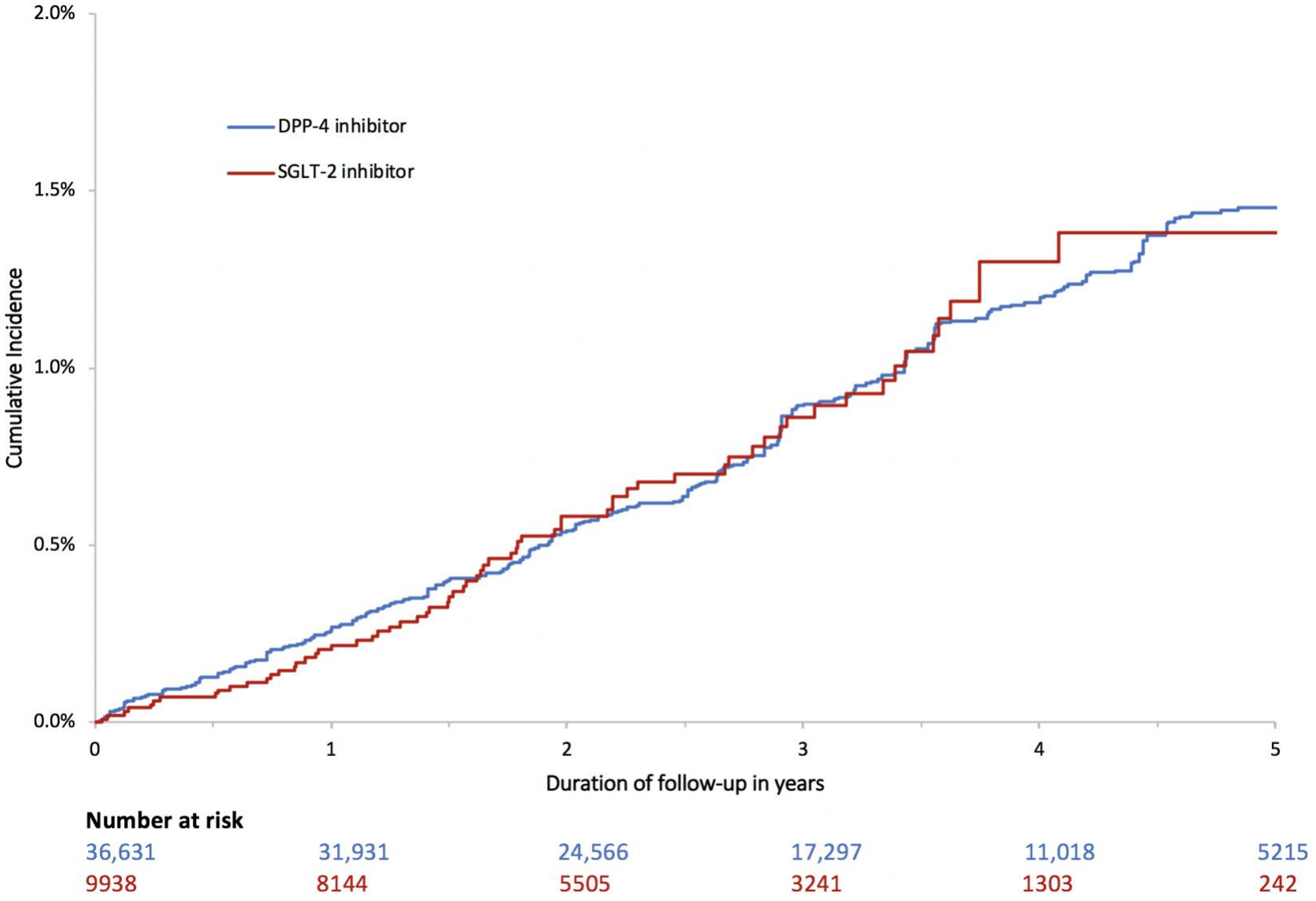


TABLE 4. Characteristics of SGLT-2 Inhibitor and DPP-4 Inhibitor Users Before and After Propensity Score Fine Stratification Weighting

