Cardiovascular Safety of Aromatase Inhibitors in Post-Menopausal Women with Breast Cancer

By: Farzin Khosrow-Khavar

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Doctor of Philosophy in Epidemiology

Department of Epidemiology, Biostatistics, and Occupational Health

McGill University

Montreal, Canada

April 2020

Abstract

Aromatase inhibitors (AIs) and tamoxifen are widely used for the treatment of estrogen-receptor positive breast cancer. Meta-analyses of randomized controlled trials (RCTs) have indicated that AIs, in comparison with tamoxifen, are associated with decreased risk of breast cancer-related mortality and better overall survival. This has led to increased treatment of estrogen-receptor positive breast cancer with AIs in the past decade. However, AIs have been associated with increased risk of musculoskeletal symptoms including osteoporosis and fractures. In addition, signals from some RCTs have indicated that AIs, in comparison with tamoxifen, may be associated with increased risk of cardiovascular outcomes. The potential cardiotoxicity of AIs is a concern given that post-menopausal women represent a patient population already at increased risk of cardiovascular disease. To date, four observational studies have examined the cardiovascular safety of AIs with discordant results. These studies had methodological limitations including confounding by indication, informative censoring, and exposure misclassification. Given the uncertainty regarding the cardiovascular risk of AIs, the overall aim of my thesis was to determine whether AIs are associated with increased risk of cardiovascular events in treatment of post-menopausal women with breast cancer.

The objective of the first manuscript was to determine whether AIs are associated with increased risk of cardiovascular outcomes by conducting a systematic review and meta-analysis of RCTs. Overall, a total of 19 RCTs were identified which assessed the efficacy of AIs and reported on cardiovascular outcomes. These RCTs compared AIs directly with tamoxifen in adjuvant setting, AIs with placebo or no-treatment in the extended-adjuvant setting (i.e. after 5 years of treatment with tamoxifen), or tamoxifen with placebo or no treatment in adjuvant or extended adjuvant setting. Overall, in RCTs comparing AIs with tamoxifen, AIs were associated

with a 19% increased risk of cardiovascular events (risk ratio [RR]: 1.19, 95% CI: 1.07-3.14). However, in the extended adjuvant setting, AIs were not associated with an increased risk of cardiovascular outcomes in comparison with placebo or no treatment (RR: 1.01, 95% CI: 0.85-1.20). Finally, tamoxifen was associated with a 33% reduction (RR: 0.67, 95% CI: 0.45-0.98) in risk of cardiovascular outcomes compared with placebo or no treatment. Consistent results were found when examining RCTs reporting ischemic heart disease. The findings from this study suggest that the increased risk of cardiovascular outcomes observed with AIs when compared with tamoxifen in RCTs may be due to cardioprotective effects of tamoxifen. This is consistent with the beneficial effect of tamoxifen in reduction of total cholesterol and low-density lipoprotein (LDL) cholesterol observed in RCTs. These findings are also consistent with a rebound effect observed in an RCT where the levels of LDL and total cholesterol after treatment cessation with tamoxifen were shown to reach the average level observed in post-menopausal women. However, there were limitations to assessing cardiovascular safety of AIs from RCTs. First, RCTs were designed to assess efficacy and not cardiovascular safety. Second, composite endpoints were used to define cardiovascular disease with heterogeneity in the outcome definition. Third, there was heterogeneity regarding duration of follow-up, patient recruitment periods, and patient characteristics. Finally, RCTs included a healthier patient population than those treated in clinical setting.

The objective of the second manuscript was to determine whether upfront AIs, in comparison with upfront tamoxifen, are associated with increased risk of cardiovascular outcomes in women with breast cancer in the real-world setting. A population-based cohort study was conducted using the United Kingdom Clinical Practice Research Datalink linked to the Hospital Episode Statistics and Office for National Statistics. The study population consisted of

women newly diagnosed with breast cancer and newly treated with either upfront AIs or tamoxifen (8,139 and 9,783, respectively). Overall, AIs were associated with an increased risk of heart failure (HR: 1.86, 95% CI: 1.14-3.03) and cardiovascular-mortality (HR: 1.50, 95% CI: 1.11-2.04), when compared with tamoxifen. AIs were also associated with a trend towards an increased risk of myocardial infarction (HR: 1.37, 95% CI: 0.88-2.13) and ischemic stroke (HR: 1.19, 95% CI: 0.82-1.72). Thus, the findings from this observational study indicate that upfront AIs, in comparison with upfront tamoxifen, are associated with an increased risk of clinically relevant cardiovascular outcomes in setting of clinical practice.

The objective of the third manuscript was to determine whether AIs in sequential treatment with tamoxifen, when compared with upfront treatment with tamoxifen, are also associated with increased risk of cardiovascular outcomes. Current clinical guidelines recommend treatment of post-menopausal women with breast cancer with upfront AIs, upfront tamoxifen, or sequential treatment of AIs with tamoxifen in the adjuvant setting. These recommendations are based on results from RCTs showing better efficacy with upfront or sequential AIs treatment when compared with upfront tamoxifen treatment. Treatment switch from tamoxifen to AIs may improve efficacy when compared with upfront tamoxifen while allowing favourable effects of tamoxifen such as lower risk of musculoskeletal symptoms when compared with upfront AIs. However, there is limited data regarding the risk of cardiovascular outcomes with AIs in the sequential setting with tamoxifen. This potential safety concern is important when weighing the net clinical benefit of sequential treatment strategy for patients with estrogen-receptor positive breast cancer. To address this question, a retrospective cohort study was conducted where 1,962 patients who switched from tamoxifen to AIs were matched to 3,874 patients on upfront tamoxifen treatment. Patients were matched on duration of tamoxifen

use and time-conditional propensity scores. Overall, the use of AIs in the sequential setting, compared with upfront tamoxifen, was associated with an increased risk of myocardial infarction (HR: 2.08, 95% CI: 1.02-4.27) and a trend towards an increased risk of ischemic stroke (HR: 1.58, 95% CI: 0.85-2.93) and heart failure (HR: 1.69, 95% CI: 0.79-3.62) but not cardiovascular mortality (HR: 0.87, 95% CI: 0.49-1.54). The results from this study indicate that AIs in sequential treatment with tamoxifen are also associated with increased risk of cardiovascular outcomes compared with upfront tamoxifen treatment. However, these results may also be due to cardioprotective effects of tamoxifen.

Overall, the findings from this thesis provide important knowledge regarding cardiovascular safety of AIs in treatment of hormone-receptor positive breast cancer. Specifically, these results suggest that AIs as upfront or in sequential treatment with tamoxifen are associated with increased risk of clinically relevant cardiovascular outcomes when compared with upfront tamoxifen use in in the real-world setting. However, the results from the systematic review and meta-analysis of RCTs suggest that cardioprotective effects of tamoxifen may account for the observed increased risk associated with AIs. Nevertheless, the different effects of AIs and tamoxifen on risk of cardiovascular outcomes should be considered when considering the net clinical benefit of these drugs in the treatment of women with estrogen-receptor positive breast cancer. Further studies are required to establish potential cardioprotective mechanisms of tamoxifen.

Résumé

Les inhibiteurs de l'aromatase (IA) et le tamoxifène sont largement utilisés dans le traitement du cancer du sein avec récepteurs aux œstrogènes positifs. Les méta-analyses d'essais cliniques randomisés (ECR) ont indiqué que les IA, comparativement au tamoxifène, sont associés à une diminution du risque de mortalité liée au cancer du sein et à une meilleure survie globale. Au cours de la dernière décennie, cela a mené à une augmentation du traitement du cancer du sein avec récepteurs d'œstrogènes positifs par des IA. Toutefois, les IA ont été associées à un risque accru de symptômes musculo-squelettiques, y compris d'ostéoporose et de fractures. De plus, des signaux provenant de certains ECR ont indiqué que les IA, comparativement au tamoxifène, peuvent être associés à un risque accru de maladies cardiovasculaires. La cardiotoxicité potentielle des IA est préoccupante étant donné que les femmes ménopausées représentent une population de patientes ayant déjà un risque accru de maladies cardiovasculaires. Jusqu'à présent, quatre études observationnelles ont examiné l'innocuité cardiovasculaire des IA, et les résultats sont discordants. Ces études présentaient des limites méthodologiques, notamment la confusion par indication, la censure informative et la mauvaise classification de l'exposition. Compte tenu de l'incertitude entourant le risque cardiovasculaire des IA, l'objectif global de ma thèse était de déterminer si les IA sont associés à un risque accru d'événements cardiovasculaires dans le traitement des femmes ménopausées atteintes du cancer du sein.

L'objectif du premier manuscrit était de déterminer si les IA sont associées à un risque accru de maladies cardiovasculaires en effectuant une revue systématique et une méta-analyse des ECR. Dans l'ensemble, un total de 19 ECR ont été identifiés pour évaluer l'efficacité des IA et faire rapport sur les résultats cardiovasculaires. Ces ECR ont comparé directement les IA avec

le tamoxifène en milieu adjuvant, les IA avec placebo ou sans traitement en milieu adjuvant prolongé (c.-à-d. après 5 ans de traitement par le tamoxifène) ou le tamoxifène avec placebo ou sans traitement en milieu adjuvant ou adjuvant prolongé. Dans l'ensemble, dans les ECR comparant les IA au tamoxifène, les IA étaient associés à un risque accru d'événements cardiovasculaires de 19% (Risque Relatif [RR]: 1.19, Intervalle de Confiance [IC] 95%: 1.07-3.14). Cependant, dans le cadre d'un traitement adjuvant prolongé, les IA n'étaient pas associés à un risque accru de complications cardiovasculaires comparativement au placebo ou à l'absence de traitement (RR: 1.01, IC 95%: 0.85-1.20). Enfin, le tamoxifène était associé à une réduction de 33% (RR: 0.67, IC 95%: 0.45-0.98) du risque de complications cardiovasculaires comparativement au placebo ou à l'absence de traitement. Des résultats similaires ont été obtenus pour les ECR rapportant l'issue de cardiopathie ischémique. Les résultats de cette étude suggèrent que le risque accru d'issues cardiovasculaires observé avec les IA comparativement au tamoxifène dans les ECR pourrait être attribuable aux effets cardioprotecteurs du tamoxifène. Ceci est conforme à l'effet bénéfique du tamoxifène sur la réduction du cholestérol total et des lipoprotéines de basse densité (LDL) observée dans les ECR. Ces résultats concordent également avec un effet de rebond observé dans un ECR où les taux de LDL et de cholestérol, après l'arrêt du traitement au tamoxifène, avaient atteint le taux moyen observé chez les femmes ménopausées. Toutefois, l'évaluation de l'innocuité cardiovasculaire des IA à partir des ECR comporte des limites. Premièrement, les ECR ont été conçus pour évaluer l'efficacité et non l'innocuité cardiovasculaire. Deuxièmement, des critères d'évaluation composites ont été utilisés pour définir les maladies cardiovasculaires avec une hétérogénéité dans la définition des issues rapportées. Troisièmement, il y avait de l'hétérogénéité quant à la durée du suivi, aux périodes de

recrutement des patients et aux caractéristiques des patients. Enfin, les ECR portaient sur une population de patients en meilleure santé que ceux traités en milieu clinique.

L'objectif du deuxième manuscrit était de déterminer si le traitement initial avec les IA, comparativement au traitement initial avec le tamoxifène, est associé à un risque accru de complications cardiovasculaires chez les femmes atteintes de cancer du sein dans la population générale. Une étude de cohorte populationnelle a été menée à l'aide de la base de données « Clinical Practice Research Datalink » du Royaume-Uni, ainsi que les données de « Hospital Episode Statistics » pour les données d'hospitalisations et les données de « Office for National Statistics » pour les données d'état civil. La population à l'étude se composait de femmes nouvellement diagnostiquées d'un cancer du sein et nouvellement traitées soit par des IA, soit par le tamoxifène (8,139 et 9,783, respectivement). Dans l'ensemble, les IA étaient associés à un risque accru d'insuffisance cardiaque (Hazard Ratio [HR]: 1.86, IC 95%: 1.14-3.03) et de mortalité cardiovasculaire (HR: 1.50, IC 95%: 1.11-2.04), comparativement au tamoxifène. Les IA étaient également associés à une tendance à l'augmentation du risque d'infarctus du myocarde (HR: 1.37, IC 95%: 0.88-2.13) et d'accident ischémique cérébral (HR: 1.19, IC 95%: 0.82-1.72). Ainsi, les résultats de cette étude observationnelle indiquent que le traitement initial avec les IA, comparativement au traitement initial avec le tamoxifène, est associé à un risque accru de maladies cardiovasculaires cliniquement pertinentes dans le contexte de la pratique clinique courante.

L'objectif du troisième manuscrit était de déterminer si les IA en traitement séquentiel au tamoxifène, comparativement au traitement initial au tamoxifène, sont également associés à un risque accru de complications cardiovasculaires. Les lignes directrices cliniques actuelles recommandent le traitement des femmes ménopausées atteintes d'un cancer du sein initialement

par des IA, par le tamoxifène ou le traitement séquentiel avec les IA et le tamoxifène dans un contexte adjuvant. Ces recommandations sont fondées sur les résultats d'ECR montrant une meilleure efficacité avec un traitement initial ou séquentiel des IA comparativement au traitement initial au tamoxifène. Le passage du tamoxifène aux IA peut améliorer l'efficacité du traitement par rapport au tamoxifène initial, tout en profitant des effets favorables du tamoxifène, tels qu'un risque moindre de symptômes musculo-squelettiques, comparativement aux IA comme premier traitement. Toutefois, on dispose de peu de données sur le risque de complications cardiovasculaires associées aux IA dans le contexte d'un traitement séquentiel avec le tamoxifène. Ce problème d'innocuité potentiel est important afin d'évaluer l'avantage clinique net d'une stratégie de traitement séquentiel pour les patientes atteintes d'un cancer du sein à récepteurs d'œstrogènes positifs. Pour répondre à cette question, une étude de cohorte rétrospective a été menée auprès de 1,962 patientes qui sont passés du tamoxifène aux IA et de 3, 874 patientes sous traitement initial au tamoxifène. Les patientes ont été appariées en fonction de leur durée d'utilisation du tamoxifène et des scores de propension pouvant varier dans le temps. Dans l'ensemble, l'utilisation des IA en traitement séquentiel comparativement au tamoxifène initial était associée à un risque accru d'infarctus du myocarde (HR: 2.08, IC 95%: 1.02-4.27) et à une tendance vers un risque accru d'accident ischémique cérébral (HR: 1.58, IC 95%: 0.85 - 2.93) et d'insuffisance cardiaque (HR: 1.69, IC 95%: 0.79-3.62), mais n'était pas associé au risque de décès cardiovasculaire (HR: 0.87, IC 95%: 0.49-1.54). Les résultats de cette étude indiquent que les IA en traitement séquentiel au tamoxifène sont également associés à un risque accru de complications cardiovasculaires comparativement au traitement initial au tamoxifène. Toutefois, ces résultats peuvent également être dus aux effets cardioprotecteurs du tamoxifène.

Dans l'ensemble, les résultats de cette thèse fournissent des connaissances importantes sur l'innocuité cardiovasculaire des IA dans le traitement du cancer du sein à récepteurs d'œstrogènes positifs. Plus précisément, ces résultats suggèrent que les IA comme traitement initial ou séquentiel au tamoxifène sont associés à un risque accru de maladies cardiovasculaires cliniquement pertinentes comparativement à l'utilisation initiale du tamoxifène. Toutefois, les résultats de la revue systématique et de la méta-analyse des ECR suggèrent que les effets cardioprotecteurs du tamoxifène pourraient expliquer le risque accru observé associé aux IA. Néanmoins, les différents effets des IA et du tamoxifène sur le risque de maladies cardiovasculaires devraient être pris en compte lorsque l'on considère le bénéfice clinique net de ces médicaments dans le traitement des femmes atteintes d'un cancer du sein à récepteurs d'œstrogènes positifs. D'autres études sont nécessaires pour établir les mécanismes cardioprotecteurs potentiels du tamoxifène.

Acknowledgements

I would like to take this opportunity to express gratitude to all individuals who have helped me throughout my doctoral thesis training.

First, I would like to thank my thesis supervisors Dr. Laurent Azoulay and Dr. Samy Suissa for their guidance and support and for providing an ideal training environment as doctoral student. I could not have asked for a more supportive and rigorous training environment during my doctoral training. I would like to thank my supervisors for dedication to mentorship and guidance in all aspects of my training as a doctoral student. I also want to express my gratitude to my supervisors for providing countless opportunities to grow and learn by partaking in non-thesis projects, seminars, journal clubs, and conferences which greatly enriched my training as a doctoral student in Epidemiology. Finally, I would also like to thank my supervisors for providing constant support and advice regarding my career aspirations.

Second, I would like to thank my committee members Dr. Kristian Filion and Dr. Nathaniel Bouganim for providing input on my thesis projects. Specifically, I would like to thank Dr. Filion for also providing opportunity to partake in collaborative research projects and his thoughts and input on my thesis projects. I would also like to thank Dr. Filion for providing opportunity to engage in teaching and his support and advice in regard to my academic and career endeavors.

Third, I would like to express my gratitude to the faculty members and staff at Department of Epidemiology, Biostatistics and Occupational Health at McGill University. My time as a graduate student in this department has been special and I could not imagine being immersed in a more supportive and at the same time rigorous department. I would like to thank all the faculty members for providing a stimulating research environment as a graduate student. Specifically, I would like to acknowledge faculty members who provided solid foundation in Epidemiology and Biostatistics through their exemplary teaching including Dr. Nandi, Dr. Harper, Dr. Kaufman, Dr. Platt, Dr. Chevrier, Dr. Abrahamowicz, and Dr. Joseph. I would like to express my gratitude to André Yves Gagnon and Katherine Hayden for their support since the beginning of my graduate studies.

Fourth, I would like to thank my colleagues including fellow graduate students, post-doctoral fellows, and faculty members at Centre for Clinical Epidemiology at Jewish General Hospital. Specifically, I would like to thank Dr. Christel Renoux for our discussions, opportunity to engage in non-thesis projects, and advice regarding research, careers, and academia and Dr. Antonios Douros for our daily discussions about pharmacoepidemiology and non-thesis projects. I would also like to thank Marisa Mancini for her help with student affairs during my doctoral studies. I would also like to express my gratitude to Hui Yin and Sophie Dell'Aniello for technical assistance and for providing a supportive environment to discuss the details of data analysis. I would also like to acknowledge past and present students and post-doctoral fellows for our daily discussions which allowed me to venture outside my own thesis and develop as a researcher and pharmacoepidemiologist. Thank you for your input, encouragement, and a friendly work environment. I would like to greatly thank Vanessa Brunetti with her assistance in translation of the thesis abstract and Dr. Francois Montastruc for collaboration on non-thesis projects.

Fifth, I would like to acknowledge my PhD cohort members, classmates, and friends at Department of Epidemiology, Biostatistics, and Occupational Health for their support. I would like to thank Dr. Oliver Lasry for always pushing the envelope to think deeply about the classroom content during early years in the program while we were building the foundation in Epidemiology. His exemplary work ethic and strive for excellence has been a great example and a source of motivation. I would also like to greatly thank Dr. Sonia Grandi for the discussions and her support through our journey as graduate students from the very beginning in the program to the comprehensive exam, protocol defense, and transition to our careers after completion of our studies at McGill. I look forward to continuing our friendships and seeing the progression of your careers.

Finally, I would like to dedicate my thesis to my family including my parents and siblings for their unwavering support and encouragement throughout the past six years and throughout my life. I could not have done this without you!

Contributions of Authors

Manuscript 1: Cardiotoxicity of aromatase inhibitors and tamoxifen in post-menopausal women with breast cancer: a systematic review and meta-analysis of randomized controlled trials. *Ann Oncol.* 2017; 28(3): 487-496.

I developed the research question with guidance from my supervisors Dr. Azoulay and Dr. Suissa. I was responsible for conducting the search and screening the articles. Data extraction and quality assessment of RCTs was conducted in parallel with Dr. Shatha Al-Qurashi at Department of Oncology. I conducted the data synthesis and meta-analysis and drafted the manuscript. All co-authors provided input in regard to methods and interpretation of results, reviewed the manuscript, and provided input during manuscript revision.