	Before Weighting			After Weighting [a]		
Characteristics	**SGLT2i users**	**DPP4i users**	**Sd. Diff.**	**SGLT2i users**	**DPP-4i users**	**Sd. Diff.**
Total	9938	36,631		9938	36,631	
Age, years, mean (SD)	57.6 (9.4)	66.1 (12.3)	0.78	57.6 (9.4)	57.6 (9.6)	0.00
Body mass index, n (%)						
< 30 kg/m2	1738 (17.5)	13,805 (37.7)	0.46	1738 (17.5)	6486 (17.7)	0.01
≥ 30 kg/m2	8063 (81.1)	22,402 (61.2)	0.45	8063 (81.1)	29,688 (81.1)	0.00
Unknown	137 (1.4)	424 (1.2)	0.02	137 (1.4)	458 (1.3)	0.01
Alcohol related disorders, n (%)	435 (4.4)	1550 (4.2)	0.01	435 (4.4)	1553 (4.2)	0.01
Smoking status, n (%)						
Never	2794 (28.1)	10,306 (28.1)	0.00	2794 (28.1)	10,288 (28.1)	0.00
Ever	7127 (71.7)	26,260 (71.7)	0.00	7127 (71.7)	26,281 (71.7)	0.00
Unknown	17 (0.2)	65 (0.2)	0.00	17 (0.2)	64 (0.2)	0.00
Duration of diabetes, years, mean (SD)	8.7 (6.3)	9.0 (6.6)	0.05	8.7 (6.3)	8.6 (6.3)	0.01
Hemoglobin A1c, n (%)						
≤ 7 %	617 (6.2)	3491 (9.5)	0.12	617 (6.2)	2272 (6.2)	0.00
7.1-8.0%	2120 (21.3)	10,365 (28.3)	0.16	2120 (21.3)	7795 (21.3)	0.00
> 8.0%	7159 (72.0)	22,577 (61.6)	0.22	7159 (72.0)	26,415 (72.1)	0.00
Unknown	42 (0.4)	198 (0.5)	0.01	42 (0.4)	151 (0.4)	0.00
Nephropathy, n (%)	43 (0.4)	233 (0.6)	0.03	43 (0.4)	165 (0.5)	0.01
Neuropathy, n (%)	2145 (21.6)	8466 (23.1)	0.04	2145 (21.6)	7713 (21.1)	0.01
Retinopathy, n (%)	3512 (35.3)	13,381 (36.5)	0.03	3512 (35.3)	12,889 (35.2)	0.00
Peripheral vascular disease, n (%)	581 (5.9)	2939 (8.0)	0.08	581 (5.9)	2172 (5.9)	0.00
Myocardial infarction, n (%)	353 (3.6)	1908 (5.2)	0.08	353 (3.6)	1283 (3.5)	0.01
Stroke, n (%)	300 (3.0)	2156 (5.9)	0.14	300 (3.0)	1142 (3.12)	0.01
Antidiabetic drugs, n (%) [b]						
Metformin	9698 (97.6)	34822 (95.1)	0.13	9698 (97.6)	35,776 (97.7)	0.01
Sulfonylureas	5211 (52.4)	20469 (55.9)	0.07	5211 (52.4)	19,289 (52.7)	0.01
Thiazolidinediones	1859 (18.7)	5647 (15.4)	0.09	1859 (18.7)	6962 (19.0)	0.01

Characteristics	Before Weighting			After Weighting [a]		
	SGLT2i users	DPP4i users	Sd. Diff.	SGLT2i users	DPP-4i users	Sd. Diff.
Meglitinides	163 (1.6)	434 (1.2)	0.03	163 (1.6)	620 (1.7)	0.01
Alpha-glucosidase inhibitors	184 (1.9)	484 (1.3)	0.05	184 (1.9)	649 (1.8)	0.01
GLP-1 receptor agonists	2187 (22.0)	1155 (3.2)	0.59	2187 (22.0)	8119 (22.2)	0.00
Insulin	2770 (27.9)	4147 (11.3)	0.43	2770 (27.9)	10,200 (27.8)	0.00
Breast cancer related						
Oophorectomy, n (%)	383 (3.9)	1424 (3.9)	0.00	383 (3.9)	1366 (3.7)	0.01
Cancer, n (%)	465 (4.7)	2556 (7.0)	0.10	465 (4.7)	1753 (4.8)	0.00
Congestive heart failure, n (%)	163 (1.6)	1710 (4.7)	0.18	163 (1.6)	591 (1.6)	0.00
Coronary artery disease, n (%)	1238 (12.5)	6741 (18.4)	0.16	1238 (12.5)	4674 (12.8)	0.01
Chronic kidney disease, n (%)	737 (7.4)	9432 (25.8)	0.51	737 (7.4)	2816 (7.7)	0.01
Antihypertension drugs, n (%)						
Beta-blockers	3508 (35.3)	15675 (42.8)	0.15	3508 (35.3)	12,918 (35.3)	0.00
Calcium channel blockers	3620 (36.4)	17406 (47.5)	0.23	3620 (36.4)	13,644 (37.3)	0.02
Angiotensin converting enzyme inhibitors	5806 (58.4)	23377 (63.8)	0.11	5806 (58.4)	21,279 (58.1)	0.01
Angiotensin receptor blockers	2173 (21.9)	9900 (27.0)	0.12	2173 (21.9)	8237 (22.5)	0.01
Diuretics	3394 (34.2)	15574 (42.5)	0.17	3394 (34.2)	12,608 (34.4)	0.00
Other drugs, n (%)						
Non-steroidal anti-inflammatory drugs	8130 (81.8)	29385 (80.2)	0.04	8130 (81.8)	30,064 (82.1)	0.01
Acetylsalicylic acid	3617 (36.4)	16993 (46.4)	0.20	3617 (36.4)	13,399 (36.6)	0.00
Other antiplatelets	759 (7.6)	4305 (11.8)	0.14	759 (7.6)	2798 (7.6)	0.00
Statins	8118 (81.7)	31453 (85.9)	0.11	8118 (81.7)	29,818 (81.4)	0.01
Hormone replacement therapy	3246 (32.7)	11490 (31.4)	0.03	3246 (32.7)	12,144 (33.2)	0.01
Oral anticoagulants	503 (5.1)	3576 (9.8)	0.18	503 (5.1)	1912 (5.2)	0.00
Mammography	1910 (19.2)	5136 (14.0)	0.14	1910 (19.2)	7135 (19.5)	0.01

Abbreviations: Sd. Diff., standardized difference (absolute). SGLT-2i, sodium-glucose cotransporter-2 inhibitor. DPP-4i, dipeptidyl peptidase-4 inhibitor.

[a] Baseline characteristics of the DPP-4 inhibitor users were weighted using propensity score fine stratification.

[b] Non-mutually exclusive categories; measured ever before cohort entry.

TABLE 5. Hazard Ratios for Breast Cancer Comparing Use of SGLT-2 inhibitors with Use of DPP-4 Inhibitors

Exposure	No. of patients	Events	Person-years	Incidence rate (95% CI) [a]	Crude HR (95% CI)	Weighted HR (95% CI) [b]
Intention-to-treat analysis						
DPP-4 inhibitor	36,631	382	103,228	3.7 (3.3-4.1)	1.00 [Reference]	1.00 [Reference]
SGLT-2 inhibitor	9938	67	23,621	2.8 (2.2-3.6)	0.78 (0.60-1.01)	1.00 (0.76-1.30)
As-treated analysis						
DPP-4 inhibitor	36,631	290	76,246	3.8 (3.4-4.3)	1.00 [Reference]	1.00 [Reference]
SGLT-2 inhibitor	9938	47	17,579	2.7 (2.0-3.6)	0.72 (0.53-0.98)	0.92 (0.67-1.26)

Abbreviations: HR, hazard ratio; CI, confidence interval; SGLT, sodium-glucose cotransporter; DPP, dipeptidyl peptidase

[a] Per 1000 person-years.

[b] Propensity score fine stratification weighting.