Manuscript 2: Aromatase Inhibitors and Risk of Cardiovascular Outcomes in Women with Breast Cancer: A Population-Based Cohort Study. *Circulation* 2020; 141(7): 549-559.

I conceptualized and developed of the objective of this manuscript with Dr. Azoulay and Dr. Suissa. I drafted the protocol for data request and ethics approval from UK Clinical Practice Research Datalink independent Scientific Advisory and Jewish General Hospital. I was responsible for data management and cohort construction, data analysis, and drafting of the manuscript. All authors contributed to study design and methodology, interpretation of the results, and revision of the manuscript.

Manuscript 3: Cardiotoxicity of Sequential Aromatase Inhibitors Therapy in Women with Breast Cancer. *American Journal of Epidemiology,* In Press.

I conceptualized and developed the objective for this project with Dr. Azoulay and Dr. Suissa. I drafted the protocol for data request and ethical approval from UK Clinical Practice Research Datalink independent Scientific Advisory, McGill University and Jewish General Hospital. I was responsible for data management and cleaning, construction of the cohort, and performing all data analyses, interpreting the results, and drafting the manuscript. All co-authors were also involved in reviewing the manuscript for intellectual content and interpretation of the results.

Statement of Contribution of Original Knowledge

The research presented in this thesis consists of original contributions which further knowledge of cardiovascular safety of aromatase inhibitors (AIs) and tamoxifen in treatment of post-menopausal women with hormone receptor-positive breast cancer. Previous systematic review and meta-analysis of randomized controlled trials (RCTs) had assessed the cardiovascular safety of AIs based on data from trials directly comparing AIs with tamoxifen. Based on this evidence, regulatory agencies such as US Food and Drug administration have indicated ischemic heart disease as a potential harm associated with AIs. In the first objective, we conducted a systematic review and meta-analysis of RCTs which included evidence from RCTs directly comparing AIs with tamoxifen, tamoxifen with placebo, and AIs with placebo or no treatment in patients previously treated with five years of tamoxifen in post-menopausal women with breast cancer. When considering the totality of evidence, we also found that AIs, in comparison with tamoxifen, are associated with increased risk of cardiovascular outcomes. However, we also found this increased risk in the direct comparison may be at least partially due to cardioprotective effects of tamoxifen.

The aim of the second and third objectives in this thesis were to determine whether AIs administered as upfront treatment or in sequential treatment after tamoxifen are associated with increased risk of cardiovascular outcomes, when compared with upfront tamoxifen, in the setting of clinical practice. Current clinical guidelines recommend treatment of post-menopausal women with hormone receptor positive breast cancer with AIs either as upfront therapy or in sequential treatment with tamoxifen for up to ten years. This is based on results from RCTs that have demonstrated similar efficacy when comparing sequential treatment with tamoxifen and AIs to upfront treatment with AIs. Overall, we found that in the setting of routine clinical practice, AIs

in the upfront or sequential setting with tamoxifen, when compared with upfront tamoxifen, were associated with increased risk of cardiovascular outcomes. The observational study in Objective 2 addressed the limitations in design and methodology of previous observational studies and was the first study to comprehensively examine risk of serious cardiovascular outcomes including MI, ischemic stroke, heart failure, and cardiovascular mortality. The observational study in objective 3 was the first observational studies to have considered the cardiovascular risk of the sequential treatment strategy. Overall, this thesis provides an important addition regarding the cardiovascular safety of AIs and tamoxifen and demonstrates differential cardiovascular effects of these drugs in RCTs and in the setting of clinical practice. The differential effects of AIs and tamoxifen on cardiovascular outcomes could be an important consideration when deciding on the optimal treatment strategy for patients with hormone receptor positive breast cancer.

I declare that I received guidance from my supervisors in regard to my thesis objectives and input from my thesis committee members concerning methodological and substantive aspects of my thesis. The conception, execution, and drafting of the manuscript and thesis were entirely my own.

Statement of Financial Support

I would like to thank various sources of funding and institutions that have provided support throughout my doctoral studies. This includes stipend from McGill Department of Epidemiology, Biostatistics and Occupational Health and studentships from McGill Faculty of Medicine and Lady Davis Institute. In addition, I was supported by a training award from Canadian Institutes for Health Research Drug Safety and Effectiveness Safety Program, Doctoral award from Fonds de Recherche du Québec Santé, and Novartis Young Oncology Investigator Award. Finally, I have also received GREAT travel award from McGill Department of Epidemiology, Biostatistics, and Occupational Health and travel awards from International Society for Pharmacoepidemiology. The projects in this thesis were generously supported by a foundation grant from Canadian Institutes for Health Research.

Table of Contents

List of Tables

List of Figures

List of Acronyms and Abbreviations

AI	Aromatase inhibitor
ABCSG	Austrian Breast and Colorectal Cancer Study Group
ARNO	Arimidex-Novaldex
ASA	Acetylsalicylic acid
ASCO	American Society of Clinical Oncology
ATAC	Anastrozole, Tamoxifen, Alone or in Combination
ATENA	Adjuvant post-Tamoxifen Exemestane versus Nothing Applied
ATLAS	Adjuvant Tamoxifen: Longer Against Shorter
BIG	The Breast International Group
BRCA1	Breast cancer type 1 susceptibility protein
BRCA2	Breast cancer type 2 susceptibility protein
CPRD	Clinical Practice Research Datalink
CVA	Cerebrovascular accident
CVD	Cardiovascular disease
CVE	Cardiovascular event
CYP	Cytochrome P450
ER	Estrogen Receptor
ESMO	European Society of Medical Oncology
FACE	Femara Versus Anastrozole Clinical Evaluation
FDA	Food and Drug Administration
FE	Fixed Effects
FRQS	Fonds de recherche du Québec - Santé
GP	General practitioner
HDL	High density lipoprotein
HER2	Human epidermal growth factor 2 receptor
HES	Hospital Episode Statistics
HF	Heart failure
HR	Hazard ratio
IBIS	International Breast Cancer Intervention Study
ICD	International Classification of Diseases
ICTRP	International Clinical Trials Registry Platform
IES	Intergroup exemestane study
IGF	Insulin-like growth factor
IMS	Intercontinental marketing system
IPCW	Inverse probability of censoring weighting

IPTW	Inverse probability of treatment weighting
IRR	Incidence rate ratio
ITA	Italian tamoxifen anastrozole
LDL	Low-density lipoprotein
MI	Myocardial infarction
MRC	Medical research council
MRI	Magnetic resonance imaging
NA	Not available
NC	Not classified
NCIC	National Cancer Institute of Canada
NHS	National health services
NOS	Not other otherwise specified
NSABP	National surgical adjuvant breast and bowel project
ONS	Office for National Statistics
OPCS	Office of Population, Censuses and Surveys Classification of Surgical Operations and Procedures
OR	Odds ratio
PR	Progesterone receptor
QOF	Quality Outcomes Framework
RCT	Randomized controlled trial
RD	Risk difference
RR	Relative risk
SEER	Surveillance, Epidemiology, and End Results program
SERM	Selective estrogen receptor modulator
SITAM-01	Italian Study of Adjuvant Treatment in Breast Cancer 01 (SITAM-01)
TEAM	Tamoxifen Exemestane Adjuvant Multinational trial
TGF	Transforming growth factor
TIA	Transient ischemic attack
UK	United Kingdom
US	United States
UTS	Up to standard
VTE	Venous thromboembolism
WHO	World health organization

Chapter 1. Introduction

1.1 Overview

Breast cancer is the second most commonly diagnosed malignancy and the second leading cause of cancer-related mortality among females globally.[1,2] As estrogens play a key role in breast cancer carcinogenesis,[3] drugs have been developed to block the effects of these hormones in women with breast cancer. These drugs include tamoxifen, which has been used for over 30 years, as well as third generation aromatase inhibitors (AIs) consisting of anastrozole, letrozole, and exemestane which are rapidly replacing tamoxifen in post-menopausal women with early hormone-sensitive breast cancer.[4,5]

In Canada, provincial health ministries recommend the treatment of post-menopausal women with early stage estrogen-receptor positive breast cancer with tamoxifen or AIs for up to five years in the adjuvant setting (i.e. after primary treatment with surgery).[6-8] AIs may be administered as upfront or sequential therapy with tamoxifen. In upfront treatment, patients initiate and complete five years of treatment with AIs as monotherapy. In sequential treatment, patients initiate treatment with tamoxifen and switch to AIs after 2-3 years.[9-11] These guidelines are based on findings from randomized controlled trials (RCTs) that demonstrated AIs were associated with an increased disease-free survival (defined as local or distant recurrence, new primary breast cancer, or death from any cause), distant disease-free survival, and decreased incidence of contralateral breast cancers in comparison with tamoxifen.[12-16] In addition, a recent individual patient-data meta-analysis demonstrated that AIs are superior to tamoxifen in reducing breast cancer-related and all-cause mortality.[17] The two treatment strategies with AIs (upfront AIs or sequential AIs) have similar efficacy in reducing breast cancer recurrence, breast cancer related mortality and all-cause mortality.[17]

AIs and tamoxifen have been associated with different adverse outcomes in RCTs. Tamoxifen has consistently been associated with an increased risk of endometrial cancer and venous thromboembolism,[18-23] whereas AIs have been associated with an increased risk of fractures.[12,13,22-25] Meta-analyses of RCTs comparing AIs with tamoxifen have also suggested that AIs may be associated with an increased risk of cardiovascular events.[23,26,27] Majority of these RCTs compared upfront treatment with AIs with upfront treatment with tamoxifen. There was sparse data regarding cardiovascular safety of sequential treatment with tamoxifen and AIs. Nevertheless, based on these results from RCTs, current guidelines from the American Society of Clinical Oncology (ASCO) identify ischemic heart disease as a potential harm associated with AIs.[10] Some experts have argued that the lack of overall survival benefit observed in RCTs comparing AIs with tamoxifen may be due to cardiotoxicity of AIs.[4,28] However, these conclusions are controversial as the increased risk of cardiovascular outcomes associated with AIs may be due to comparison with tamoxifen, which may have cardioprotective effects.[29-31] The potential cardiotoxicity of AIs is a concern given that post-menopausal women represent a patient population already at increased risk of cardiovascular disease and a higher risk of mortality is attributable to cardiovascular disease in breast cancer survivors than those without breast cancer.[32]

1.2 Research Objectives

The overall purpose of my thesis is to address important gaps in knowledge regarding the cardiovascular safety of AIs. There are three objectives which address this overarching aim:

1. To determine the risk of cardiovascular outcomes when comparing AIs with tamoxifen, AIs with placebo or no treatment in patients previously treated with five years of tamoxifen, and tamoxifen with placebo in post-menopausal women with breast cancer by conducting a systematic review and meta-analysis of RCTs.

2. To determine whether treatment with upfront AIs, compared with upfront tamoxifen, is associated with an increased risk of cardiovascular outcomes, including myocardial infarction (MI), ischemic stroke, heart failure, and cardiovascular mortality, in post-menopausal women with breast cancer in the setting of routine clinical practice.

3. To determine whether sequential treatment with tamoxifen and AIs, compared with continuous tamoxifen treatment, is associated with an increased risk of cardiovascular outcomes in post-menopausal women with breast cancer in setting of routine clinical practice.

1.3 Thesis Organization

This thesis is manuscript based. Chapter 2 presents the background for my overarching research question, including the epidemiology of breast cancer, diagnosis of breast cancer, and treatments for breast cancer. In addition, the efficacy and toxicity of AIs and tamoxifen is described, including the cardiovascular safety of AIs and tamoxifen in RCTs and observational studies. Chapter 3 provides an overview of primary data sources used in the observational studies conducted to address Objectives 2 and 3 and a description of the United Kingdom Clinical Practice Research Datalink (CPRD), the Hospital Episode Statistics (HES), and Office for National Statistics (ONS). Chapter 4 consists of a systematic review and meta-analysis of RCTs assessing the risk of cardiovascular outcomes of AIs and tamoxifen in post-menopausal women with breast cancer. Chapter 5 presents an observational study assessing the risk of cardiovascular outcomes, including MI, ischemic stroke, heart failure, and cardiovascular mortality, comparing upfront treatment with AIs with upfront treatment with tamoxifen. Chapter 6 consists of an observational study assessing the risk of cardiovascular outcomes when comparing sequential treatment with tamoxifen and AIs in comparison with continuous tamoxifen treatment in post-menopausal women with breast cancer. Finally, Chapter 7 summarizes findings from three

manuscripts and provides a discussion of the overall strengths and limitations, overall clinical and public health implications, and discussion of further research in this domain. References for Chapters 1-3 and Chapter 7 are provided at the end of the thesis. References for Chapters 3-5 (manuscripts) are presented in the corresponding chapters.

Chapter 2. Background

2.1 Breast Cancer Epidemiology

Breast cancer is the second most commonly diagnosed cancer and is the second leading cause of cancer mortality among women globally.[1,2] In 2015, approximately 25,000 women in Canada were diagnosed with breast cancer, with 5,000 breast cancer-related deaths.[33] The risk of breast cancer increases with age, with approximately 82% of incident cases occurring in women over the age of 50.[33] In Canada, one in nine women is expected to develop breast cancer during her lifetime, and one in thirty women is expected to die from this disease[33] and the average five-year survival rate for invasive breast cancer is 88%.[34] Globally, there are approximately 2.1 million incident cases of breast cancer annually, 627,000 breast-cancer related deaths, and the five-year prevalence of breast cancer is approximately 6.9 million patients.[1] The incidence of breast cancer varies by region, with highest incidence observed in United States and Northern Europe followed by Southern and Eastern Europe, South America, and Asia.[1]

Non-modifiable risk factors for breast cancer include age, race, timing of menarche, timing of menopause, and physiological factors.[35] Breast cancer incidence and mortality increase with age, with risks peaking at approximately 60 years of age. In United States, the incidence of breast cancer is higher among non-Hispanic Caucasian women when compared with African-American women, Hispanics, American-Indians, and Asians.[36] Younger age at menarche and older age at menopause have also been associated with increased risk of breast cancer.[35,37] Family history of breast cancer has been associated with increased risk of breast cancer while genetic factors such as mutations in tumour suppressor genes breast cancer type 1 susceptibility protein (BRCA1) and breast cancer type 2 susceptibility protein (BRCA2) are accountable for 5% to 10% of all breast cancers.[2,38] Patients diagnosed with non-invasive ductal or lobular carcinoma in situ have been show to have augmented risk of breast cancer.[39] In addition, physiological factors such as higher

circulating levels of endogenous estrogens, prolactin, and insulin-like growth factor 1 (IGF-1) in post-menopausal women have been associated with increased risk of breast cancer.[40-43]

Modifiable risk factors for breast cancer include socioeconomic status, reproductive patterns, lifestyle factors, exogenous hormones, and environmental exposures.[35] Studies have indicated that nulliparity is associated with higher risk of breast cancer while having a first child at younger age, higher number of births, less time between births, and breastfeeding are associated with lower risk of breast cancer.[35,44] One study has shown that the risk of pre-menopausal breast cancer increases by 5% for each year of delay in first birth whereas a one year delay in menopause is associated with a 3% increased risk.[45,46] Longer duration of breastfeeding has also been shown to decrease the risk of breast cancer.[47,48] Lifestyle factors including high levels of alcohol and caffeine consumption, high body mass index, and lack of physical activity may also increase the risk of breast cancer.[35,49] In addition, results from the Women's Health Initiative randomized trial indicate that oral hormonal therapy consisting of estrogen and progesterone may increase the risk of breast cancer.[50] Finally, exposure to diagnostic radiation and organic chlorides (which act as estrogenic agents) have also been associated with increased risk of breast cancer.[35,51]

2.2 Breast Cancer Detection and Diagnosis

2.2.1 Screening

Breast cancer is commonly detected through routine screening using mammography or as a result of presentation of symptoms (such as pain or palpable mass).[52] Healthy women are screened to detect tumours at an earlier stage, leading to more successful treatment and improved survival.[53] Mammography is the mainstay modality of screening and associated with an approximately 20% reduction in breast-cancer related mortality for women at average risk of breast cancer when compared with no screening.[53] However, this benefit is variable depending on age,

with less pronounced benefits for women in their 40s due to increased risk of false positives in women who have higher breast density.[52] In addition, screening with mammography can lead to overdiagnosis, leading to unnecessary treatment and psychological distress.[52] Current clinical guidelines in Canada recommend screening once every two to three years for women aged 50 to 74 years who are not at increased risk of breast cancer years.[52] Mammography may be combined with other detection modalities including magnetic resonance imaging (MRI) or ultrasound in women at high risk of breast cancer.[52] The use of MRI and ultrasound with mammography has been shown to increase sensitivity of detection of malignant breast cancer. However, MRI and ultrasound are also susceptible to high false positive rate.[54] Thus, Canadian guidelines do not recommend use of MRI or ultrasound in women who are not at increased risk of breast cancer.[52]

2.2.2 Pathological Evaluation and Tumour Grade and Stage

Fine-needle aspiration, core biopsy, or surgical excision are used to obtain biospecimen of the tumour for diagnosis of breast cancer. Subsequently, histological cross-sections are combined with immunohistochemistry and molecular-based diagnostic tests for breast cancer diagnosis. The progression of breast cancer is classified into five anatomical stages.[55] In Stage 0 breast cancer, which corresponds to carcinoma in situ, cancer cells are non-invasive. In Stage 1 breast cancer, tumours are less than 2 cm in size and some cells may have infiltrated the lymph nodes. In Stage 2 breast cancer, the tumour can be up to 5 cm or lager with cancer cells that have spread to up to 9 axillary lymph nodes and internal mammary lymph nodes. In Stage 3, the tumour can be larger than 5 cm with cancer cells that may have spread to more than 10 axillary lymph nodes, internal mammary lymph nodes, supraclavicular (above collar bone) lymph nodes, or infraclavicular (below collar bone) lymph nodes.[55] Finally, in Stage 4, the tumour has spread to other body parts (metastasis), including bone, liver, lungs, or brain.[55] Overall, the survival rate of patients with

breast cancer differs by stage of diagnosis. In United States, the overall 5-year probability of breast cancer survival for localized breast cancer is 99% (which corresponds to 62% of all diagnoses of breast cancer).[56] For cancer that has spread to regional lymph nodes, the 5-year survival is 85%, while 5-year survival probability is only 26% for metastatic breast cancer.[56]

Tumour grade describes the morphology of the cancer cells and how quickly these cells can grow and spread. Grade 1 corresponds to slow growing cancer cells that appear small and uniform. Grade 2 corresponds to cancer cells that do not appear like normal cells and are faster growing, and Grade 3 cancer cells appear different and are fastest-growing.[57]

2.2.3 Breast Cancer Histological Subtypes

Breast cancer is classified as invasive ductal carcinoma or invasive lobular carcinoma depending on the origin of the site of the tumour. Ductal carcinomas originate in the milk ducts and account for 80% of all malignant breast cancer.[58] In contrast, invasive lobular carcinoma occurs in lobules in the breast and accounts for 10% of patients with malignant breast.[58] Some studies have indicated that in post-menopausal women, patients with diagnosis of invasive lobular carcinoma have higher survival and better prognosis in comparison with patients with ductal carcinoma.[59,60] Ductal and lobular carcinoma in situ are common forms of non-invasive breast cancer that are confined to the ducts and lobules, respectively.[61]

2.2.4 Breast Cancer Molecular Subtypes and Receptor Status

Breast cancers are characterized by differential expression of receptors status of the tumour: estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) positive, or triple negative (not positive for the three receptors).[62] Approximately 75% of all breast cancers are classified as ER positive (ER+).[4,62] Estrogen receptors are involved in regulating the growth, differentiation, and function of the mammary gland.[63]