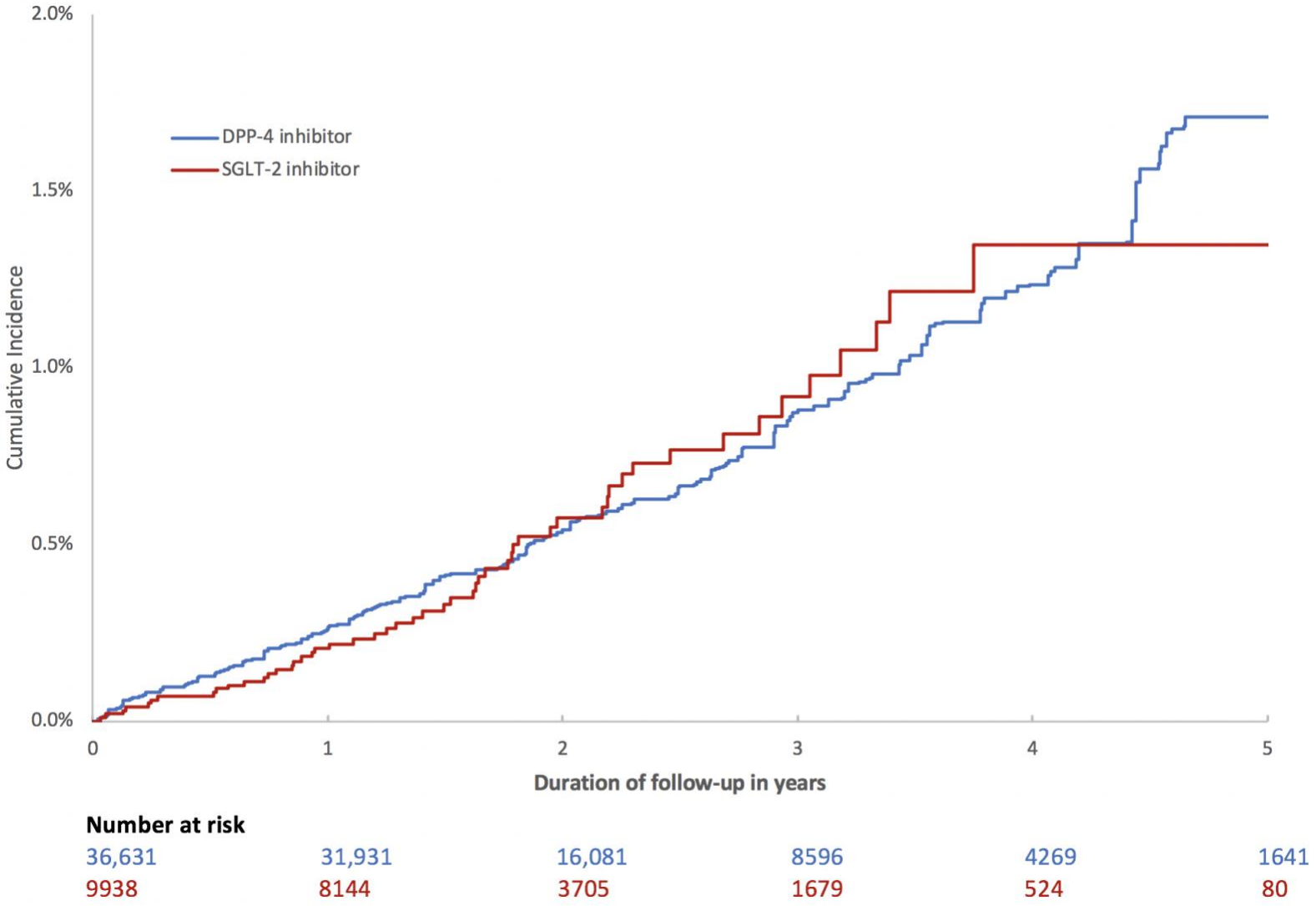
5.8 SUPPLEMENTAL FIGURES AND TABLES

SUPPLEMENTAL FIGURES LEGEND

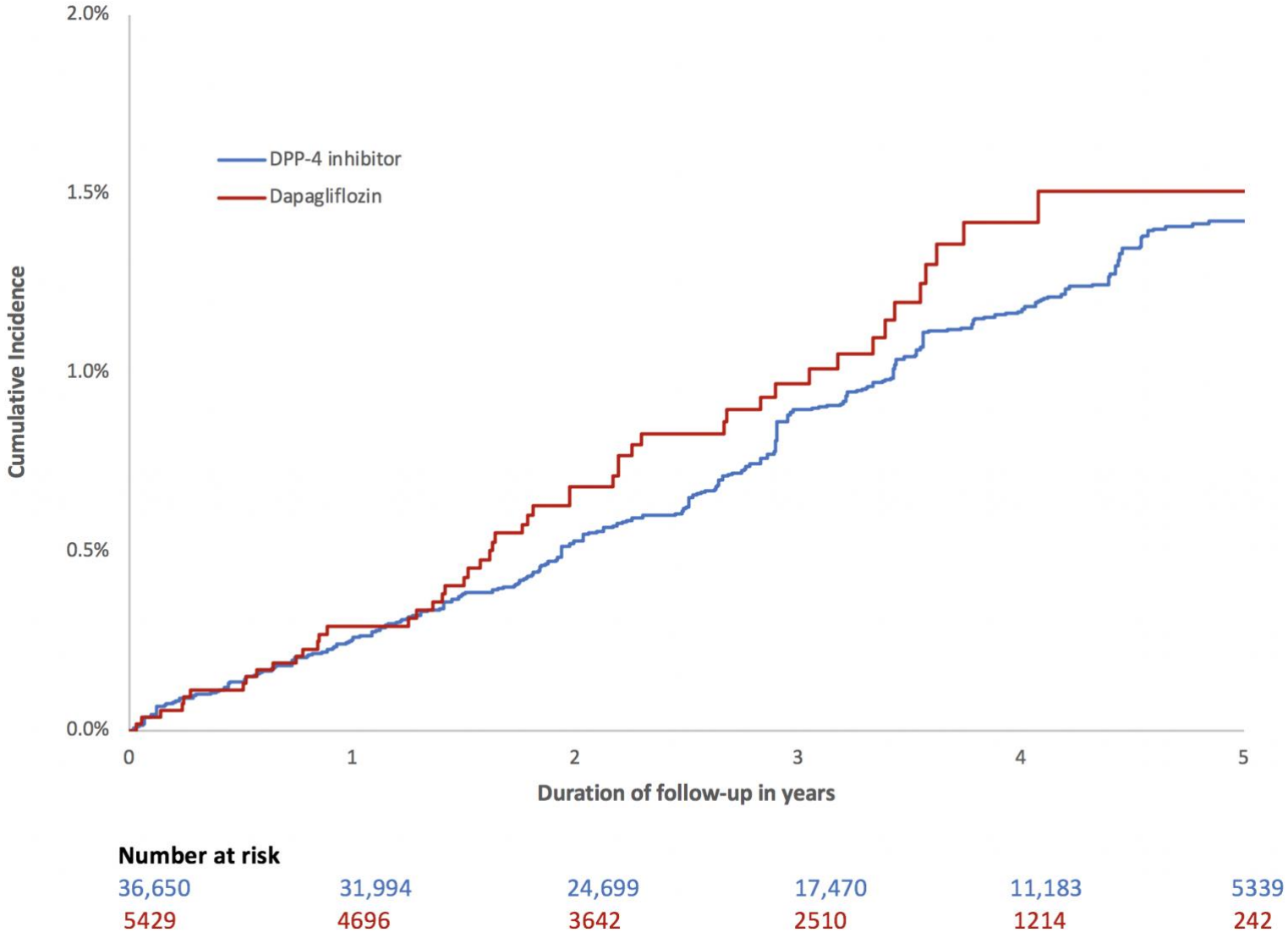
Supplemental Figure 1. Kaplan Meier curve of cumulative incidence of breast cancer with use of SGLT-2 inhibitors (as treated)

Supplemental Figure 2. Kaplan Meier curve of cumulative incidence of breast cancer with use of dapagliflozin (intention to treat)