Prolonged exposure of the ER to estrogen can lead to abnormal cell growth and development of breast cancer.[63,64] In contrast, tumorigenesis in ER-negative type breast cancers is estrogen independent and also less common.[65] Thus, anti-estrogenic drugs are used to treat patients with ER-positive breast tumours as ER negative tumours are unaffected by the action of these drugs.[4] Similarly, human epidermal growth factor receptor 2 (HER2) is a protein that initiates signalling pathways leading to cell-cycle progression, proliferation, and differentiation. However, this receptor is overexpressed in 15-30% of invasive breast cancer, and overexpression of this receptor has been shown to lead to tumorigenesis.[66] A recent observational study using Surveillance, Registries, and End Results Registries (SEER) found that among patients with invasive breast cancer and with known HR/HER2 receptor status (i.e. 88%), approximately 73% were ER+/PR+ and HER2-, 10% were HR+/HER+, 12% were triple negative (did not express ER, PR, or HR), and 5% were HR- and HER2+.[67] Breast cancer is classified into four subtypes.[68] Luminal A refers to hormone receptor positive, HER2 negative breast cancer with low expression of Ki-67 protein which controls the growth of cancer cells. Luminal A breast cancers are slow progressing and have the best prognosis. [68] Luminal B breast cancers are faster growing, hormone receptor positive, with presence or absence of HER2 and high expression of Ki-67.[68] HER2-enriched breast cancers are hormone-receptor negative, and HER2 positive cancers tend to grow faster and have worse prognosis in comparison with luminal cancers.[68] Finally, triple negative cancers are fastest growing and are most common among patients with mutations in BRCA1 genes and African-American women.[68]

2.3 Breast Cancer Treatment

The treatment strategy for early stage breast cancer is outlined in Figure 2.1. For early stage non-metastatic breast cancer, the objective of treatment is to surgically remove the tumour

from breast and regional lymph nodes and eradicate the tumour using chemotherapy or radiation therapy. Surgical removal of the tumour is the primary treatment for breast cancer and consists of either mastectomy, where the entire breast is removed, or breast-conserving surgery (also known as lumpectomy or partial mastectomy), where only the tumour and surrounding breast tissue is removed.[69] Breast conserving surgery is combined with radiation therapy depending on tumour size and location.[70] Long-term follow-up of patients has shown radiation therapy combined with lumpectomy decreases breast cancer recurrence by 50% and breast-cancer related death by one sixth when compared to patients who did not receive radiotherapy.[71] Breast conserving surgery combined with radiation therapy has been shown to have similar outcomes in regards to relapse free and overall survival when compared with mastectomy.[72] Axillary lymph node dissection is also the standard of care for the removal of cancerous cells for patients with node positive breast cancer.[70]

Chemotherapy may be administered in the neoadjuvant setting (prior to surgery) to decrease tumour size and enable breast conserving surgery or in the adjuvant setting (after surgery) to eradiate residual tumour cells. Common chemotherapeutic regimens include combinations of anthracyclines, taxanes, 5-fluouracil, cyclophosphamide, methotrexate, and carboplatin.[73-75] For patients with HER2 receptor-positive breast cancer, trastuzumab in combination with chemotherapy or pertuzumab is the standard adjuvant treatment.[69] Trastuzumab and pertuzumab are monoclonal antibodies that bind to human epidermal growth factor 2 receptor and inhibit the signalling pathways that lead to tumorigenesis. Trastuzumab has been associated with improved disease-free survival in comparison with chemotherapy alone in treatment of early stage breast cancer.[76] Addition of pertuzumab has been associated with modest improvement in invasive disease-free survival in comparison with treatment with trastuzumab

and chemotherapy.[77] For patients with hormone receptor positive breast cancer, which comprises approximately 75% of all new diagnoses of breast cancer, treatment with endocrine therapy (AIs or tamoxifen) for up to ten years is the standard of care to prevent breast cancer recurrence and breast-cancer related mortality.[10,78] Current guidelines recommend treatment of post-menopausal women with upfront AIs where patients initiate treatment on AIs and continue treatment for five years. Patients may also be administered AI in sequential setting with tamoxifen whereby patients switch to AIs after 2-3 or five years of tamoxifen and treated for up to five or ten years with AIs.[9-11] ASCO also recommends treatment with tamoxifen in the upfront setting for ten years as one of the treatment strategies for hormone receptor-positive breast cancer.[10]

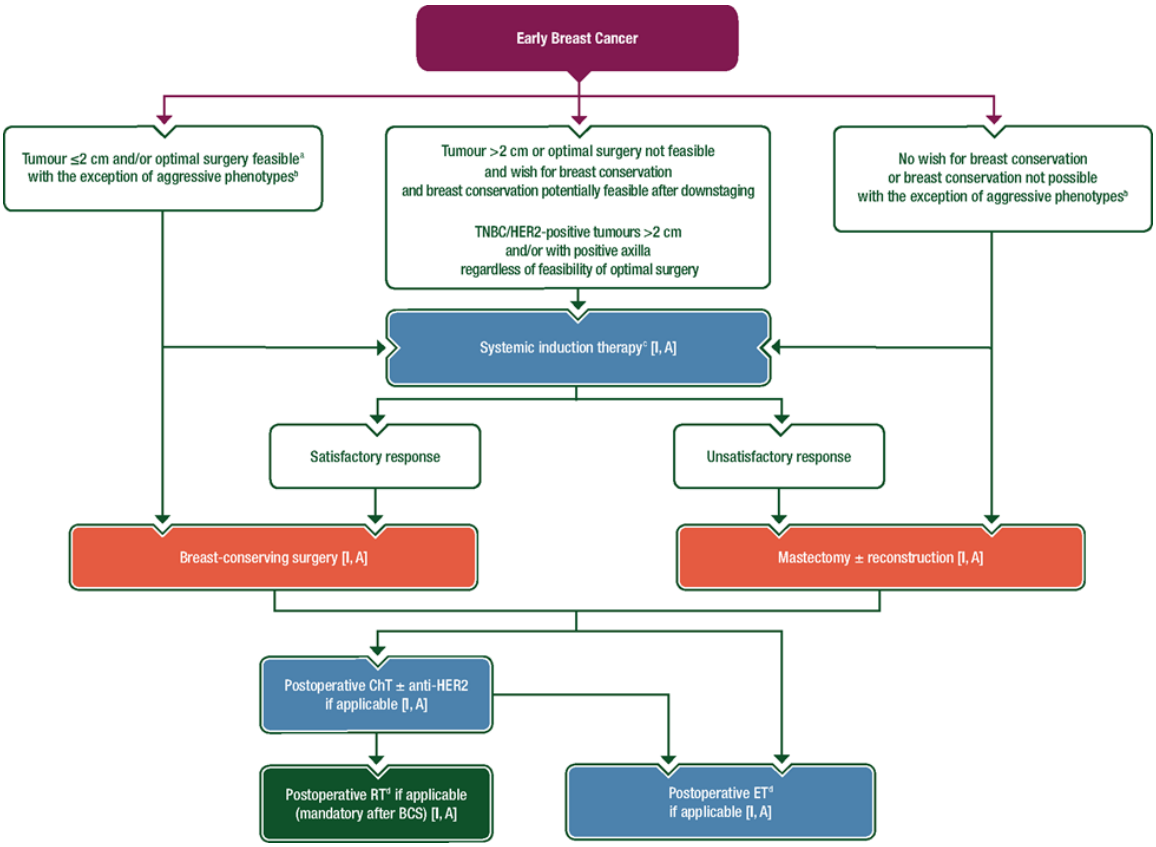


Figure 2.1 Treatment and Management of Non-metastatic Breast Cancer (Reproduced with Permission)11 BC, breast-conserving surgery; ChT, chemotherapy; ET, endocrine therapy; HER2, human epidermal growth factor receptor 2; RT, radiotherapy; TNBC, triple-negative breast cancer

2.4 Mechanism of Action of AIs and Tamoxifen

Endocrine drugs are designed to antagonize the activity of estrogen at the ER or inhibit the synthesis of estrogen. Tamoxifen is a selective estrogen receptor modulator that binds to the estrogen receptor and antagonizes transcriptional activation of genes required for tumour growth.[62] However, tamoxifen is also a partial estrogen agonist, and it stimulates cell proliferation in the endometrium, which leads to an increased risk of uterine cancer and thrombogenecity.[4,62] AIs decrease serum levels of estrogen by inhibiting the enzymatic conversion of testosterone to estradiol by the aromatase enzyme.[65] In pre-menopausal women, this conversion occurs mainly in the ovaries and the decreased negative feedback of estrogen leads to ovarian hyperstimulation by gonadotropins and increased expression of aromatase.[65] As a result, AIs are contraindicated in pre-menopausal women.[65] However, in post-menopausal women, most aromatase and residual sources of estrogen are produced in peripheral tissue, particularly subcutaneous fat. Thus, AIs inhibit aromatase directly in post-menopausal women without causing ovarian stimulation.[65] There are two classes of generation AIs: the non-steroidal inhibitors, which consist of anastrozole and letrozole, and the steroidal inhibitor exemestane. Anastrozole and letrozole bind reversibly to the aromatase complex whereas exemestane binds irreversibly.[65] First-generation AI (aminoglutethimide) and second generation AIs (fadrozole and formestane) are no longer in the US or European market due to toxicity and lack of potency respectively.[79]

2.5 Pharmacokinetics and Pharmacodynamics of AIs and Tamoxifen

AIs are administered orally as once daily doses (anastrozole 1 mg, letrozole 2.5 mg, exemestane 25 mg). The half lives of exemestane and anastrozole are approximately 27 and 41 hours respectively whereas the half life for letrozole is 4 days.[80] Exemestane suppresses estrogen conversion by 52-72%, anastrozole by 81-94%, and letrozole by 88-98% with time to peak

estradiol suppression of 2-4 days for anastrozole and letrozole and seven days for exemestane.[80] Anastrozole and exemestane achieve steady state in seven days while letrozole takes 60 days to achieve steady state. Anastrozole has been shown to inhibit CYP1A2, CYP2C8/9, and CYP3A4 while letrozole inhibits CYP2A6, CYP2C19 and CYP3A4.[80] Exemestane is metabolized by CYP3A4.[80] Tamoxifen is administered as 10 or 20 mg oral daily dose and has an elimination half life of 5-7 days with steady state levels of tamoxifen occur within four weeks.[81] Tamoxifen is a substrate of CYP450, CYP2C9, and CYP2D6 and is actively metabolized to N-desmyl tamoxifen.[81] There are known drug-drug interactions between tamoxifen and anastrozole and letrozole. [80] Co-administration of tamoxifen decreases the concentration of AIs.[80]

2.6 Utilization of AIs and Tamoxifen in Clinical Practice

In a longitudinal, cross-national drug utilization study, a downward trend in tamoxifen use and upward trend in AI use was observed across eight European countries and Australia between 2001 and 2012.[5] AIs constituted the majority of total endocrine therapy use in all countries (as high as 74% and 80% in France and Denmark, respectively) in 2012.[5] Three observational studies have examined persistence to endocrine therapy in clinical practice among women diagnosed with breast cancer.[82-84] In a study in the UK, 19% of the women with breast cancer on AI therapy compared with 31% of women on tamoxifen had discontinued their treatment within the first five years of follow-up.[84] Approximately 14% of patients had switched between AIs and tamoxifen. In a study using French health insurance database, 31% of endocrine therapy users were identified as non-persistent.[82] Initiators of AIs were less likely to discontinue treatment in comparison with tamoxifen users (HR: 0.62, 95% CI: 0.46-0.83). However, in a study using the IMS health data (database consisting of 10 million patients from Germany, the UK, France, and Austria), a similar

rate of discontinuation was found between AIs and tamoxifen users within first three years of initiation (52.2% tamoxifen, 47% anastrozole, 44.3% letrozole, and 55.1% exemestane).[83]

2.7 Efficacy of AIs and Tamoxifen in Randomized Controlled Trials

Three major RCTs have compared the efficacy of third generation AIs with tamoxifen in post-menopausal women with early-stage breast cancer in adjuvant setting. In the Breast Cancer International Group (BIG) 1-98 trial, patients were randomized to five years of treatment with letrozole or tamoxifen in the adjuvant setting.[85] After a median of 4.3 years, disease-free survival decreased by 18% (hazard ratio (HR): 0.82, 95% confidence interval (CI): 0.71-0.95) in letrozole group when compared with tamoxifen. However, there was no significant decrease in overall survival (HR: 0.91, 95% CI: 0.75-1.11).[85] In the Anastrozole, Tamoxifen, Alone or in Combination (ATAC) RCT, patients were randomized to five years of adjuvant treatment with anastrozole or tamoxifen. After a median of 10 years, anastrozole improved disease-free survival by 14% (HR: 0.86, 95% CI: 0.78-0.95), with no differences in overall survival between the two arms (HR: 0.95, 95% CI: 0.84-1.06).[86] In the Intergroup Exemestane Study (IES) trial, patients were randomized to two to three years of exemestane or tamoxifen after two years of prior tamoxifen treatment. After a median follow-up of 7.6 years, AIs were associated with a significant decrease in all-cause mortality favoring exemestane (HR: 0.86, 95% CI: 0.75-0.99).[25] An individual patient data meta-analysis of RCTs demonstrated that AIs reduced breast cancer-mortality (incidence rate ratio (IRR): 0.86, 95% CI: 0.80-0.94) and all-cause mortality (IRR: 0.88, 95% CI: 0.82-0.94) when combining on-treatment and off-treatment periods.[17]

In the MA.17 trial, patients were randomized to letrozole or placebo after five years of initial treatment with tamoxifen (i.e., extended-adjuvant setting).[24] AIs were associated with 43% decreased risk of breast cancer recurrence (HR: 0.57, 95% CI: 0.43-0.75) and a non-significant

24% decreased in all-cause mortality (HR: 0.76, 95% CI: 0.48-1.21). Similarly, in the MA.17R RCT, patients were randomized to five years of treatment with letrozole or placebo after five years of initial treatment with letrozole. After a median of 6.3 years, AIs were associated with 34% decreased risk of breast cancer recurrence (HR; 0.66, 95% CI: 0.48-0.91) with no difference on overall survival (HR: 0.97, 95% CI: 0.73-1.28).[22]

To date, two RCTs have directly compared the efficacy of different AIs. In the Femara Versus Anastrozole Clinical Evaluation (FACE) Trial, five years of treatment with letrozole was not associated with significant improvement in disease free survival when compared with five years of anastrozole treatment (HR: 0.93, 95% CI: 0.80-1.07).[87] Similarly, the National Cancer Institute of Canada (NCIC) MA.27 RCT did not show a significant improvement in disease free survival (HR: 0.93, 95% CI: 0.70-1.24) or overall survival when comparing five years of adjuvant treatment with exemestane to anastrozole (HR: 0.93, 95% CI: 0.77-1.13).[88]

2.8 AIs and Tamoxifen Non-Cardiotoxic Adverse Events in Randomized Controlled Trials

Meta-analyses of RCTs have consistently shown that AIs, in comparison with tamoxifen, increase the risk of fractures, whereas tamoxifen increases the risk of endometrial cancer and venous thromboembolism.[17,23,26,89] In the BIG 1-98 RCT, letrozole had a lower risk of thromboembolic events than tamoxifen (2.6% vs 4.3%), but a higher risk of musculoskeletal events including fractures (10% vs 6.7%), arthralgia (22.5% vs 16.6%), myalgia (8.4% vs 7%), and osteoporosis (5.1% vs 2.2%) after 6.2 years of follow-up.[13] Similarly, in ATAC RCT, anastrozole had a lower risk of thromboembolic events than tamoxifen (2.1% vs 3.5%) but a higher risk of fractures (5.9% vs 3.7%).[90] The risk of endometrial cancer, although rare, was lower in the anastrozole group (0.1% vs 0.5%) at 2.8 years of follow-up.[90] Similarly, exemestane, compared with tamoxifen, was associated with a numerically increased risk of fractures (6.8% vs 5.7%) and

myalgia (0.9% vs 0.5%) but not arthralgia (6.4% vs 6.4%), arthritis (8.7% vs 8.2%), or osteoarthritis (5.7% vs 5.8%).[25] The risk of endometrial hyperplasia was lower in the exemestane group (0.8% vs 0.6%) compared with tamoxifen.[25]

In the MA.17R trial comparing letrozole to no treatment in extended adjuvant setting, the event rate for clinical fractures (5.3% vs 4.6%) and osteoporosis (8.1% vs 6.0%) was higher when comparing letrozole to placebo with modest differences for arthritis (6% vs 5%), arthralgia (25% vs 21%), and myalgia (15% vs 12%) at 2.4 years of follow-up.[24] In the Adjuvant Tamoxifen Longer Against Shorter (ATLAS) RCT, in comparison with placebo, longer duration tamoxifen in the extended adjuvant setting was associated with increased risk of endometrial cancer (116/6454 vs 63/6440, RR: 1.74: 1.30-2.34) but not fractures (62/6454 vs 70/6440).[18]

2.9 Cardiotoxicity of AIs and Tamoxifen in Randomized Controlled Trials

While AIs have been shown to improve cancer-related outcomes in patients with breast cancer, there are concerns that these drugs may increase the risk of adverse cardiovascular outcomes. Indeed, in the 6.2 years follow-up of BIG 1-98, letrozole was associated with more cardiac events in comparison with tamoxifen (6.9% vs 6.2%).[13] In the ATAC RCT, there was a non-significant increase in incidence of ischemic cardiovascular events observed between women who were randomized to anastrozole in comparison with women who were randomized to tamoxifen at 5.7 years of follow-up (RR: 1.22, 95% CI: 0.95-1.58).[91] Similarly, in the IES trial, the incidence of cardiovascular events was higher in patients treated with exemestane compared with tamoxifen at 7.6 years of follow-up (RR: 1.19, 95% CI: 1.00-1.41).[25] In the MA.17 and MA.17R trials, similar incidences of cardiovascular events were observed when comparing the treatment of letrozole with placebo after five years of initial adjuvant treatment with tamoxifen (MA.17) or letrozole (MA.17R).[22,92] Results from previous systematic review and meta-analysis

of RCTs have been divergent with some studies indicating that AIs in comparison with tamoxifen increase the risk of composite cardiovascular outcomes in post-menopausal women with breast cancer[23,26] while other studies did not show a clinically significant increased risk associated with AIs.[93] These studies only assessed evidence from RCTs directly comparing AIs with tamoxifen with heterogeneity in definition of composite cardiovascular outcomes used across RCTs.