SUPPLEMENTAL FIGURE 1. Kaplan Meier curve of cumulative incidence of breast cancer with use of SGLT-2 inhibitors (as treated)



SUPPLEMENTAL FIGURE 2. KAPLAN MEIER CURVE OF CUMULATIVE INCIDENCE OF BREAST CANCER WITH USE OF DAPAGLIFLOZIN (INTENTION TO TREAT)



SUPPLEMENTAL TABLES LEGEND

Supplemental Table 1: Breast cancer codes

Supplemental Table 2: Hazard Ratios for the Association Between Drug Specific SGLT-2 inhibitors and Early Breast Cancer

Supplemental Table 3: Hazard Ratios for the Association Between SGLT-2 inhibitors and Early Breast Cancer (Duration Analysis)

Supplemental Table 4: Hazard Ratios for the Association Between SGLT-2 inhibitors and Early Breast Cancer (Interaction with BMI)

Supplemental Table 5: Hazard Ratios for the Association Between SGLT-2 inhibitors and Early Breast Cancer (Interaction with age group)

Supplemental Table 6: Hazard Ratios for the Association Between SGLT-2 inhibitors and Early Breast Cancer (Lag 1 year)

Supplemental Table 7: Hazard Ratios for the Association Between SGLT-2 inhibitors and Early Breast Cancer (Lag 2 years)

Supplemental Table 8: Hazard Ratios for the Association Between SGLT-2 inhibitors and Early Breast Cancer (Inverse probability of censoring weighting)

SUPPLEMENTAL TABLE 1. Breast cancer codes

Read term	Med Code	Read Code
[X]Other carcinoma in situ of breast	53803	ByuFG00
Benign neoplasm of breast	5411	B77..00
Benign neoplasm of female breast	19138	B770.00
Benign neoplasm of breast NOS	28870	B77z.00
Carcinoma in situ of skin of breast	8647	B825000
Carcinoma in situ of breast and genitourinary system	45681	B83..00
Carcinoma in situ of breast	7833	B830.00
Lobular carcinoma in situ of breast	10387	B830000
Intraductal carcinoma in situ of breast	18694	B830100
Neoplasm of uncertain behaviour of breast	41232	B933.00
Neoplasm of unspecified nature of breast	45906	BA03.00
Unspecified lump in breast		N63

SUPPLEMENTAL TABLE 2. Hazard Ratios for the Association Between Drug Specific SGLT-2 inhibitors and Early Breast Cancer

Exposure	Number of patients	Events	Person-years	Incidence rate (95% CI) [a]	Crude HR (95% CI)	Weighted HR (95% CI) [b]
Intention to treat						
DPP-4 inhibitor	35,380	369	100,523	3.7 (3.3-4.1)	1.00 [Reference]	1.00 [Reference]
Canagliflozin	1726	7	4083	1.7 (0.7-3.5)	0.48 (0.23-1.02)	0.62 (0.29-1.31)
DPP-4 inhibitor	36,650	384	103,289	3.7 (3.4-4.1)	1.00 [Reference]	1.00 [Reference]
Dapagliflozin	5429	50	15,111	3.3 (2.5-4.4)	0.89 (0.66-1.19)	1.16 (0.86-1.57)
DPP-4 inhibitor	36,044	377	101,978	3.7 (3.3-4.1)	1.00 [Reference]	1.00 [Reference]
Empagliflozin	2771	10	4405	2.3 (1.1-4.2)	0.66 (0.35-1.24)	0.83 (0.44-1.56)
As treated						
DPP-4 inhibitor	35,380	278	73,970	3.8 (3.3-4.2)	1.00 [Reference]	1.00 [Reference]
Canagliflozin	1726	3	2985	1.0 (0.2-2.9)	0.28 (0.09-0.87)	0.37 (0.12-1.15)
DPP-4 inhibitor	36,650	292	76,307	3.8 (3.4-4.3)	1.00 [Reference]	1.00 [Reference]
Dapagliflozin	5429	35	10,287	3.4 (2.4-4.7)	0.90 (0.63-1.28)	1.18 (0.83-1.69)
DPP-4 inhibitor	36,044	286	75,246	3.8 (3.4-4.3)	1.00 [Reference]	1.00 [Reference]
Empagliflozin	2771	7	3732	1.9 (0.8-3.9)	0.53 (0.25-1.13)	0.66 (0.31-1.40)

Abbreviations: HR, hazard ratio; CI, confidence interval; SGLT, sodium-glucose cotransporter; DPP, dipeptidyl peptidase

[a] Per 1000 person-years.

[b] Propensity score fine stratification weighting.