2.10 Cardiotoxicity of AIs and Tamoxifen in Observational Studies

Four observational studies have examined the cardiovascular and cerebrovascular safety of AIs and tamoxifen (Table 2.1).[94-97] In one study using the Ontario provincial health insurance databases, AIs were associated with a doubling of the risk of MI (HR: 2.02, 95% CI: 1.16 to 3.53), when compared with tamoxifen.[94] However, in an observational study conducted using Kaiser Permanente Health Insurance Databases, similar risks of cardiac ischemia (HR: 0.97, 95% CI: 0.78-1.22) and stroke (HR: 0.97, 95% CI: 0.70-1.33) were reported when comparing use of AIs with tamoxifen in post-menopausal women without history of cardiovascular disease.[95] Similarly, no association was observed between AIs and MI in a study using the SEER-Medicare database (HR: 1.01, 95% CI: 0.72 to 1.42).[96] In a study conducted using US HealthCore Integrated Research Databases, there was also no association found between AIs and MI (HR: 0.90, 95% CI: 0.65 to 1.25)[97] and no increased risk of ischemic stroke (HR: 0.71, 95% CI: 0.49 to 1.03) in comparison with no endocrine treatment in patients without a diagnosis of breast cancer.[97] The different results across these studies may be due to heterogeneity in the study populations with the inclusion of patients with or without a history of cardiovascular disease. These studies also had methodological limitations, including the use of an intention-to-treat exposure definition which may lead to non-differential exposure misclassification and a dilution

of the effect estimate,[94,95] informative censoring due to discontinuation and switching between treatments,[96] and confounding by indication.[97]

2.11 Effect of AIs and Tamoxifen on Serum Lipid Levels

Some RCTs have shown the use of anastrozole and letrozole, when compared with tamoxifen, were associated with increased risk of hypercholesterolemia.[14,98,99] In the ATAC trial, there was a higher risk hypercholesterolemia when comparing anastrozole with tamoxifen (AI: 9.0% vs tam: 3.0%) at 5.7 years of follow-up.[91] In the Italian Tamoxifen Anastrozole (ITA) RCT, patients switching to anastrozole where also shown to have greater hypercholesterolemia in comparison with tamoxifen (8.1% vs 2.7%).[100] Similarly, higher risks of lipid metabolism disorders were reported for letrozole in the BIG 1-98 RCT (50.6% vs 24.6%)[14] and for exemestane in the IES trial (8.8% vs 6.6%).[16] However, other RCTs have not observed important changes in cholesterol levels with anastrozole, letrozole, or exemestane when assessing patients' baseline cholesterol levels during follow-up.[101-104] Furthermore, in extended adjuvant trials, no differences in low-density lipoprotein (LDL) cholesterol or triglyceride levels where reported when comparing AIs with placebo or no treatment.[93,102,103,105] Thus, the observed increase in hypercholesterolemia in direct comparison of AIs with tamoxifen may be due to the lipid lowering effects of tamoxifen. Consistent with this hypothesis, tamoxifen has been shown to decreases levels of total cholesterol by up to 39mg/dL and LDL cholesterol by 31mg/dL when comparing baseline to three months of follow-up, with the effect persisting up to one year after treatment initiation.[31,104,106-109] There is evidence for a rebound effect of tamoxifen after treatment in an RCT where lipid levels after discontinuation of five years of tamoxifen treatment returned to the average observed in post-menopausal women.[110] The favorable effect on

tamoxifen on cholesterol level may occur through inhibition of enzymes involve in cholesterol metabolism pathway including sterol-Δ8,7 isomerase and acetyl-coenzyme A acetyltransferase.[29]

2.12 Effect of AIs and Tamoxifen on Cardiovascular Biomarkers

Tamoxifen has been shown to have anti-inflammatory effects and lowers C-reactive protein and fibrinogen levels which are strong predictors of cardiovascular disease.[31,111-114] The anti-inflammatory effects of tamoxifen may also be mediated through cytokine transforming growth factor (TGF)-β, which maintains vessel wall structure during atherogenesis.[29,115] Finally, *in vitro* studies have shown that tamoxifen may have antioxidant properties which prevents LDL cholesterol from harmful oxidation.[116,117] There are discrepant findings regarding the effect of AIs and tamoxifen on endothelia function. Some studies suggest that tamoxifen increases flow-mediated dilation leading to decrease in carotid intima-media thickness and improvement in endothelial function.[109,118] However, a recent study suggest that AIs may be associated with vascular injury and attenuated peripheral endothelial function.[119]

2.13 Intersection of Breast Cancer and Cardiovascular Disease

The potential cardiotoxicity of AIs in comparison in tamoxifen is of great concern as post-menopausal women with breast cancer represent a patient population at an increased risk of cardiovascular disease.[32] Breast cancer and cardiovascular disease share common risk factors (**Figure 2.2**). Post-menopausal women with breast cancer are more likely to die of non-breast cancer related conditions than underlying breast cancer, with cardiovascular disease as the leading cause of mortality in this patient population.[32] Majority of breast cancer survivors are above the age of 65.[32] In post-menopausal women with breast cancer, mortality attributable to cardiovascular disease is higher in breast cancer survivors than women without history of breast cancer.[32] Thus, the identification and management of risk factors for cardiovascular disease is

paramount as cardiovascular disease poses a greater health risk than underlying breast cancer itself.

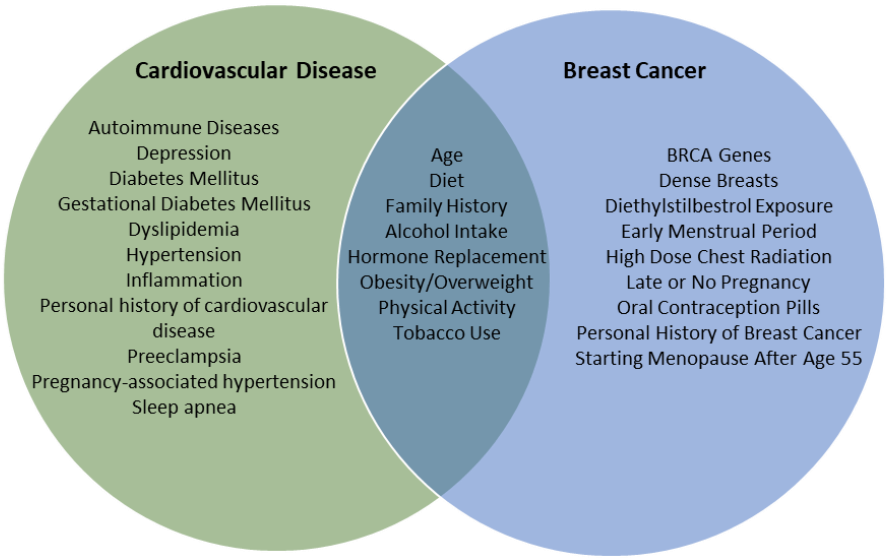


Figure 2.2 Risk Factors for Breast Cancer and Cardiovascular Disease (figure adapted)[32]

2.14 Clinical Regulatory Agencies Guidelines Regarding Efficacy and Toxicity of AIs and Tamoxifen

The American Society of Clinical Oncology (ASCO) and European Society for Medical Oncology (ESMO) currently recommend treatment of post-menopausal women with hormone-receptor positive breast cancer for five years in the upfront setting with AIs or up to ten years in sequential treatment with tamoxifen.[9-11] ASCO also recommends upfront treatment with tamoxifen for ten years.[9] ASCO indicates venous thromboembolism and uterine cancer as major harms associated with tamoxifen, while osteoporosis and ischemic heart disease are indicated as harms associated with AIs.[10] Similarly, the United States Food and Drug Administration product label for anastrozole indicates ischemic heart disease as a potential harm associated with anastrozole.[120] In Canada, provincial health authorities recommend treatment of post-menopausal women with breast cancer with 5 years of upfront treatment with AIs or sequentially starting with 2-3 years or five years of tamoxifen and switching to AIs thereafter.[6-8,121] Cardiovascular

disease is not stated as an explicit risk associated with AIs in product labels by Health Canada and provincial health agencies.[6-8] Similarly, the European Medicine Agency have not indicated cardiovascular adverse events in their product assessment and ESMO does not indicate cardiovascular adverse events as a safety concern with AIs.[11,122,123]

2.15 Summary

AIs and tamoxifen are widely administered as adjuvant therapy for hormone receptor positive breast cancer. AIs have demonstrated better efficacy when compared with tamoxifen in RCTs and thus are preferentially prescribed by oncologists and general practitioners.[5] However, there is uncertainty regarding the cardiovascular safety of these drugs. Signals from RCTs have demonstrated that AIs may increase the risk of cardiovascular outcomes in comparison with tamoxifen.[23,26] The elevated cardiovascular outcomes associated with AIs from RCTs is alarming because post-menopausal women with breast cancer are at increased risk of cardiovascular disease and cardiovascular mortality is leading cause of mortality in this population.[32] To date, no meta-analysis has considered the totality of evidence from RCTs including RCTs comparing AIs to tamoxifen, placebo controlled tamoxifen RCTs, and RCTs comparing AIs to no-treatment in extended adjuvant setting. The results from observational studies assessing cardiovascular safety of AIs in the real-world setting have been divergent with methodological limitations that limit the interpretability of these results. Finally, there are limited data regarding the cardiovascular safety of AIs in the sequential treatment strategy with tamoxifen, a treatment strategy that has shown similar efficacy to upfront treatment with AIs.[17] This thesis aims to address these gaps in knowledge and determine the cardiovascular safety of AIs and tamoxifen by first reviewing evidence from RCTs and evaluating this risk with upfront and sequential treatment strategies with AIs by conducting observational studies in setting of clinical practice.

Table 2.1 Summary of observational studies assessing the cardiovascular safety of aromatase inhibitors and tamoxifen

Study	Data Source	Study Population	Exposure/Comparator	Outcome definition	Exposure definition	Summary of Findings
Kamaraju S et al. 2018[96]	SEER-Medicare	Women ≥ 67 years diagnosed with breast cancer (2006-2008)	AIs (n=4,690) vs tamoxifen (n=958)	MI	As-treated	HR: 1.01 (95% CI: 0.72-1.42)
Haque R et al. 2016[95]	Kaiser Permanente Health Insurance Database	Post-menopausal women with diagnosis of breast cancer (1991-2010) and without diagnosis of CVD	AIs (n=4,207) vs tamoxifen (n=3,807)	i) Cardiac ischemia ii) Stroke iii) Combined endpoint of heart failure and cardiomyopathy iv) Combined endpoint of dysrhythmia, valvular dysfunction, pericarditis	Intention to treat	i) Cardiac ischemia (HR: 0.97, 95% CI: 0.78-1.22) ii) Stroke (HR: 0.97, 95% CI: 0.70-1.33) iii) Combined endpoint of heart failure and cardiomyopathy (HR: 1.10, 95% CI: 0.86-1.40) iv) Combined endpoint of dysrhythmia, valvular dysfunction, and pericarditis (HR: 1.29, 95% CI: 1.11-1.50)
Abdel-Qadir H et al. 2016[94]	Ontario health insurance databases	Women >65 years with diagnosis of stage I to III breast cancer (2005-2010)	AIs (n=7,408) vs tamoxifen (n=1,941)	MI	Patients classified as AI or tamoxifen based on ≥90% days of drug dispensation	HR: 2.02, 95% CI: 1.16-3.53
Ligibel et al. 2012[97]	HealthCore integrated research databases	Women at least 50 years of age with more than two diagnosis codes for breast cancer (2001-2007)	AIs vs non-endocrine use in patients without breast cancer (n=44,026)	i) MI ii) Ischemic stroke	As-treated	i) MI (HR: 0.90, 95% CI: 0.65-1.25) ii) Ischemic stroke (HR: 0.71, 95% CI: 0.49-1.03)

Chapter 3. Data Sources and Methodology

To address Objectives 2 and 3, cohort studies were conducted by linking the United Kingdom (UK) Clinical Practice Research Datalink (CPRD) with the Hospital Episode Statistics (HES) and the Office for National Statistics (ONS) databases. These data sources are described in this chapter.

3.1 Data Sources

3.1.1 The Clinical Practice Research Datalink

The National Health Service (NHS) provides comprehensive, universal, and free healthcare services to citizens of United Kingdom (UK).[124] In UK, over 98% of the population are registered with a primary care general practitioners (GPs) who act as the gatekeeper of the healthcare system. GPs are the primary point of contact for non-emergency health conditions which may be managed by the GPs or referred to secondary care when needed. Each patient in UK has a unique NHS number, and patient data are recorded on a routine basis by practice staff.[124]

The CPRD is one of the largest primary care databases in the world and captures over four million active patients in the United Kingdom which account for 7% of the UK population.[124] This database has been extensively used for observational studies including pharmacoepidemiologic studies examining the effectiveness and safety of drugs.[124] The CPRD captures patient level on a routine basis across 700 practices that have consented to participate at practice level and provide data on a monthly basis. The practices in CPRD are distributed across England, Wales, Scotland, and Northern Island.[124] The CPRD captures anonymous information on medical diagnoses, procedures and prescriptions written by general practitioners. Clinical diagnoses and procedures are classified according to the Read code classification system. Read

codes are a hierarchical clinical classification system consisting of 96,000 codes.[125] They consist of five character alpha numerical codes and are organized by chapters. Chapters 1-9 of the Read system correspond to history, examination, procedures, and administration while chapters A to Z correspond to conditions, diagnosis, and injuries. Within chapters, terms are organized hierarchically from more general to more specific terms.[125] Prescriptions are classified according to the UK Pricing Authority Dictionary. Prescriptions by GPs include information on product names and associated product and British National Formulary code, quantity, and dose.

A major strength of CPRD is that it contains information on lifestyle variables and anthropometric measures including smoking, blood pressure, alcohol intake, and body mass index. The recording of lifestyle variables for patients recorded in the CPRD has increased over time, with majority of patients having information for these variables.[124] The increase in completeness of recording for these variables is due to the implementation of the Quality and Outcomes Framework (QOF), an incentive program for GPs which provided practices with substantial financial rewards for achievement of quality indicators in ten chronic conditions and lifestyle measures such as smoking.[126] The CPRD has been shown to be representative of UK population with respect to key characteristics such as age, ethnicity, and body mass index.[124]

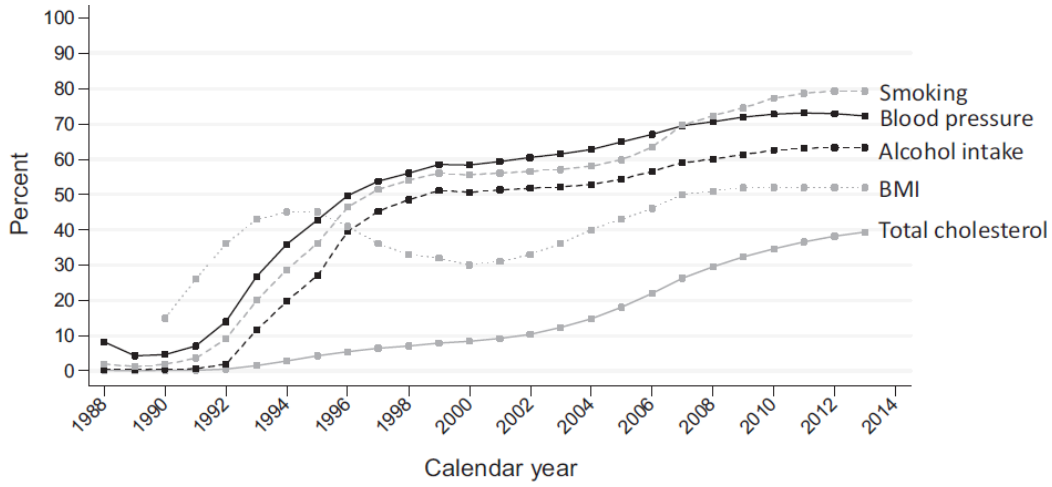


Figure 3.1 The recording of key lifestyle variables in the past three years for patients registered with the CPRD by calendar year[124]

The CPRD provides quality assurance metrics including up to standard (UTS) dates for practices and acceptability flags (for research purposes) for patients. The UTS metric is a practice-based quality metric based on continuity of recording and number of expected deaths reported for each practice. The UTS corresponds to the latest date at which practices meet these minimum quality criteria. The acceptable metric is based on registration status, recording of events, and having a valid age and gender. Patient data are deemed unacceptable if there is any of the following:

i) Missing or invalid first registration date or current registration date.

ii) Missing record year of birth

iii) A first registration date or current registration date before year of birth

iv) A transfer out date with missing reason or transfer out date before current or first registration date

v) A gender excluding Female/Male/Indeterminate or age of greater than 115 at end of follow up

vi) Recorded health care episodes in years prior to birth year

vii) All recorded health care episodes have missing or invalid event dates

viii) Registration status of temporary patients

Diagnoses have been shown to be valid in the CPRD.[127] A systematic review of 182 diagnoses in CPRD validated with diagnostic algorithms, questionnaires and record requests to GPs, manual reviews of anonymized text, and external UK-based data sources confirmed approximately 89% of all diagnoses.[127] A similar finding was found in an independent systematic review with high concordance rate between disease prevalence in CPRD and other primary care databases and national statistics.[128] The diagnosis of breast cancer has been shown to be valid in CPRD with high concordance rates with the UK cancer registry.[129] Validation studies have found 96-97% concordance rates between breast cancer diagnoses recorded in the CPRD compared with the National Cancer Data Repository (96-97%) and medical profile reviews (98%).[130-132]

CPRD data also have some potential limitations. First, CPRD captures prescriptions written by general practitioners and not specialists. Thus, this database is amenable to studies examining conditions that are routinely managed and treated by general practitioners. Nevertheless, in the United Kingdom, GPs serve as the gatekeeper of the healthcare system and are extensively involved in management and treatment of patients with various acute and chronic conditions (including breast cancer).[124,133,134] Second, the CPRD does not capture information on over-the-counter drugs and has no information on adherence of patients to treatment which may introduce exposure misclassification. Finally, variables such as tumour grade and stage are not captured within CPRD and require linkage to national cancer data repository.

3.1.2 Hospital Episode Statistics and Office for National Statistics

Approximately 75% of English practices have consented to participate in the CPRD linkage scheme with patient-level data linkage to Hospital Episode Statistics (HES), Office for National Statistics (ONS), and Index of Multiple Deprivation Score and Townsend Scores and disease registries.[133,134] The HES is a repository which captures all inpatient and outpatient hospital admissions. Primary and secondary diagnoses are recorded in the HES using the International Classification of Disease 10th revision [ICD-10] codes) and procedures are recorded using the Office of Population Censuses and Surveys classification of interventions and procedures (4th revision).[135] In Objective 2 and 3, HES was used to identify incident cases of MI, ischemic stroke, and heart failure. These outcomes have been shown to be well recorded in the HES, with MI having a positive predictive value of 92%, diagnoses of coronary heart disease having a specificity and negative predictive value of 96%, and stroke having a 100% specificity and negative predictive value.[136,137]

The ONS database in UK includes the electronic death certificates of all residents in the UK and includes primary and secondary causes of death recorded using ICD-9 and ICD-10 codes.[138] The ONS is used to identify cardiovascular-associated death in Objective 2 and 3. The CPRD is also linked to the Townsend deprivation index which is a proxy for socioeconomic status.[139] The Townsend deprivation index is calculated at a population level for a region uses census data and incorporates unemployment (percentage of adults aged 16 and over who are unemployed), non-car ownership (% of households), non-home ownership (% households), and household overcrowding with a greater score corresponding to greater deprivation and lower socioeconomic status.[139]

3.2 Methodology

3.2.1 Base Cohort Formation

For Objectives 2 and 3**,** a cohort of women, at least 50 years of age, with a first diagnosis of breast cancer was identified between April 1, 1998 and February 29, 2016 using the UK CPRD. The diagnostic Read codes for breast cancer are shown in Table 3.1. We excluded patients with less than one year of medical history before their first breast cancer diagnosis and those with evidence of metastatic disease (using diagnostic Read codes corresponding to secondary malignancy, recurrence, or metastases). Additionally, we excluded patients with prescriptions of AIs or tamoxifen before their breast cancer diagnosis to minimize the inclusion of prevalent users.[140] This base cohort was subsequently used for the formation of the study populations in Objectives 2 and 3. Additional details regarding specific cohort formation and corresponding flow diagram for Objectives 2 and 3 are outlined in the manuscripts in Chapters 5 and 6.

3.2.2 Exposure definition

For Objectives 2 and 3, an *as-treated* exposure definition was used where patients were followed while they were continuously exposed to the AIs or tamoxifen (description of relevant product codes in Table 3.2). Patients were considered exposed if the duration of one prescription plus a 30-day grace period overlapped with the date of the next prescription of the same drug class. Thus, treatment discontinuation corresponded to the end of a 30-day grace period or at the date of a switch between prescriptions from different drug classes (tamoxifen to AI or vice versa). In Objective 2, an *as-treated* exposure definition was used to compare risk of cardiovascular outcomes between patients who initiated treatment on AIs when compared with

tamoxifen. In Objective 3, an *as-treated* exposure definition was used when comparing patients on sequential AI treatment in comparison with patients on continuous tamoxifen treatment.

3.2.3 Outcome definition

For Objectives 2 and 3**,** we considered the following four outcomes, which were assessed independently in the analyses, with separate follow-up durations determined for each: MI, ischemic stroke, heart failure, and cardiovascular mortality (ICD-9 and ICD-10 codes provided in Table 3.3). Thus, patients were followed until one of the study outcomes (defined below), treatment discontinuation or switch (end of a 30-day grace period or date of a switch between prescriptions from different drug classes), non-cardiovascular death, end of registration with the general practice, or end of the study period (February 29, 2016).

3.2.4 Confounders

For Objectives 2 and 3, the following potential confounders were measured at cohort entry: age, body mass index (<25, 25-30, ≥30 kg/m^2), Townsend Deprivation Score (quantiles), ethnicity (Caucasian, other, unknown), smoking status (current, past, never, unknown), and alcohol-related disorders.