SUPPLEMENTAL TABLE 3. Hazard Ratios for the Association Between SGLT-2 inhibitors and Early Breast Cancer (Duration Analysis)

Exposure	Number of patients	Events	Person-years	Incidence rate (95% CI) [a]	Crude HR (95% CI)	Weighted HR (95% CI) [b]
Overall						
DPP-4 inhibitor	36,631	382	103,228	3.7 (3.3-4.1)	1.00 [Reference]	1.00 [Reference]
SGLT-2 inhibitor	9938	67	23,621	2.8 (2.2-3.6)	0.78 (0.60-1.01)	1.00 (0.77-1.30)
Cumulative duration of SGLT-2 inhibitor use [c]						
≤1 year	-	40	15,733	2.5 (1.8-3.5)	0.73 (0.52-1.02)	0.94 (0.67-1.32)
1.1-2 years	-	23	5318	4.3 (2.7-6.5)	1.13 (0.74-1.74)	1.46 (0.94-2.25)
>2 years	-	4	2570	1.6 (0.4-4.0)	0.37 (0.14-1.00)	0.45 (0.17-1.23)
Time since initiation of SGLT-2 inhibitor use [c]						
≤1 year	-	19	9368	2.0 (1.2-3.2)	0.60 (0.37-0.97)	0.78 (0.48-1.29)
1.1-2 years	-	25	6828	3.7 (2.4-5.4)	1.04 (0.67-1.61)	1.31 (0.84-2.05)
>2 years	-	23	7424	3.1 (2.0-4.6)	0.76 (0.49-1.17)	0.96 (0.62-1.49)
Dapagliflozin						
DPP-4 inhibitor	36,650	384	103,289	3.7 (3.4-4.1)	1.00 [Reference]	1.00 [Reference]
Dapagliflozin	5429	50	15,111	3.3 (2.5-4.4)	0.89 (0.66-1.19)	1.16 (0.86-1.57)
Cumulative duration of Dapagliflozin use [c]						
≤1 year	-	31	9846	3.1 (2.1-4.5)	0.88 (0.61-1.27)	1.16 (0.80-1.69)
1.1-2 years	-	16	3401	4.7 (2.7-7.6)	1.22 (0.73-2.03)	1.58 (0.95-2.64)
>2 years	-	3	1864	1.6 (0.3-4.7)	0.38 (0.12-1.20)	0.48 (0.15-1.51)

Time since initiation of Dapagliflozin use [c]						
≤1 year	-	15	5180	2.9 (1.6-4.8)	0.85 (0.50-1.45)	1.15 (0.66-1.99)
1.1-2 years	-	16	4177	3.8 (2.2-6.2)	1.08 (0.63-1.83)	1.40 (0.82-2.41)
>2 years	-	19	5754	3.3 (2.0-5.2)	0.80 (0.50-1.28)	1.02 (0.63-1.65)

Abbreviations: HR, hazard ratio; CI, confidence interval; SGLT, sodium-glucose cotransporter; DPP, dipeptidyl peptidase

[a] Per 1000 person-years.

[b] Propensity score fine stratification weighting.

[c] Time-dependent exposure.

SUPPLEMENTAL TABLE 4. Hazard Ratios for the Association Between SGLT-2 inhibitors and Early Breast Cancer (Interaction with BMI)

Exposure	BMI < 30 kg/m2	BMI ≥ 30 kg/m2
Overall		
DPP-4 inhibitor	1.00 [Reference]	1.00 [Reference]
SGLT-2 inhibitor	1.68 (0.99-2.83)	0.87 (0.64-1.19)
		p-interaction: 0.03
Dapagliflozin		
DPP-4 inhibitor	1.00 [Reference]	1.00 [Reference]
Dapagliflozin	2.35 (1.33-4.13)	0.95 (0.66-1.35)
		p-interaction: 0.01

Abbreviations: HR, hazard ratio; CI, confidence interval; SGLT, sodium-glucose cotransporter; DPP, dipeptidyl peptidase
Propensity score fine stratification weighting.

SUPPLEMENTAL TABLE 5. Hazard Ratios for the Association Between SGLT-2 inhibitors and Early Breast Cancer (Interaction with age group)

Exposure	Age ≤ 70	Age > 70
Overall		
DPP-4 inhibitor	1.00 [Reference]	1.00 [Reference]
SGLT-2 inhibitor	1.03 (0.78-1.36)	0.79 (0.35-1.77)
		p-interaction: 0.55
Dapagliflozin		
DPP-4 inhibitor	1.00 [Reference]	1.00 [Reference]
Dapagliflozin	1.15 (0.84-1.58)	1.21 (0.51-2.89)
		p-interaction: 0.91

Abbreviations: HR, hazard ratio; CI, confidence interval; SGLT, sodium-glucose cotransporter; DPP, dipeptidyl peptidase
Propensity score fine stratification weighting.

SUPPLEMENTAL TABLE 6. Hazard Ratios for the Association Between SGLT-2 inhibitors and Early Breast Cancer (Lag 1 year)

Exposure	Number of patients	Events	Person-years	Incidence rate (95% CI) [a]	Crude HR (95% CI)	Weighted HR (95% CI) [b]
Overall						
DPP-4 inhibitor	30,620	262	67,937	3.9 (3.4-4.4)	1.00 [Reference]	1.00 [Reference]
SGLT-2 inhibitor	8143	48	14,250	3.4 (2.5-4.5)	0.88 (0.65-1.20)	1.14 (0.83-1.56)
Dapagliflozin						
DPP-4 inhibitor	30,390	261	67,531	3.9 (3.4-4.4)	1.00 [Reference]	1.00 [Reference]
Dapagliflozin	4695	35	9928	3.5 (2.5-4.9)	0.91 (0.64-1.29)	1.19 (0.83-1.70)

Abbreviations: HR, hazard ratio; CI, confidence interval; SGLT, sodium-glucose cotransporter; DPP, dipeptidyl peptidase

[a] Per 1000 person-years.