We also included the following comorbidities measured at any time before cohort entry: previous MI, stroke or transient ischemic attack, heart failure, peripheral vascular disease, venous thromboembolism, chronic obstructive pulmonary disease, chronic kidney disease, cancers (other than breast and non-melanoma skin cancer), and non-breast cancer surgeries in the year prior to cohort entry. We expanded the look-back period to all available data to improve sensitivity and minimize misclassification.[141]

The following prescription drugs were measured in the year before cohort entry: anticoagulants, antidepressants, antidiabetic drugs, antihypertensive drugs, bisphosphonates,

non-steroidal anti-inflammatory drugs, opioids, acetylsalicylic acid (ASA), non-ASA antiplatelets, statins, and hormone replacement therapy. Prescriptions were captured in the year prior to cohort entry to improve specificity. Given that the acute nature of the outcomes of interest, a one-year look back period improves sensitivity and specificity by allowing for a biologically relevant time window of the effect of prescriptions on cardiovascular outcomes.

Finally, the model included breast cancer-related variables, including receipt of chemotherapy and radiation therapy in the year prior and breast cancer surgery prior to treatment with AIs or tamoxifen and time since the breast cancer diagnosis (defined as the time between the first breast cancer diagnosis and cohort entry). Age and time since breast cancer diagnosis were modelled flexibly as restricted cubic splines with five interior knots.[142] In Objective 3, we also assessed the duration of previous tamoxifen treatment. The definition of covariates, data sources used to define covariates, and corresponding covariate assessment windows are shown in Table 3.4

Table 3.1 Read codes for diagnosis of breast cancer

Read Codes	Description
B34..00	Malignant neoplasm of female breast
B34..11	Ca female breast
B340.00	Malignant neoplasm of nipple and areola of female breast
B340000	Malignant neoplasm of nipple of female breast
B340100	Malignant neoplasm of areola of female breast
B340z00	Malignant neoplasm of nipple or areola of female breast NOS
B341.00	Malignant neoplasm of central part of female breast
B342.00	Malignant neoplasm of upper-inner quadrant of female breast
B343.00	Malignant neoplasm of lower-inner quadrant of female breast
B344.00	Malignant neoplasm of upper-outer quadrant of female breast
B345.00	Malignant neoplasm of lower-outer quadrant of female breast
B346.00	Malignant neoplasm of axillary tail of female breast
B347.00	Malignant neoplasm, overlapping lesion of breast
B34y.00	Malignant neoplasm of other site of female breast
B34y000	Malignant neoplasm of ectopic site of female breast
B34yz00	Malignant neoplasm of other site of female breast NOS
B34z.00	Malignant neoplasm of female breast NOS
BB91.00	[M]Infiltrating duct carcinoma
BB91.11	[M]Duct carcinoma NOS
BB91100	[M]Infiltrating duct and lobular carcinoma
BB94.00	[M]Juvenile breast carcinoma
BB94.11	[M]Secretory breast carcinoma
BB9F.00	[M]Lobular carcinoma NOS
BB9G.00	[M]Infiltrating ductular carcinoma
BB9J.00	[M]Paget's disease, mammary
BB9J.11	[M]Paget's disease, breast
BB9K.00	[M]Paget's disease and infiltrating breast duct carcinoma
BB9K000	[M]Paget's disease and intraductal carcinoma of breast
Byu6.00	[X]Malignant neoplasm of breast

Table 3.2 Product codes for aromatase inhibitors for tamoxifen

Product Code	Product Name	Drug Substance
2815	Anastrozole 1mg tablets	Anastrozole
4789	Arimidex 1mg tablets (AstraZeneca UK Ltd)	Anastrozole
46036	Nastrosa 1mg tablets (Discovery Pharmaceuticals)	Anastrozole
47656	Anastrozole 1mg tablets (Teva UK Ltd)	Anastrozole
58870	Anastrozole 1mg tablets (APC Pharmaceuticals & Chemicals	Anastrozole
62952	Anastrozole 1mg tablets (Alliance Healthcare (Distribution)	Anastrozole
6779	Letrozole 2.5mg tablets	Letrozole
10121	Femara 2.5mg tablets (Novartis Pharmaceuticals UK Ltd)	Letrozole
58587	Letrozole 2.5mg tablets (A H Pharmaceuticals Ltd)	Letrozole
61583	Letrozole 2.5mg tablets (Actavis UK Ltd)	Letrozole
64465	Letrozole 2.5mg tablets (Sandoz Ltd)	Letrozole
5940	Exemestane 25mg tablets	Exemestane
17079	Aromasin 25mg tablets (Pfizer Ltd)	Exemestane
62792	Exemestane 25mg tablets (Accord Healthcare Ltd)	Exemestane
62985	Exemestane 25mg tablets (Teva UK Ltd)	Exemestane
300	Tamoxifen 10mg tablets	Tamoxifen citrate
1416	Tamoxifen 20mg tablets	Tamoxifen citrate
3648	Tamoxifen -20 Tablet (Pharmacia Ltd)	Tamoxifen citrate
7346	Nolvadex D 20mg tablets (AstraZeneca UK Ltd)	Tamoxifen citrate
7936	Nolvadex 10mg tablets (AstraZeneca UK Ltd)	Tamoxifen citrate
8490	Tamoxifen 40mg tablets	Tamoxifen citrate
9075	Nolvadex -forte 40mg Tablet (AstraZeneca UK Ltd)	Tamoxifen citrate
11377	Soltamox 10mg/5ml oral solution (Rosemont Pharmaceuticals	Tamoxifen citrate
11389	Tamoxifen 10mg/5ml oral solution	Tamoxifen Citrate
15038	Tamoxifen -10 10mg Tablet (Pharmacia Ltd)	Tamoxifen citrate
21887	Oestrifen 20mg Tablet (Ashbourne Pharmaceuticals Ltd)	Tamoxifen citrate
21888	Oestrifen 10mg Tablet (Ashbourne Pharmaceuticals Ltd)	Tamoxifen citrate
22013	Fentamox 20mg Tablet (Opus Pharmaceuticals Ltd)	Tamoxifen citrate
23972	Tamoxifen -40 Tablet (Pharmacia Ltd)	Tamoxifen citrate
24217	Noltam 10mg Tablet (Wyeth Pharmaceuticals)	Tamoxifen citrate
24238	Noltam 20mg Tablet (Wyeth Pharmaceuticals)	Tamoxifen citrate
26224	Fentamox 10mg Tablet (Opus Pharmaceuticals Ltd)	Tamoxifen citrate
26227	Emblon 10mg tablets (Teva UK Ltd)	Tamoxifen citrate
26273	Emblon 20mg Tablet (Berk Pharmaceuticals Ltd)	Tamoxifen citrate
26306	Oestrifen 40mg Tablet (Ashbourne Pharmaceuticals Ltd)	Tamoxifen citrate
26956	Tamoxifen 20mg tablets (Kent Pharmaceuticals Ltd)	Tamoxifen citrate
29607	Tamoxifen 20mg tablets (Wockhardt UK Ltd)	Tamoxifen citrate
30919	Tamoxifen 20mg tablets (Actavis UK Ltd)	Tamoxifen citrate
33470	Tamoxifen 20mg tablets (Teva UK Ltd)	Tamoxifen citrate
33670	Tamoxifen 20mg tablets (IVAX Pharmaceuticals UK Ltd)	Tamoxifen citrate
34010	Tamoxifen 10mg tablets (Wockhardt UK Ltd)	Tamoxifen citrate
34513	Tamoxifen 20mg tablets (Mylan Ltd)	Tamoxifen citrate

Product Code	Product Name	Drug Substance
41590	Tamoxifen 20mg tablets (A H Pharmaceuticals Ltd)	Tamoxifen citrate
41598	Tamoxifen 10mg tablets (Actavis UK Ltd)	Tamoxifen citrate
42098	Fentamox 40mg Tablet (Opus Pharmaceuticals Ltd)	Tamoxifen citrate
45330	Tamoxifen 20mg Tablet (Sandoz Ltd)	Tamoxifen citrate
47841	Tamoxifen 20mg Tablet (Co-Pharma Ltd)	Tamoxifen citrate
50847	Tamoxifen 10mg/5ml oral solution sugar free	Tamoxifen citrate
55963	Tamoxifen 10mg tablets (Teva UK Ltd)	Tamoxifen citrate
60586	Tamoxifen 20mg tablets (Alliance Healthcare (Distribution)	Tamoxifen citrate
61675	Tamoxifen 10mg tablets (Waymade Healthcare Plc)	Tamoxifen citrate
63980	Tamoxifen 20 tablets (Pfizer Ltd)	Tamoxifen citrate
18317	Tamoxifen D TAB	
57312	Tamoxifen 10mg/5ml oral suspension	

Table 3.3 ICD-9 and ICD-10 diagnostic codes for cardiovascular outcomes

Study Outcome	**ICD-9 diagnosis codes**	**ICD-10 diagnosis codes**
Myocardial infarction	410x	I21x
Ischemic stroke	433x, 434x, 436x	I63x, I64x
Congestive Heart Failure	428x	I50x
Cardiovascular Mortality	390x-398x, 401x-405x, 410x-417x, 420x-429x (excluding 427.5), 430x-438x, 440x-447x	I00x-I77x excluding I46.9

Table 3.4 Covariate definitions and assessment period

Covariate	Definition	Databases/Codes	Covariate Assessment Period	Variable Type
Demographic and lifestyle variables				
Age, mean (SD)	Cohort entry year minus year of birth	__	Cohort Entry	Continuous
Body mass index	Weight (kg)/height (m^2)	__	Latest recorded	Categorical
Townsend deprivation score	Quintiles 1-5	__	Latest recorded	Categorical
Ethnicity	Caucasian, other, unknown	__	Latest recorded	Categorical
Smoking status	Current, past, never, unknown	__	Last recorded	Categorical
Comorbidities				
Alcohol-related disorders	Present/absent	Read codes	Ever prior to cohort entry	Binary
Myocardial infarction	Present/absent	Read codes and ICD-9/10	Ever prior to cohort entry	Binary
Stroke or transient ischemic attack	Present/absent	Read codes and ICD-9/10	Ever prior to cohort entry	Binary
Heart failure	Present/absent	Read codes and ICD-9/10	Ever prior to cohort entry	Binary
Peripheral vascular disease	Present/absent	Read codes and ICD-9/10	Ever prior to cohort entry	Binary
Venous thromboembolism	Present/absent	Read codes and ICD-9/10	Ever prior to cohort entry	Binary
Chronic obstructive pulmonary disease	Present/absent	Read codes and ICD-9/10	Ever prior to cohort entry	Binary
Chronic kidney disease	Present/absent	Read codes and ICD-9/10	Ever prior to cohort entry	Binary
Other cancers	Present/absent	Read codes and ICD-9/10	Ever prior to cohort entry	Binary
Non-breast cancer surgery (Hysterectomy and oophorectomy)	Present/absent	OPCS, ICD-9/10, Read codes	Ever prior to cohort entry	Binary
Prescriptions				
Anticoagulants				
Vitamin K antagonists	Present/absent	Product codes	Year prior to cohort entry	Binary
Direct oral anticoagulants	Present/absent	Product codes	Year prior to cohort entry	Binary
Heparin	Present/absent	Product codes	Year prior to cohort entry	Binary

Covariate	Definition	Databases/Codes	Covariate Assessment Period	Variable Type
Antidepressants				
Selective serotonin reuptake inhibitors	Present/absent	Product codes	Year prior to cohort entry	Binary
Serotonin and noradrenaline reuptake	Present/absent	Product codes	Year prior to cohort entry	Binary
Tricyclic antidepressants	Present/absent	Product codes	Year prior to cohort entry	Binary
Other	Present/absent	Product codes	Year prior to cohort entry	Binary
Antidiabetic drugs				
Metformin	Present/absent	Product codes	Year prior to cohort entry	Binary
Sulfonylureas	Present/absent	Product codes	Year prior to cohort entry	Binary
Thiazolidinediones	Present/absent	Product codes	Year prior to cohort entry	Binary
Incretin-based drugs	Present/absent	Product codes	Year prior to cohort entry	Binary
Insulin	Present/absent	Product codes	Year prior to cohort entry	Binary
Other	Present/absent	Product codes	Year prior to cohort entry	Binary
Antihypertensive drugs				
Diuretics	Present/absent	Product codes	Year prior to cohort entry	Binary
Beta-blockers	Present/absent	Product codes	Year prior to cohort entry	Binary
Calcium channel blockers	Present/absent	Product codes	Year prior to cohort entry	Binary
Angiotensin converting enzyme inhibitors	Present/absent	Product codes	Year prior to cohort entry	Binary
Angiotensin II receptor blockers	Present/absent	Product codes	Year prior to cohort entry	Binary
Other	Present/absent	Product codes	Year prior to cohort entry	Binary
Other drugs				
Bisphosphonates	Present/absent	Product codes	Year prior to cohort entry	Binary
Non-steroidal anti-inflammatory drugs	Present/absent	Product codes	Year prior to cohort entry	Binary
Opioids	Present/absent	Product codes	Year prior to cohort entry	Binary
Acetylsalicylic acid	Present/absent	Product codes	Year prior to cohort entry	Binary
Non-ASA antiplatelets	Present/absent	Product codes	Year prior to cohort entry	Binary
Statins	Present/absent	Product codes	Year prior to cohort entry	Binary
Hormone replacement therapy	Present/absent	Product codes	Year prior to cohort entry	Binary

Covariate	Definition	Databases/Codes	Covariate Assessment Period	Variable Type
Breast-cancer related variables				
Chemotherapy	Present/absent	OPCS, ICD-9/10, product codes	Year prior to cohort entry	Binary
Radiation therapy	Present/absent	OPCS, ICD-9/10 codes, Read codes	Year prior to cohort entry	Binary
Breast cancer surgery (Mastectomy, Lumpectomy, Lymph node excision)	Present/absent	OPCS codes, Read codes	Ever prior to cohort entry	Binary
Time since diagnosis, months	Cohort entry date minus diagnosis date	__	At cohort entry	Continuous

Chapter 4. Manuscript 1-Cardiotoxicity of Aromatase Inhibitors and Tamoxifen in Post-Menopausal Women with Breast Cancer: A Systematic Review and Meta-Analysis of Randomized Controlled Trials

4.1 Preface

Previous meta-analyses of RCTs have indicated that AIs, in comparison with tamoxifen, are associated with an increased risk of cardiovascular outcomes. However, these studies only considered evidence from RCTs directly comparing AIs with tamoxifen. In addition, the majority of RCTs reported cardiovascular disease as composite endpoint. Thus, the aim of the first objective of this thesis was to conduct a comprehensive systematic review to captures RCTs reporting cardiovascular outcomes and comparing i) AIs with tamoxifen in the adjuvant setting ii) AIs with placebo or no-treatment in the extended adjuvant setting iii) tamoxifen with placebo to no-treatment in the adjuvant setting iv) tamoxifen with placebo or no-treatment in the extended adjuvant setting. We subsequently conducted meta-analyses stratified by the aforementioned four settings. We also conducted meta-analyses by restricting RCTs reporting on ischemic events based on safety concerns from ASCO and FDA.[10,120] The systematic review of RCTs conducted in this objective examined whether AIs increase the risk of cardiovascular outcomes, including ischemic heart disease, in comparison with tamoxifen. In addition, this study evaluated whether AIs, in comparison with placebo or no treatment in the extended adjuvant setting, increase the risk of cardiovascular outcomes. Finally, the effects of tamoxifen on cardiovascular outcomes were assessed when compared with placebo. Overall, this is the first study to comprehensively examine the cardiovascular effects of AIs and tamoxifen using evidence from all RCTs in the adjuvant and extended adjuvant setting. This manuscript was published in *Annals of Oncology* (2017; 28(3): 487-496).

4.2 Title Page

Article type: Systematic Review and Meta-Analysis

Cardiotoxicity of Aromatase Inhibitors and Tamoxifen in Post-Menopausal Women with Breast Cancer: A Systematic Review and Meta-Analysis of Randomized Controlled Trials

F. Khosrow-Khavar MSc [1,2], K.B. Filion PhD [1,2,3], S. Al-Qurashi MD [4], N. Torabi MLIS [5], N. Bouganim MD [4,6], S. Suissa PhD [1,2], L. Azoulay PhD [1,2,4]

[1] Centre for Clinical Epidemiology, Lady Davis Institute, Jewish General Hospital, Montreal, Canada

[2] Department of Epidemiology, Biostatistics, and Occupational Health, McGill University, Montreal, Canada

[3] Division of Clinical Epidemiology, Department of Medicine, McGill University, Montreal, Canada

[4] Gerald Bronfman Department of Oncology, McGill University, Montreal, Canada

[5] McGill Library, McGill University, Montreal, Canada

[6] Department of Oncology, Cedar Cancer Center, McGill University Health Center, Montreal, Canada

Dissemination: The findings of this study were presented, in part, as a poster (abstract #1573) at the American Society of Clinical Oncology 2016 Annual Meeting.

Word count: 3,522

Correspondence:
Dr. Laurent Azoulay
Centre for Clinical Epidemiology, Lady Davis Institute, Jewish General Hospital
3755 Côte Sainte-Catherine, H-425.1, Montreal, Quebec, Canada, H3T 1E2
Tel: 514.340.8222 extension 8396; Fax: 514.340.7564; Email: laurent.azoulay@mcgill.ca

4.3 ABSTRACT

Background: Aromatase inhibitors (AIs) have been associated with cardiovascular disease in adjuvant randomized controlled trials (RCTs) comparing these drugs to tamoxifen. However, it is unclear whether this risk is real or due to cardioprotective effects of tamoxifen. To address this question, we conducted a systematic review and meta-analysis of all RCTs of AIs and tamoxifen in adjuvant and extended adjuvant setting.

Patients and Methods: We searched PubMed, Embase (OVID), Cochrane CENTRAL, WHO International Clinical Trials Registry Platform, and ClinicalTrials.gov from inception to June 2016 for all RCTs comparing cardiovascular and cerebrovascular safety of AIs to tamoxifen, AIs to placebo or no-treatment, or tamoxifen to placebo or no-treatment in the adjuvant or extended adjuvant setting. Relative risks were pooled using DerSimonian and Laird random-effects models with analyses stratified by RCT design.

Results: A total of 19 RCTs were included in the meta-analysis (n=62,345). In the adjuvant setting, AIs were associated with a 19% (relative risk [RR]: 1.19, 95% confidence interval [CI]: 1.07-1.34) increased risk of cardiovascular events compared with tamoxifen. AIs were not associated with an increased risk compared with placebo in the extended-adjuvant setting (RR: 1.01, 95% CI: 0.85-1.20). In the adjuvant setting, tamoxifen was associated with a 33% (RR: 0.67, 95% CI: 0.45-0.98) decreased risk compared with placebo or no-treatment. The results from extended adjuvant RCTs comparing tamoxifen to placebo were inconclusive, but suggestive of a small protective effect (RR: 0.91, 95% CI: 0.77-1.07).

Conclusions: The increased risk of cardiovascular events with AIs relative to tamoxifen is likely the result of cardioprotective effects of the latter. This new evidence should be considered when assessing the benefits and risks of AIs in the treatment of breast cancer.

Keywords: Breast Cancer, Aromatase Inhibitors, Tamoxifen, Anastrozole, Letrozole, Exemestane

Key Message:

The increased risk of cardiovascular events associated with aromatase inhibitors in randomized controlled trials in the adjuvant setting is due to comparison to tamoxifen which is cardioprotective. This is the most comprehensive meta-analysis on the topic and the first study to consider the totality of evidence from all trials of these drugs in the adjuvant and extended-adjuvant setting.

4.4 INTRODUCTION

Third generation aromatase inhibitors (AIs) have replaced tamoxifen as the mainstay treatment of estrogen-receptor (ER) positive breast cancer in post-menopausal women.[1] According to a comprehensive individual patient data meta-analysis, AIs significantly reduce breast cancer recurrence and breast cancer-related mortality and increase overall survival in comparison with tamoxifen.[2] However, there have been concerns regarding the cardiovascular safety of AIs. Indeed, several adjuvant RCTs comparing AIs with tamoxifen have indicated that AIs increase the risk of cardiovascular disease [3-6] and as a result, current guidelines indicate that AIs are associated with increased ischemic heart disease.[7]

To date, several systematic reviews and meta-analyses have compared the cardiovascular safety of AIs to tamoxifen,[8-13] with several of these reporting increased risks with AIs.[8-10] However, previous clinical studies have demonstrated that tamoxifen may have favorable cardiovascular effects, including reducing total cholesterol levels and low-density lipoprotein cholesterol (LDL-C) levels, increasing high-density lipoprotein cholesterol (HDL-C) levels, and reducing C-protein and fibrinogen levels.[14-19] Thus, the observed increased risk of cardiovascular events associated with AIs in RCTs comparing AIs to tamoxifen may be due to cardioprotective effects of the latter.

Given the known benefits of AI therapy,[2] there is an urgent need to better understand the cardiovascular safety of these drugs. We therefore conducted a systematic review and meta-analysis of RCTs to determine whether AIs are associated with an increased risk of cardiovascular events and if present, whether this association is due to cardioprotective effects of tamoxifen.

4.5 METHODS

4.5.1 Search Strategy

We systematic searched PubMed, Embase, Cochrane CENTRAL, WHO International Clinical Trials Registry Platform (ICTRP), and ClinicalTrials.gov from inception to March 2015 for all RCTs consisting of tamoxifen or aromatase inhibitors. The electronic search was updated in June 2016. Medical Subject Heading (MeSH) terms were used in PubMed and Cochrane CENTRAL, EMTREE terms in Embase, and keyword search terms for tamoxifen and AIs (including generic and brand names) in all databases. In PubMed and Embase, the BMJ RCT filter that optimizes sensitivity and specificity was applied to restrict inclusion to RCTs.[20] The search was also restricted to articles published in English. The detailed search strategy of each electronic database is shown in Supplementary Tables 1-5. Manual searches of the bibliographies of previous systematic reviews and relevant RCTs were conducted to retrieve additional RCTs that may not have been identified in our electronic search.

4.5.2 Study Selection

The title and abstracts of identified publications were screened independently by two reviewers (FKK and SQ), with any publication deemed potentially relevant by either reviewer carried forward to full-text evaluation. Disagreements during full-text review were resolved by consensus or, when necessary, by a third independent reviewer (LA).

We restricted inclusion to phase III RCTs examining third generation AIs and tamoxifen among post-menopausal women with a diagnosis of breast cancer. These RCTs consisted of adjuvant phase III RCTs comparing AIs to tamoxifen, extended-adjuvant RCTs comparing AIs or tamoxifen to placebo or no-treatment, and adjuvant and extended adjuvant RCTs comparing

tamoxifen to placebo or no-treatment. We only included studies if cardiovascular or cerebrovascular adverse events were reported.

We excluded phase I and phase II trials of AIs and tamoxifen, RCTs of first and second-generation aromatase inhibitors or raloxifene, RCTs comparing third generation AIs in combination with other adjuvant therapy including radiation therapy or chemotherapy, cancer prevention RCTs, and RCTs administered in pre-menopausal women (defined as any study where pre-menopausal population was greater than 50% of the study population). In addition, we also excluded RCTs that reported the combined results of RCTs, those that included non-cardiovascular events as part of their composite endpoints, and those published in a language other than English. Finally, we excluded RCTs where the primary indication for use of adjuvant hormonal therapy was not breast cancer (e.g., polycystic ovarian syndrome, ovulation induction, and uterine adenomyosis).

4.5.3 Data Extraction

Data extraction was conducted independently by two reviewers (FKK and SQ) using a standardized, pilot-tested data extraction form. Disagreements between reviewers were resolved by consensus or by a third reviewer (LA). For each RCT, the following data were extracted: year of publication, total number of randomized patients, number of patients included in analysis, dosage, and duration of follow-up time. We also extracted the following baseline demographic and clinical characteristics: mean age, proportion of post-menopausal women, proportion of node-positive patients, proportion of ER /progesterone-receptor positive patients, tumor size, and previous breast cancer therapy (chemotherapy, radiation therapy, and mastectomy). Count data for all cardiovascular and cerebrovascular events were extracted from included RCTs. When

multiple follow-up periods were reported for a given RCT, we selected the trial with the most comprehensive reporting of cardiovascular and cerebrovascular events and/or the longest follow-up reported.

4.5.4 Quality Assessment

The quality of each included RCT was assessed using the Cochrane Collaboration's tool for assessing risk of bias.[21] Each RCT was evaluated for random sequence generation, allocation concealment, blinding of outcome assessment, incomplete outcome data, selective outcome reporting, and other potential sources of bias. Potential conflicts of interest were determined by considering funding sources. Each domain was assigned a "high", "low", or "unclear" risk of bias independently by two reviewers (FKK and SQ), with disagreements adjudicated by a third reviewer (LA).

4.5.5 Statistical analysis

The cardiovascular endpoints reported in RCTs of AIs and tamoxifen are presented in Supplementary Table 6 and the definition of composite endpoints and corresponding counts that we used in the quantitative analysis are reported in Supplemental Table 7. Where possible composite endpoints of cardiovascular disease reported in RCTs were used as the definition of cardiovascular events in the quantitative analysis. For trials for which composite endpoints were not reported, cardiovascular events were combined (excluding hypertension, hypercholesterolemia, and thromboembolism). Cardiovascular death was not pooled with ischemic heart disease when these endpoints were reported separately as more than half of cardiovascular deaths are attributed to ischemic heart disease, and thus these events are not

mutually-exclusive.[22] Cardiovascular death was used to define cardiovascular events when only this outcome was reported. Similarly, cerebrovascular death was not combined with stroke or transient ischemic attack when reported separately. We conducted secondary analysis using the outcome of ischemic heart disease. Similar to cardiovascular events, in the absence of a composite endpoint of ischemic heart disease, we combined myocardial infarction and angina (for RCTs that reported both events separately). Finally, we conducted sensitivity analyses for this endpoint by using myocardial infarctions only for such trials since the occurrences of these two events are not mutually exclusive.

Data were meta-analyzed across RCTs to obtained pooled relative risks (RRs) and corresponding 95% confidence intervals (CIs) using DerSimonian and Laird random-effects models with inverse variance weighting.[23] All analyses were stratified by RCT design: 1) adjuvant RCTs of upfront AIs in comparison to upfront tamoxifen; 2) sequential treatment with tamoxifen and AIs (or vice versa) in comparison to tamoxifen; 3) sequential treatment with AIs and tamoxifen (or vice versa) in comparison to AIs; 4) extended adjuvant RCTs comparing AIs to placebo; 5) adjuvant RCTs comparing tamoxifen to placebo or no treatment; 6) extended adjuvant RCTs comparing tamoxifen to placebo. The amount of heterogeneity across the RCTs was estimated using the I^2 statistic.[24] To examine the impact of our choice of meta-analytic model, we conducted sensitivity analyses using the fixed-effects models with inverse variance weighting. A continuity correction was applied in RCTs with zero events.[25] All statistical analyses were conducted using R metafor package.[26]

4.6 RESULTS

4.6.1 Search Results

The flow diagram for our electronic search strategy and study selection is shown in Figure 1. Our electronic search identified 16,697 potentially relevant publications. After removing duplicates and screening titles, abstracts, and full-texts, we identified 35 publications corresponding to 19 different RCTs that met our inclusion criteria and were included in our systematic review and meta-analysis.

4.6.2 Study and Patient Characteristics

The study design and population characteristics of included RCTs are shown in Table 1. The mean age ranged across RCTs from 55 to 71 years. Most of the RCTs were completely restricted to post-menopausal women (n=12, 63%). RCTs that did not have this restriction (n=7, 37%) predominantly randomized post-menopausal women, including BIG 1-98 (98%),[27] SITAM-01 (94%),[28] ATLAS (90%),[29] Scottish (82%),[30] NSABP-B14 (73%),[31] NSABP-B14 phase I trial (70%),[31] and UK Over 50s (52%).[32] In addition, the majority of RCTs were restricted to patients with hormone-receptor (estrogen or progesterone receptor) positive breast cancer. The tamoxifen dose was 20 mg/day in the majority of the RCTs (n=15, 88%). In terms of individual AIs, the dose was consistent across RCTs using anastrozole (1 mg/day), letrozole (2.5 mg/day), and exemestane (25 mg/day).

4.6.3 Quality Assessment

The majority of RCTs were of low risk of bias in different domains of Cochrane Collaboration's tool (Table 1 and Supplemental Table 8) and were funded by industry. In the BIG 1-98 trial, 25% of patients selectively crossed over from the tamoxifen arm to the letrozole

arm in 2005 after it was demonstrated that letrozole significantly reduces distance recurrences and improves disease-free progression in comparison to tamoxifen.[4] However, all adverse events were reported within thirty days of selective crossover from tamoxifen to letrozole, and thus the crossover did not bias the results reported.[4] Similarly, in MA.17 trial, participants were unblinded at a median 2.4 years of follow-up.[33] However, the cardiovascular and cerebrovascular events were reported at 30 months of follow-up. Finally, in the Scottish trial, 330 patients out of 656 patients randomized to the control arm received tamoxifen during the study period due to relapse or suspicion of relapse.[34] The cardiovascular and cerebrovascular events were reported by treatment allocation at randomization and patients were censored at the date of systemic relapse.[30]

4.6.4 Cardiovascular Disease

The counts for cardiovascular events for all RCTs meeting the inclusion and exclusion and included in the quantitative analysis are reported in Supplemental Tables 6 and 7. Pooled analysis of eight RCTs comparing upfront adjuvant AIs to tamoxifen showed a 19% increased risk of cardiovascular events (RR: 1.19, 95% CI: 1.07-1.34; Figure 2). Similar results were obtained among RCTs comparing AIs to tamoxifen (RR: 1.19, 95% CI: 1.02-1.39; Supplemental Figure 1) and among RCTs comparing these two drugs after initial adjuvant treatment with tamoxifen (RR: 1.20, 95% CI: 1.02-1.41; Supplementary Figure 1). These results are also consistent when examining different AIs independently (Supplementary Figure 2). Similar results were also observed when comparing sequential treatment with tamoxifen and AIs to upfront treatment with tamoxifen alone (RR: 1.23, 95% CI: 0.93-1.61; Figure 2), although the 95% CIs were wide and included unity. Pooled analyses of RCTs comparing AIs to sequential

treatment with tamoxifen and AIs (RR: 1.16, 95% CI: 1.03-1.32; Figure 2) also demonstrate an increased risk of cardiovascular events associated with AIs. RCTs comparing sequential treatment with AIs and tamoxifen to upfront treatment with tamoxifen (RR: 1.07, 95% CI: 0.80-1.42; Figure 2), and RCTs of upfront treatment with AIs to sequential treatment with AIs and tamoxifen (RR: 1.10, 95% CI: 0.84-1.45; Figure 2) were all inconclusive due to wide 95% CIs. In the MA.17 trial, patients initially treated for a median five years with tamoxifen were randomized to additional five years of additional treatment with either letrozole or placebo.[33] In the MA.17R trial, patients initially treated with 4.5-6 years of adjuvant treatment with any aromatase inhibitor (preceded in most patients with tamoxifen treatment) were randomized to letrozole of placebo for an additional five years.[35] In this extended adjuvant setting, AIs were not associated with cardiovascular events (RR: 1.01, 95% CI: 0.85-1.20; Figure 2) when pooling data across these RCTs or when considering each RCT independently. Consistent with these results, pooled estimate showed a 33% decreased risk when comparing upfront tamoxifen to placebo or no treatment in the adjuvant setting (RR: 0.67, 95% CI: 0.45-0.98; Figure 2). In Scottish trial, approximately 50% of participants in the control arm received the treatment, which could lead to a dilution of the effect. However, similar results were obtained in a sensitivity analysis that excluded this study (RR: 0.65, 95% CI: 0.28-1.49), though estimates were less precise (Supplemental Figure 3). The cardiovascular safety of AIs in RCTs comparing tamoxifen to placebo or no treatment in the extended adjuvant setting is inconclusive due to lack of precision (RR: 0.91, 95% CI: 0.77-1.07). Similar results were obtained for all the above contrasts using fixed-effects analyses (Supplemental Figure 4).

4.6.5 Ischemic Heart Disease

Restricting the definition of cardiovascular events to ischemic heart disease yielded similar results (Supplemental Figures 5-6). In RCTs comparing upfront AIs to tamoxifen in the adjuvant setting, there was a 30% increased risk of ischemic heart disease when comparing AIs to tamoxifen (RR: 1.30, 95% CI: 1.11-1.53). In the extended adjuvant setting, AIs were not associated with an increased risk of ischemic heart disease in comparison to placebo (RR: 0.82, 95% CI: 0.60-1.13). Pooled analysis showed a significant 34% decreased risk when comparing upfront tamoxifen to placebo or no-treatment in the adjuvant setting (RR: 0.66, 95% CI: 0.45-0.98). The association between tamoxifen and cardiovascular ischemic events in the extended adjuvant setting remained inconclusive due to low precision (RR: 1.21, 95% CI: 0.58-2.53) and high degree of heterogeneity (I^2 statistic: 60.6%). Similar results were also obtained when restricting the definition of ischemic heart disease to myocardial infarction in RCTs reporting myocardial infarction and angina (rather than a composite endpoint of ischemic heart disease). However, in this analysis there was loss of precision in the pooled estimate when comparing tamoxifen to placebo in the adjuvant setting and tamoxifen to placebo or no-treatment in the extended adjuvant setting (Supplemental Figures 7-8).

4.6.6 Cerebrovascular Disease

Cerebrovascular endpoints were reported inconsistently across RCTs, leading to low events rates. When data were pooled across trials, no evidence of a difference was observed (Figure 3). However, these analyses were inconclusive due to wide 95% CIs (upfront treatment with AIs versus tamoxifen: RR: 0.96, 95% CI: 0.61-1.51; AIs versus sequential treatment with tamoxifen and AIs: RR: 0.96, 95% CI: 0.53-1.73; tamoxifen versus no treatment in the extended

adjuvant setting: RR: 1.00, 95% CI: 0.44-2.24; and tamoxifen versus placebo or no treatment in the adjuvant setting: RR: 1.18, 95% CI: 0.67-2.08). Similar results were obtained using fixed-effects analysis (Supplemental Figure 9).

4.7 DISCUSSION

The risk of cardiovascular disease increases with age and is considerably higher in post-menopausal women in comparison to pre-menopausal women.[36] Thus, in post-menopausal women, excess risk of cardiovascular disease from breast cancer treatment is a major health concern. Similar to previous studies, we found that adjuvant treatment with AIs increases the risk of cardiovascular events in comparison to tamoxifen in post-menopausal women with breast cancer. However, we also found that tamoxifen is associated with 33% reduction in risk of cardiovascular events in RCTs comparing tamoxifen to placebo or no treatment. Thus, the cardioprotective effects of tamoxifen can completely account for the increase risk in cardiovascular events observed in the RCTs comparing AIs to tamoxifen. This conclusion is further supported by the MA.17 and MA.17R RCTs, where there was no association between AIs and cardiovascular event or ischemic heart disease in the extended adjuvant setting.[35,37] The cardiovascular safety of AIs may also be compared to placebo in the MAP.3 breast cancer prevention RCT.[38] In this setting, there was also no increased risk of cardiovascular events when comparing exemestane to placebo in post-menopausal women at moderate risk of breast cancer at 35 months of follow-up.[38]

Tamoxifen has been shown to decrease cardiovascular disease in previous studies. A meta-analysis of all RCTs comparing tamoxifen to placebo or no-treatment (in the presence of co-interventions) demonstrated that tamoxifen decreases the risk of myocardial infarction by 26% (RR: 0.74, 95% CI: 0.47-1.16) and the risk of myocardial infarction-associated mortality by 45% (RR: 0.55, 95% CI: 0.36-0.87) in breast cancer treatment RCTs.[39] In the Swedish Breast Cancer Group RCT, post-menopausal women with early stage breast cancer were randomized to either five or two years of treatment with tamoxifen.[40,41] In this setting, treatment with tamoxifen for five years led to lower incidence of coronary heart disease and coronary heart disease-

associated mortality in comparison to two years of treatment with tamoxifen during the treatment period.[40] There remained a decrease in the risk of coronary heart disease (HR: 0.83, 95% CI: 0.70-1.00) and coronary heart disease mortality (HR: 0.72, 95% CI: 0.53-0.97) at a median 12 years of follow-up.[40] Finally, in IBIS-I tamoxifen breast-cancer prevention RCT, there remained a non-significant decrease in the incidence of myocardial infarction when comparing tamoxifen to placebo at 16 years of median follow-up in healthy women at risk of breast cancer (OR: 0.76, CI: 0.34-1.67).[42]

A major mechanism proposed for the cardioprotective effects of tamoxifen is alterations in serum lipid levels. In RCTs comparing tamoxifen to placebo, tamoxifen decreases serum total and LDL cholesterol, while increasing apoliprotein A-I levels in post-menopausal women with breast cancer[16,19] and in healthy post-menopausal women.[18] Tamoxifen may lower LDL and total cholesterol by inhibiting enzymes involve in cholesterol metabolism pathway including sterol-Δ8,7 isomerase and Acetyl-Coenzyme A acetyltransferase.[17] In contrast, evidence from RCTs that suggests AIs do not significantly alter plasma lipoproteins. In ATENA and a MA.17L substudy, there were no differences in plasma lipoprotein between patients who received AIs and those who received placebo or no treatment.[43-45] Consistent with these results, it has been demonstrated that AIs do not systematically alter plasma lipoproteins from baseline to follow-up assessments.[12] Tamoxifen also has anti-inflammatory effects and lowers C-reactive protein and fibrinogen levels, both of which are strong predictors of cardiovascular disease.[19,46-49] The anti-inflammatory effects of tamoxifen may also be mediated through cytokine TGF-β, which maintains vessel wall structure during atherogenesis.[17,50] Finally, tamoxifen has been shown to have antioxidant properties which protect LDL cholesterol from harmful oxidation.[51,52]

Previous systematic reviews and meta-analyses have compared the cardiovascular effects of AIs to tamoxifen with discordant results.[8-13] The discordance between these studies may be due to consideration of trials comparing AIs to tamoxifen in adjuvant setting only,[8] absence of a systematic search or limited search of electronic databases,[9,10,13] and qualitative assessment of evidence.[12] This is the first study to date to additionally include adjuvant and extended-adjuvant RCTs comparing the cardiovascular effects of tamoxifen to placebo or no treatment. We have also included up-to-date results from adjuvant RCTs comparing AIs to tamoxifen and the extended-adjuvant RCTs comparing AIs to placebo. Thus, the major strength of this study is the consideration of the totality of evidence from all RCTs of AIs and tamoxifen in the adjuvant and extended-adjuvant setting.

There are also some limitations to this study. First, there was heterogeneity in reporting of cardiovascular and cerebrovascular endpoints between studies. However, we additionally used ischemic heart disease as definition of cardiovascular event in our secondary analysis and found similar results in comparison to using composite endpoint of cardiovascular events. Second, there was some heterogeneity present among RCTs with respect to duration of follow-up, patient recruitment periods, and patient characteristics. Nevertheless, results were consistent when analysis was conducted across trial subtypes. We used random-effects models to account for between-study heterogeneity and found results were consistent with fixed-effects analysis. Efficacy was the primary endpoint of RCTs of AIs and tamoxifen included in this study and thus publication bias in regards to cardiotoxicity of these drugs is not anticipated. In addition, there was not sufficient information to assess risk of cardiovascular events by patients' baseline cardiovascular disease risk. We were also not able to conduct analysis for cardiovascular mortality as this endpoint was reported inconsistently across trials. Pharmacoepidemiologic

studies will need to address whether AIs increase the risk of cardiovascular-associated mortality in comparison to tamoxifen.