[b] Propensity score fine stratification weighting.

SUPPLEMENTAL TABLE 7. Hazard Ratios for the Association Between SGLT-2 inhibitors and Early Breast Cancer (Lag 2 years)

Exposure	Number of patients	Events	Person-years	Incidence rate (95% CI) [a]	Crude HR (95% CI)	Weighted HR (95% CI) [b]
Overall						
DPP-4 inhibitor	22,854	167	41,292	4.0 (3.5-4.7)	1.00 [Reference]	1.00 [Reference]
SGLT-2 inhibitor	5503	23	7422	3.1 (2.0-4.6)	0.76 (0.49-1.18)	1.00 (0.64-1.56)
Dapagliflozin						
DPP-4 inhibitor	22,614	166	40,939	4.1 (3.5-4.7)	1.00 [Reference]	1.00 [Reference]
Dapagliflozin	3641	19	5754	3.3 (2.0-5.2)	0.80 (0.50-1.29)	1.07 (0.66-1.74)

Abbreviations: HR, hazard ratio; CI, confidence interval; SGLT, sodium-glucose cotransporter; DPP, dipeptidyl peptidase

[a] Per 1000 person-years.

[b] Propensity score fine stratification weighting.

SUPPLEMENTAL TABLE 8. Hazard Ratios for the Association Between SGLT-2 inhibitors and Early Breast Cancer (Inverse probability of censoring weighting)

Exposure	Number of patients	Events	Person-years	Incidence rate (95% CI) [a]	Crude HR (95% CI)	Weighted HR (95% CI) [b]
Overall						
DPP-4 inhibitor	36,631	382	103,228	3.7 (3.3-4.1)	1.00 [Reference]	1.00 [Reference]
SGLT-2 inhibitor	9938	67	23,621	2.8 (2.2-3.6)	0.78 (0.60-1.01)	1.00 (0.76-1.30)
Dapagliflozin						
DPP-4 inhibitor	36,650	384	103,289	3.7 (3.4-4.1)	1.00 [Reference]	1.00 [Reference]
Dapagliflozin	5429	50	15,111	3.3 (2.5-4.4)	0.89 (0.66-1.19)	1.16 (0.86-1.56)

Abbreviations: HR, hazard ratio; CI, confidence interval; SGLT, sodium-glucose cotransporter; DPP, dipeptidyl peptidase

[a] Per 1000 person-years.

[b] Propensity score fine stratification and inverse probability of censoring weighting.

CHAPTER 6: DISCUSSION

6.1 SUMMARY OF FINDINGS

The aim of this thesis was to determine whether SGLT-2 inhibitors are associated with an increased risk of early breast cancer among women with type 2 diabetes. This thesis study was conducted in response to signals of a possible association between SGLT-2 inhibitors and breast cancer were first observed in premarketing trials of dapagliflozin. In these premarketing trials, there were numerical imbalances in breast cancer events in patients randomized to the dapagliflozin group compared with placebo, all occurring within one year of randomization (rate ratio 2.47, 95% CI 0.64-14.10).[70] In contrast, no imbalances were observed in subsequent large cardiovascular outcome trials of dapagliflozin and other SGLT-2 inhibitors. [18-20]

To our knowledge, the study described in this thesis is the first to investigate the association between SGLT-2 inhibitors and early breast cancer risk. Using a population-based cohort study from the UK CPRD, we found that use of SGLT-2 inhibitors was not associated with an overall early increased risk of breast cancer among women with type 2 diabetes, when compared with use of DPP-4 inhibitors. While we observed similar findings for empagliflozin and canagliflozin, dapagliflozin was associated with a moderately elevated hazard ratio, but with a wide CI that included the null value (HR 1.16, 95% CI 0.86 to 1.56) and with cumulative incidence curves diverging at around 1.5 years after treatment initiation.

While the discrepant findings between the pre- and post-marketing trials could be due to chance, one hypothesis is that the early breast cancer imbalances are the result of an accelerated tumor promoting effect of SGLT-2 inhibitors. However, this is unlikely given that sodium-glucose cotransporter proteins are not expressed in mammary tissue, and animal studies have failed to

demonstrate that SGLT-2 inhibitors have any neoplastic activity.[93] Another hypothesis relates to the weight-lowering effects of SGLT-2 inhibitors which could facilitate the detection of existing breast lumps, thus leading to a transient over-detection of breast cancer. However, additional studies are required to further assess this association and elucidate the potential biological mechanisms, particularly for dapagliflozin. In addition, as SGLT-2 inhibitors are a relatively new class of drugs, the follow-up period of our study was limited (median 2.6 years). However, this study was designed to assess an early effect of SGLT-2 inhibitors on breast cancer incidence given the early imbalances observed in the premarketing trials (all trials were of short durations, ranging from 24 to 208 weeks).