4.8 CONCLUSIONS

RCTs directly comparing AIs to tamoxifen suggest that AIs are associated with an increased risk of cardiovascular events. As a result, current clinical practice guidelines indicate that ischemic heart disease is a major adverse event associated with AIs.[7] However, the results from this study demonstrate that the cardiovascular events associated with AIs in RCTs directly comparing AIs to tamoxifen may be accounted for by the cardioprotective effects of tamoxifen. Concordant with these results, AIs are not associated with cardiovascular events when compared to placebo in the extended-adjuvant setting. The results from this study are consistent with the putative mechanisms for cardioprotective actions of tamoxifen in previous studies. The findings of this systematic review and meta-analysis should be considered when assessing the benefits and risks of AIs in treatment of breast cancer in post-menopausal women.

Acknowledgements

Dr. Filion holds a Canadian Institutes of Health Research New Investigator Award. Dr. Samy Suissa is the recipient of the James McGill Chair. Dr. Laurent Azoulay is the recipient of a Chercheur-Boursier career award from the Fonds de la recherche en santé du Québec.

Funding

This study was supported by a Foundation Grant from the Canadian Institutes of Health Research (FDN-333744). The funding source has no role design and conduct of the study, collection, management, analysis, writing, review, or approval of the manuscript.

Disclosure: NB served as a consultant from Amgen, Novartis, Amgen, and Roche. SS has received research funding, participated in advisory board meetings or as a speaker for AstraZeneca, Boehringer-Ingelheim, Novartis, Pfizer and Merck. All other authors have no conflicts of interest to declare.

4.9 REFERENCES

1. Kelly E, Lu CY, Albertini S, Vitry A. Longitudinal trends in utilization of endocrine therapies for breast cancer: an international comparison. *Journal of clinical pharmacy and therapeutics.* 2015;40(1):76-82.

2. Early Breast Cancer Trialists' Collaborative G, Dowsett M, Forbes JF, et al. Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. *Lancet.* 2015;386(10001):1341-1352.

3. Comprehensive side-effect profile of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: long-term safety analysis of the ATAC trial. *Lancet Oncol.* 2006;7(8):633-643.

4. Colleoni M, Giobbie-Hurder A, Regan MM, et al. Analyses adjusting for selective crossover show improved overall survival with adjuvant letrozole compared with tamoxifen in the BIG 1-98 study. *J Clin Oncol.* 2011;29(9):1117-1124.

5. Bliss JM, Kilburn LS, Coleman RE, et al. Disease-related outcomes with long-term follow-up: an updated analysis of the intergroup exemestane study. *J Clin Oncol.* 2012;30(7):709-717.

6. Van De Velde CJ, Rea D, Seynaeve C, et al. Adjuvant tamoxifen and exemestane in early breast cancer (TEAM): A randomised phase 3 trial. *The Lancet.* 2011;377(9762):321-331.

7. Burstein HJ, Temin S, Anderson H, et al. Adjuvant endocrine therapy for women with hormone receptor-positive breast cancer: american society of clinical oncology clinical practice guideline focused update. *J Clin Oncol.* 2014;32(21):2255-2269.

8. Amir E, Seruga B, Niraula S, Carlsson L, Ocana A. Toxicity of adjuvant endocrine therapy in postmenopausal breast cancer patients: a systematic review and meta-analysis. *J Natl Cancer Inst.* 2011;103(17):1299-1309.

9. Aydiner A. Meta-analysis of breast cancer outcome and toxicity in adjuvant trials of aromatase inhibitors in postmenopausal women. *Breast.* 2013;22(2):121-129.

10. Cuppone F, Bria E, Verma S, et al. Do adjuvant aromatase inhibitors increase the cardiovascular risk in postmenopausal women with early breast cancer? Meta-analysis of randomized trials. *Cancer.* 2008;112(2):260-267.

11. Josefsson ML, Leinster SJ. Aromatase inhibitors versus tamoxifen as adjuvant hormonal therapy for oestrogen sensitive early breast cancer in post-menopausal women: meta-analyses of monotherapy, sequenced therapy and extended therapy. *Breast.* 2010;19(2):76-83.

12. Younus M, Kissner M, Reich L, Wallis N. Putting the cardiovascular safety of aromatase inhibitors in patients with early breast cancer into perspective: a systematic review of the literature. *Drug Saf.* 2011;34(12):1125-1149.

13. Ryden L, Heibert Arnlind M, Vitols S, Hoistad M, Ahlgren J. Aromatase inhibitors alone or sequentially combined with tamoxifen in postmenopausal early breast cancer compared with tamoxifen or placebo - Meta-analyses on efficacy and adverse events based on randomized clinical trials. *Breast.* 2016;26:106-114.

14. Bruning PF, Bonfrer JM, Hart AA, et al. Tamoxifen, serum lipoproteins and cardiovascular risk. *British journal of cancer.* 1988;58(4):497-499.

15. Cuzick J, Allen D, Baum M, et al. Long term effects of tamoxifen. Biological effects of Tamoxifen Working Party. *European journal of cancer.* 1992;29A(1):15-21.

16. Dewar JA, Horobin JM, Preece PE, Tavendale R, Tunstall-Pedoe H, Wood RA. Long term effects of tamoxifen on blood lipid values in breast cancer. *BMJ.* 1992;305(6847):225-226.

17. Grainger DJ, Schofield PM. Tamoxifen for the prevention of myocardial infarction in humans: preclinical and early clinical evidence. *Circulation.* 2005;112(19):3018-3024.

18. Guetta V, Lush RM, Figg WD, Waclawiw MA, Cannon RO, 3rd. Effects of the antiestrogen tamoxifen on low-density lipoprotein concentrations and oxidation in postmenopausal women. *Am J Cardiol.* 1995;76(14):1072-1073.

19. Love RR, Wiebe DA, Feyzi JM, Newcomb PA, Chappell RJ. Effects of tamoxifen on cardiovascular risk factors in postmenopausal women after 5 years of treatment. *J Natl Cancer Inst.* 1994;86(20):1534-1539.

20. British Medical Journal Evidence Center Information Specialists. Study Design Search Filters. *BMJ Clinical Evidence* http://clinicalevidence.bmj.com/x/set/static/ebm/learn/665076.html.

21. Higgins JP, Altman DG, Gotzsche PC, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ.* 2011;343:d5928.

22. Center for Disease Control and Prevention. Heart Disease Facts. 2015; http://www.cdc.gov/heartdisease/facts.htm.

23. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials.* 1986;7(3):177-188.

24. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ.* 2003;327(7414):557-560.

25. Friedrich JO, Adhikari NK, Beyene J. Inclusion of zero total event trials in meta-analyses maintains analytic consistency and incorporates all available data. *BMC Med Res Methodol.* 2007;7:5.

26. Viechtbauer W. Conducting meta-analysis in R with metafor Package. *Journal of Statistical Software.* 2010;36(3):1-48.

27. Thurlimann B, Keshaviah A, Coates AS, et al. A comparison of letrozole and tamoxifen in postmenopausal women with early breast cancer. *New England Journal of Medicine.* 2005;353(26):2747-2757.

28. Sacco M, Valentini M, Belfiglio M, et al. Randomized trial of 2 versus 5 years of adjuvant tamoxifen for women aged 50 years or older with early breast cancer: Italian Interdisciplinary Group for Cancer Evaluation Study of Adjuvant Treatment in Breast Cancer 01. *Journal of Clinical Oncology.* 2003;21(12):2276-2281.

29. Davies C, Pan H, Godwin J, et al. Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial. *The Lancet.* 2013;381(9869):805-816.

30. McDonald CC, Alexander FE, Whyte BW, Forrest AP, Stewart HJ. Cardiac and vascular morbidity in women receiving adjuvant tamoxifen for breast cancer in a randomised trial. The Scottish Cancer Trials Breast Group. *Bmj.* 1995;311(7011):977-980.

31. Fisher B, Dignam J, Bryant J, et al. Five versus more than five years of tamoxifen therapy for breast cancer patients with negative lymph nodes and estrogen receptor-positive tumors. *Journal of the National Cancer Institute.* 1996;88(21):1529-1542.

32. Hackshaw A, Roughton M, Forsyth S, et al. Long-term benefits of 5 years of tamoxifen: 10-Year follow-up of a large randomized trial in women at least 50 years of age with early breast cancer. *Journal of Clinical Oncology.* 2011;29(13):1657-1663.

33. Goss PE, Ingle JN, Martino S, et al. A Randomized Trial of Letrozole in Postmenopausal Women after Five Years of Tamoxifen Therapy for Early-Stage Breast Cancer. *N Engl J Med.* 2003;349(19):1793-1802.

34. Stewart HJ, Prescott RJ, Forrest AP. Scottish adjuvant tamoxifen trial: a randomized study updated to 15 years. *J Natl Cancer Inst.* 2001;93(6):456-462.

35. Goss PE, Ingle JN, Pritchard KI, et al. Extending Aromatase-Inhibitor Adjuvant Therapy to 10 Years. *N Engl J Med.* 2016.

36. Rosano GM, Vitale C, Marazzi G, Volterrani M. Menopause and cardiovascular disease: the evidence. *Climacteric.* 2007;10 Suppl 1:19-24.

37. Goss PE, Ingle JN, Martino S, et al. Randomized trial of letrozole following tamoxifen as extended adjuvant therapy in receptor-positive breast cancer: Updated findings from NCIC CTG MA.17. *J Natl Cancer Inst.* 2005;97(17):1262-1271.

38. Goss PE, Ingle JN, Ales-Martinez JE, et al. Exemestane for breast-cancer prevention in postmenopausal women. *N Engl J Med.* 2011;364(25):2381-2391.

39. Braithwaite RS, Chlebowski RT, Lau J, George S, Hess R, Col NF. Meta-analysis of vascular and neoplastic events associated with tamoxifen. *Journal of general internal medicine.* 2003;18(11):937-947.

40. Rosell J, Nordenskjold B, Bengtsson NO, et al. Effects of adjuvant tamoxifen therapy on cardiac disease: Results from a randomized trial with long-term follow-up. *Breast Cancer Research and Treatment.* 2013;138(2):467-473.

41. Randomized trial of two versus five years of adjuvant tamoxifen for postmenopausal early stage breast cancer. Swedish Breast Cancer Cooperative Group. *Journal of the National Cancer Institute.* 1996;88(21):1543-1549. http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/032/CN-00133032/frame.html.

42. Cuzick J, Sestak I, Cawthorn S, et al. Tamoxifen for prevention of breast cancer: extended long-term follow-up of the IBIS-I breast cancer prevention trial. *Lancet Oncol.* 2015;16(1):67-75.

43. Markopoulos C, Chrissochou M, Michailidou A, et al. Effect of exemestane on the lipidemic profile of post-menopausal operable breast cancer patients following 5-7 years of adjuvant tamoxifen: Preliminary results of the ATENA substudy. *Anti-cancer drugs.* 2005;16(8):879-883.

44. Markopoulos C, Dafni U, Misitzis J, et al. Extended adjuvant hormonal therapy with exemestane has no detrimental effect on the lipid profile of postmenopausal breast cancer patients: final results of the ATENA lipid substudy. *Breast Cancer Res.* 2009;11(3):R35.

45. Wasan KM, Goss PE, Pritchard PH, et al. The influence of letrozole on serum lipid concentrations in postmenopausal women with primary breast cancer who have completed 5 years of adjuvant tamoxifen (NCIC CTG MA.17L). *Ann Oncol.* 2005;16(5):707-715.

46. Bonanni B, Johansson H, Gandini S, et al. Effect of tamoxifen at low doses on ultrasensitive C-reactive protein in healthy women. *J Thromb Haemost.* 2003;1(10):2149-2152.

47. Cushman M, Costantino JP, Tracy RP, et al. Tamoxifen and cardiac risk factors in healthy women: Suggestion of an anti-inflammatory effect. *Arterioscler Thromb Vasc Biol.* 2001;21(2):255-261.

48. Stec JJ, Silbershatz H, Tofler GH, et al. Association of fibrinogen with cardiovascular risk factors and cardiovascular disease in the Framingham Offspring Population. *Circulation.* 2000;102(14):1634-1638.

49. Emerging Risk Factors C, Kaptoge S, Di Angelantonio E, et al. C-reactive protein, fibrinogen, and cardiovascular disease prediction. *N Engl J Med.* 2012;367(14):1310-1320.

50. Grainger DJ, Witchell CM, Metcalfe JC. Tamoxifen elevates transforming growth factor-beta and suppresses diet-induced formation of lipid lesions in mouse aorta. *Nat Med.* 1995;1(10):1067-1073.

51. Wiseman H. Tamoxifen as an antioxidant and cardioprotectant. *Biochem Soc Symp.* 1995;61:209-219.

52. Wiseman H, Laughton MJ, Arnstein HR, Cannon M, Halliwell B. The antioxidant action of tamoxifen and its metabolites. Inhibition of lipid peroxidation. *FEBS Lett.* 1990;263(2):192-194.

53. Baum M, Buzdar AU, Cuzick J, et al. Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early breast cancer: First results of the ATAC randomised trial. *Lancet.* 2002;359(9324):2131-2139.

54. Abo-Touk NA, Sakr HA, Abd El-Lattef A. Switching to letrozole versus continued tamoxifen therapy in treatment of postmenopausal women with early breast cancer. *J Egypt Natl Canc Inst.* 2010;22(1):79-85.

55. Aihara T, Takatsuka Y, Ohsumi S, et al. Phase III randomized adjuvant study of tamoxifen alone versus sequential tamoxifen and anastrozole in Japanese postmenopausal women with hormone-responsive breast cancer: N-SAS BC03 study. *Breast Cancer Research and Treatment.* 2010;121(2):379-387.

56. Boccardo F, Rubagotti A, Puntoni M, et al. Switching to anastrozole versus continued tamoxifen treatment of early breast cancer: Preliminary results of the Italian Tamoxifen Anastrozole Trial. *Journal of Clinical Oncology.* 2005;23(22):5138-5147.

57. Kaufmann M, Jonat W, Hilfrich J, et al. Improved overall survival in postmenopausal women with early breast cancer after anastrozole initiated after treatment with tamoxifen compared with continued tamoxifen: The ARNO 95 study. *Journal of Clinical Oncology.* 2007;25(19):2664-2670.

58. Coombes RC, Hall E, Gibson LJ, et al. A Randomized Trial of Exemestane after Two to Three Years of Tamoxifen Therapy in Postmenopausal Women with Primary Breast Cancer. *N Engl J Med.* 2004;350(11):1081-1092.

59. Paridaens RJ, Dirix LY, Beex LV, et al. Phase III study comparing exemestane with tamoxifen as first-line hormonal treatment of metastatic breast cancer in postmenopausal women: The European Organisation for Research and Treatment of Cancer Breast Cancer Cooperative Group. *Journal of Clinical Oncology.* 2008;26(30):4883-4890.

60. Takei H, Ohsumi S, Shimozuma K, et al. Health-related quality of life, psychological distress, and adverse events in postmenopausal women with breast cancer who receive tamoxifen, exemestane, or anastrozole as adjuvant endocrine therapy: National Surgical Adjuvant Study of Breast Cancer 04 (N-SAS BC 04). *Breast Cancer Res Treat.* 2012;133(1):227-236.

61. Preliminary results from the cancer research campaign trial evaluating tamoxifen duration in women aged fifty years or older with breast cancer. Current Trials working Party of the Cancer Research Campaign Breast Cancer Trials Group. *J Natl Cancer Inst.* 1996;88(24):1834-1839.

62. Adjuvant tamoxifen in the management of operable breast cancer: the Scottish Trial. Report from the Breast Cancer Trials Committee, Scottish Cancer Trials Office (MRC), Edinburgh. Lancet. 1987;2(8552):171-175. http://onlinelibrary.wiley.com/o/cochrane/clcentral/articles/771/CN-00048771/frame.html.

63. Cummings FJ, Gray R, Tormey DC, et al. Adjuvant tamoxifen versus placebo in elderly women with node-positive breast cancer: long-term follow-up and causes of death. *J Clin Oncol.* 1993;11(1):29-35.

4.10 FIGURE LEGENDS

Table 4.1 Patient characteristics at baseline in randomized controlled trials of aromatase inhibitors and tamoxifen included in the study.

Abbreviations: A-Anastrozole, E-Exemestane HR-Hormone-receptor (estrogen or progesterone) positive, L-Letrozole, NT-No Treatment, P-Placebo, PM-post-menopausal, T-Tamoxifen

Figure 4.1 PRISMA flow diagram describing systematic search for RCTs of aromatase inhibitors and tamoxifen.

Figure 4.2 Forest plot of relative risks of cardiovascular events with AIs and tamoxifen by trial design. Pooled relative risks and confidence intervals were obtained using DerSimonian and Laird random-effects models.

Abbreviation: ARNO 95: German Adjuvant Breast Cancer Group/Arimidex-Novaldex 95, ATAC: Anastrozole, Tamoxifen, Alone or in Combination, ATLAS: Adjuvant Tamoxifen, Longer Against Shorter trial, BIG 1-98: Breast International Group 1-98, IES: Intergroup Exemestane Study, ITA: Italian Tamoxifen Anastrozole trial, NSABP-B14-National Surgical Adjuvant Breast and Bowel Project B14, N-SAS BC03: National Surgical Adjuvant Study Breast Cancer 03 trial, N-SAS BC04: National Surgical Adjuvant Study Breast Cancer 04 trial, SITAM-01: Italian Study of Adjuvant Treatment in Breast Cancer-01, TEAM: Tamoxifen Exemestane Adjuvant Multinational trial, AI: Aromatase Inhibitors, CI: Confidence Interval, NT: No treatment, P: placebo, RR: Relative risk, T: Tamoxifen. Arrow indicates switch between endocrine therapy.

Figure 4.3 Forest plot of relative risks of cerebrovascular events with AIs and tamoxifen by trial design. Pooled relative risks and confidence intervals were obtained using DerSimonian and Laird random-effects models.

Abbreviations: ARNO 95: German Adjuvant Breast Cancer Group/Arimidex-Novaldex 95, ATAC: Anastrozole, Tamoxifen, Alone or in Combination, ATLAS: Adjuvant Tamoxifen, Longer Against Shorter trial, BIG 1-98: Breast International Group 1-98, ITA: Italian Tamoxifen Anastrozole trial, NSABP B-14: National Surgical Adjuvant Breast and Bowel Project B-14, N-SAS BC03: National Surgical Adjuvant Study Breast Cancer 03 trial, N-SAS BC04: National Surgical Adjuvant Study Breast Cancer 04 trial, SITAM-01: Italian Study of Adjuvant Treatment in Breast Cancer-01, TEAM: Tamoxifen Exemestane Adjuvant Multinational trial, AI: Aromatase Inhibitors, CI: Confidence Interval, NT: No treatment, P: placebo, RR: Relative risk, T: Tamoxifen. Arrow indicates switch between endocrine therapy.

Table 4.1 Patient characteristics at baseline in randomized controlled trials of aromatase inhibitors and tamoxifen included in the study.