6.2 IMPLICATIONS OF FINDINGS

Since their approval, SGLT-2 inhibitors have significantly changed the therapeutic landscape for patients with type 2 diabetes. These drugs effectively reduce A1C levels, induce weight loss, and lower blood pressure.[17 66] Importantly, they have been shown to significantly reduce the risk of cardiovascular disease related outcomes.[14] However, there drugs have also been associated with several important adverse events, including diabetic ketoacidosis,[67] genital infections[68] and lower extremity amputations.[69] While there have been concerns that these drugs may be associated with urinary tract infections and fractures, recent studies failed to find any associations with these possible adverse events.[112-114] Additionally, there have been ongoing concerns regarding the potential carcinogenicity of these drugs, particularly with regards to bladder and breast cancer,[18 66 70] although these safety concerns have not been investigated in observational studies until now.

Our study addressed an important knowledge gap with regards to the safety profile of SGLT-2 inhibitors and their effect on breast cancer incidence. It should be noted that almost half of Canadians with type 2 diabetes use second-to-third line antidiabetic drugs.[115] Given the cardiovascular benefits of SGLT-2 inhibitors[14] and their increasing use in patients with type 2 diabetes,[24] a significant number of these patient could be exposed to these drugs. Thus, the findings of this thesis will provide the concerned stakeholders with valuable information that can help better assess the risks and benefits of SGLT-2 inhibitors.

6.3 The role of pharmacoepidemiology

This thesis highlights the important role of pharmacoepidemiology for the assessment of relatively rare outcomes, such as breast cancer. It is important to note that while an RCT would provide the most definitive answer to this safety question, the feasibility of such a study would be severely hindered by important ethical and logistical issues. Thus, well-conducted observational pharmacoepidemiologic studies remain the best means of addressing rare and long-term effects of prescription drugs. With respect to SGLT-2 inhibitors, it will be necessary to reassess their long-term effects after they have been on the market for a significantly long period time (for example, at least 10 years, or around the year 2023). Moreover, it will be critical for these studies to determine whether an association between SGLT-2 inhibitors and breast cancer incidence is the result of tumour promotion or detection bias. Thus, while this study provides some reassurance on the relatively short-term effects of SGLT-2 inhibitors on breast cancer risk, further research will be needed to assess the long-term effects on the incidence of this malignancy.

6.4 FUTURE DIRECTIONS

While the findings of this thesis indicate that there is no association between SGLT-2 inhibitors and early breast cancer, a secondary analysis revealed that dapagliflozin may be associated with an increased risk. However, it should be noted that our study was not designed to assess dapagliflozin specifically, and such secondary analyses should be interpreted with caution. That being said, the risk imbalance observed in our study appears to mirror the imbalances observed in premarketing RCTs. Indeed, canagliflozin and empagliflozin did not generate imbalances with respect to breast cancer when compared to placebo in RCTs,[85 86] while the signal appeared limited to dapagliflozin. This discrepancy was noted by the US FDA and the EMA in their reviews of the dapagliflozin trials. Specifically, they noted that patients randomized to dapagliflozin had a higher number of bladder and breast cancers, compared to those randomized to placebo.[70 87-89] Interestingly, no imbalances were observed in subsequent large cardiovascular outcome trials of dapagliflozin and other SGLT-2 inhibitors.[18-20] Thus, additional observational studies, with larger sample sizes, are needed to investigate the association between dapagliflozin and breast cancer incidence. Finally, while our study provides important information on the safety of SGLT-2 inhibitors, gaps in knowledge remain. To date, no studies have investigated the effect of SGLT-2 inhibitors on bladder cancer, a disease with high relapse rate and poor survival.[116] This remains a contentious issue that will need to be investigated in future studies.

CHAPTER 7: CONCLUSION

The availability of SGLT-2 inhibitors in the past seven years has transformed the therapeutic landscape of type 2 diabetes. These drugs are highly effective for glycemic control and have been shown to offer significant cardiovascular benefits, greatly decreasing the risk of death and heart failure. Since the release of these drugs, there has been extensive research from both RCTs and observational studies on their various benefits and adverse effects. However, their possible association with cancer incidence has not yet been studied in observational studies, in part because they have been recently introduced on the market. The study described in this thesis is the first observational study to investigate the association between SGLT-2 inhibitors and cancer following signals from pre-marketing RCTs. Our findings suggest that SGLT-2 inhibitors are not associated with an increased risk of breast cancer, but that a signal with dapagliflozin may be present. Thus, while this thesis provides an important addition to the growing body of literature surrounding the safety and effectiveness of SGLT-2 inhibitors, large pharmacoepidemiologic studies with longer periods of follow-up will be needed to determine whether these drugs have any neoplastic activity, particularly with breast and bladder cancer. Such endeavors would provide the concerned stakeholders with the necessary information to better understand the risks and benefits on these drugs.

CHAPTER 8: REFERENCES

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