Trial	Trial Funding	Trial arm (randomized)	Age (mean)	PM (%)	Node positive (%)	HR-positive (%)	Tumor size >2cm (%)	Primary treatment		
								Mastectomy (%)	Radiotherapy (%)	Chemotherapy (%)
ATAC[53]	Industry	A-3125	A-64	A-100	A-35	A-84	A-35	A-48	A-63	A-22
		T-3116	T-64	T-100	T-34	T-83	T-36	T-47	T-63	T-21
BIG 1-98[27]	Industry &	L-4003	L-61*	L-99	L-42	L-~100	L-37	L-44	L-72	L-25
	Nonprofit	T-4007	T-61*	T-98	T-41	T-~100	T-38	T-42	T-72	T-25
Abo-Touk N et al.[54]	NA	L-60	L-NA	L-100	L-48	L-100	L-87	L-70	L-95	L-NA
		T-60	T-NA	T-100	T-35	T-100	T-83	T-68	T-93	T-NA
N-SAS BC03[55]	Industry &	A-347	A-60	A-100	A-41	A-100	A-21‡	A-48	A-NA	A-54
	Nonprofit	T-349	T-60	T-100	T-40	T-100	T-21‡	T-48	T-NA	T-53
ITA[56]	Industry	A-223	A-63*	A-100	A-100	A-91	A-24	A-52	A-54	A-67
		T-225	T-63*	T-100	T-100	T-86	T-19	T-55	T-49	T-67
ARNO95[57]	Industry	A-489	A-61	A-100	A-26	A-97	A-36	A-33	A-67	A-NA
		T-490	T-61	T-100	T-27	T-96	T-37	T-30	T-68	T-NA
IES[58]	Industry &	E-2352	E-64	E-100	E-44	E-81†	E-NA	E-52	E-NA	E-32
	Nonprofit	T -2372	T-64	T-100	T-44	T-81†	T-NA	T-52	T-NA	T-32
Paridaens RJ et al.[59]	Industry &	E-190	E-63*	E-100	E-NA	E-92	E-NA	E-NA	E-41	E-30
	Nonprofit	T-192	T-62*	T-100	T-NA	T-94	T-NA	T-NA	T-42	T-33
TEAM[6]	Industry	E-4904	E-64*	E-100	E-47	E-100	E-42	E-44	E-69	E-36
		T→E-4875	T→E-64*	T→E-100	T→E-47	T→E-100	T→E-41	T→E-45	T→E-68	T→E-36
N-SAS BC04[60]	Industry &	A-55	A-63	A-100	A-66	A-95†	A-NA	A-33	A-62	A-38
	Nonprofit	E-55	E-63	E-100	E-62	E-96†	E-NA	E-27	E-64	E-38
MA.17[33]	Industry &	L-2593	L-62*	L-100	L-46	L-98	L-NA	L-50	L-60	L-46
	Nonprofit	P-2594	P-62*	P-100	P-46	P-98	P-NA	P-50	P-59	P-46
MA.17R[35]	Industry &	L-959	L-66*	L-100	L-51	L-99	L-9	L-48	L-NA	L-59
	Non-profit	P-959	P-65*	P-100	P-52	P-99	P-8	P-49	P-NA	P-58
ATLAS[29]	Industry &	T-6454	T-NA	T-90	T-41	T-53†	T-52	T-72	T-NA	T-NA
	Nonprofit	NT-6440	NT-NA	NT-90	NT-40	NT-53†	NT-52	NT-71	NT-NA	NT-NA
SITAM-01[28]	Industry	T-943	T-61	T-94	T-44	T-59†	T-45	T-63	T-40	T-11
		NT-958	NT-61	NT-95	NT-43	NT-61†	NT-43	NT-64	NT-39	NT-9
NSABP B-14[31]	Nonprofit	T-583	T-56	T-73	T-0	T-100†	T-32	T-56	T-NA	T-NA
		P-570	P-56	P-74	P-0	P-100†	P-35	P-56	P-NA	P-NA
UK Over 50s[32,61]	Nonprofit	T-1725	T-62*	T-53	T-25	T-NA	T-NA	T-38	T-62	T-NA
		NT-1724	NT-62*	NT-52	P-26	NT-NA	NT-NA	NT-37	NT-62	NT-NA
Scottish[62]	Industry &	T-661	T-59	T- 82	T -32	T-41†	T-68	T-100	T-32	T-NA
	Nonprofit	NT-651	NT-59	NT-82	NT-33	NT-39†	NT-71	NT-100	NT-31	NT-NA
NSABP-B14 phase I[31]	Nonprofit	T-1404	T-55	T-71	T-0	T-100†	T-43	T-62	T-NA	T-NA
		P-1414	P-55	P-68	P-0	P-100†	P-41	P-62	P-NA	P-NA
Cummings FJ et al.[63]	Nonprofit	T-85	T-71*	T-100	T-100	T-86†	T-33‡	T-100	T-NA	T-NA
		P-83	P-70*	P-100	P-100	P-84†	P-28‡	P-100	P-NA	P-NA

Symbols: * median age, † Estrogen-receptor positive, ‡ ≥3cm, Proportions do not include patients with unknown, uncertain, or other status, arrow indicates switch between endocrine therapy

Figure 4.1 PRISMA flow diagram describing systematic search for RCTs of aromatase inhibitors and tamoxifen.

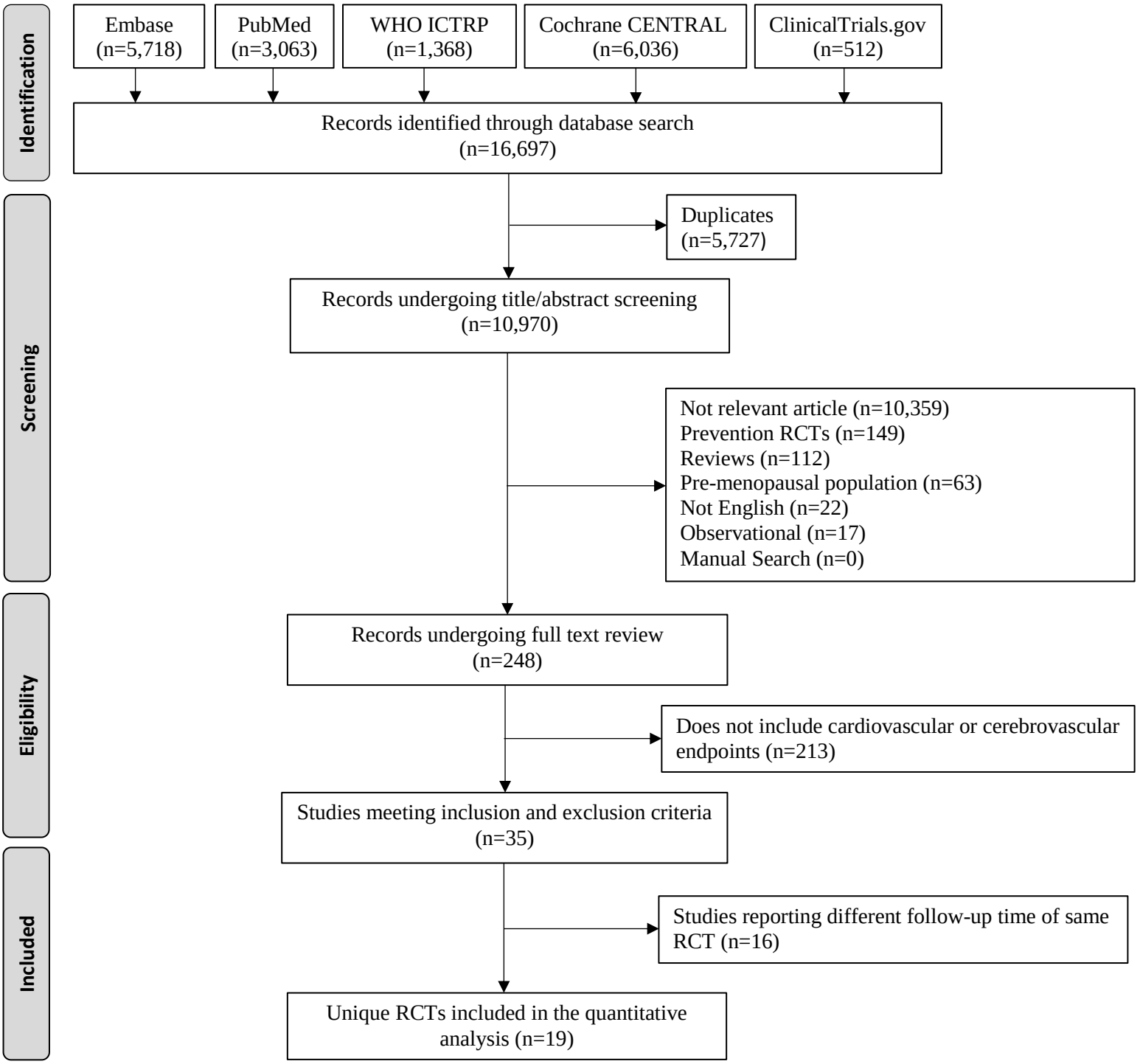


Figure 4.2 Forest plot of relative risks of cardiovascular events with AIs and tamoxifen by trial design. Pooled relative risks and confidence intervals were obtained using DerSimonian and Laird random-effects models.

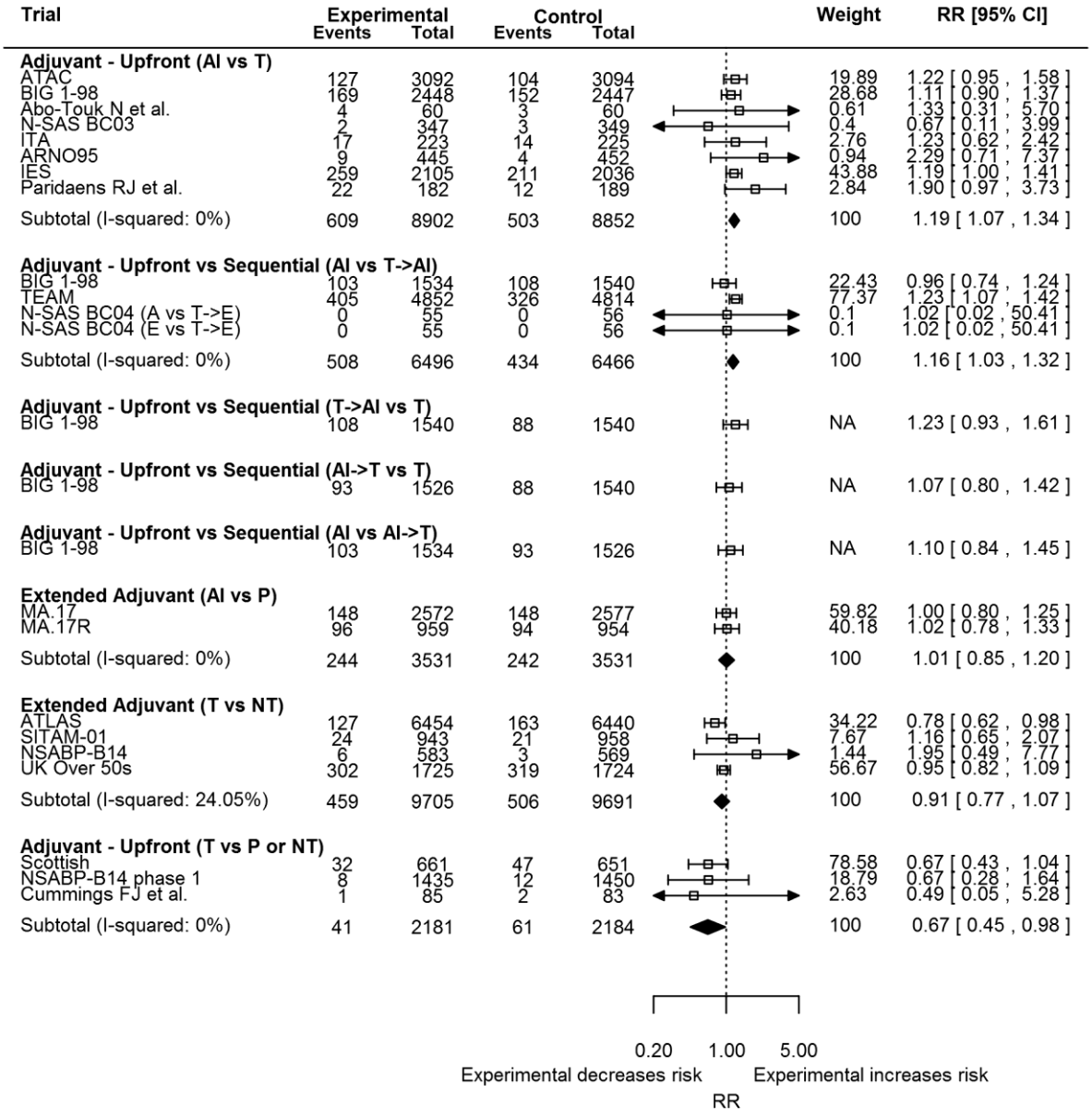
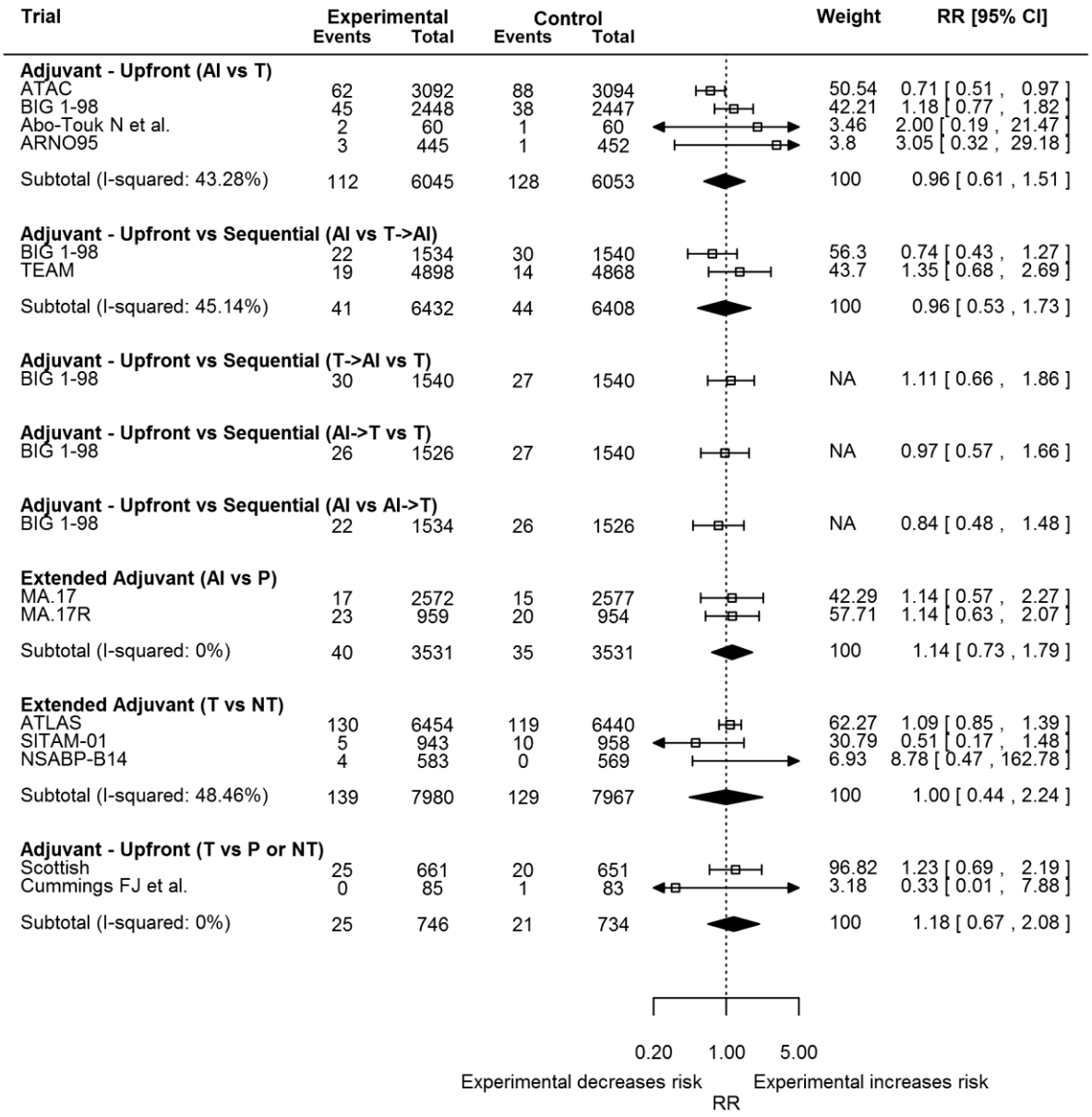


Figure 4.3 Forest plot of relative risks of cerebrovascular events with AIs and tamoxifen by trial design. Pooled relative risks and confidence intervals were obtained using DerSimonian and Laird random-effects models.



4.11 Online Supplementary Material

Supplemental Table 4.1 Embase (OvidSP) search strategy.

Supplemental Table 4.2 PubMed search strategy.

Supplemental Table 4.3 Cochrane Central Register of Controlled Trials (CENTRAL) search strategy.

Supplemental Table 4.4 ClincialTrials.gov search strategy.

Supplemental Table 4.5 World Health Organization International Clinical Trials Registry Platform search strategy.

Supplemental Table 4.6 Cardiovascular and cerebrovascular events reported in randomized controlled trials included in the quantitative analysis.

Supplemental Table 4.7 Enumeration of cardiovascular and cerebrovascular events in randomized controlled trials included in the quantitative analysis.

Supplemental Table 4.8 Quality assessment of randomized controlled trials included in the quantitative analysis using Cochrane Collaboration tool for assessing risk of bias [1].

Supplemental Figure 4.1 Forest plot of relative risks of cardiovascular events in randomized controlled trials comparing aromatase inhibitor to tamoxifen when separating trials with no inclusion criteria for treatment with tamoxifen prior to randomization (upper panel) and trials including patients receiving 2-3 years of previous tamoxifen treatment (lower panel). Pooled relative risks and confidence intervals were obtained using DerSimonian and Laird random-effects models.

Supplemental Figure 4.2 Forest plot of relative risks of cardiovascular events in randomized controlled trials comparing AIs to tamoxifen in adjuvant setting stratified by drug molecule (A: anastrozole, L: letrozole, E: exemestane, T: tamoxifen). Pooled relative risks and confidence intervals were obtained using DerSimonian and Laird random-effects models.

Supplemental Figure 4.3 Forest plot of relative risks of cardiovascular events in randomized controlled trials comparing tamoxifen to placebo or no-treatment when excluding Scottish trial. Pooled relative risks and confidence intervals were obtained using DerSimonian and Laird random-effects models.

Supplemental Figure 4.4 Forest plot of relative risks of cardiovascular events by trial design. Pooled relative risks and confidence intervals were obtained using inverse-variance fixed-effects models.

Supplemental Figure 4.5 Forest plot of relative risks of ischemic cardiovascular events by trial design. Pooled relative risks and confidence intervals were obtained using DerSimonian and Laird random-effects models.

Supplemental Figure 4.6 Forest plot of relative risks of ischemic cardiovascular events by trial design. Pooled relative risks and confidence intervals were obtained using inverse-variance fixed-effects models.

Supplemental Figure 4.7 Forest plot of relative risks of ischemic cardiovascular events by trial design when restricting outcome definition to myocardial infarction in trials reporting myocardial infarction and angina. Pooled relative risks and confidence intervals were obtained using DerSimonian and Laird random-effects models.

Supplemental Figure 4.8 Forest plot of relative risks of ischemic cardiovascular adverse events by trial design when restricting outcome definition to myocardial infarction in trials reporting myocardial infarction and angina. Pooled relative risks and confidence intervals were obtained using inverse-variance fixed-effects models.

Supplemental Figure 4.9 Forest plot of relative risks of cerebrovascular events of AIs and tamoxifen by trial design. Pooled relative risks and confidence intervals were obtained using inverse-variance fixed-effects models.

Supplemental Table 4.1 Embase (OvidSP) search strategy.

#	Search Statement	Results
1	aromatase inhibitors.mp. or exp aromatase inhibitor/	25,694
2	(aromatase inhibitor or estrogen synthetase inhibitor).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]	13,117
3	AIs.ti,ab.	10,662
4	Anastrozole.mp. or anastrozole/	7,922
5	Arimidex.mp.	1,644
6	Letrozole.mp. or letrozole/	8,871
7	Femara.mp.	1,046
8	Exemestane.mp. or exemestane/	4,946
9	Aromasin.mp.	495
10	tamoxifen/	52,045
11	Tamoxifen.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]	55784
12	selective estrogen receptor modulator/	6,671
13	SERM.mp.	1,831
14	SERMs.mp.	1,881
15	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14	83,828
16	(random$ or placebo$ or single blind$ or double blind$ or triple blind$).ti,ab.	1,224,244
17	RETRACTED ARTICLE/	8,238
18	16 or 17	1,232,275
19	(animal$ not human$).sh,hw.	4,074,268
20	(book or conference paper or editorial or letter or review).pt. not exp randomized controlled trial/	4,381,400
21	(random sampl$ or random digit$ or random effect$ or random survey or random regression).ti,ab. not exp randomized controlled trial/	73,686
22	19 or 20 or 21	8,307,747
23	18 not 22	953,328
24	15 and 23	5,996
25	limit 24 to English	5,718

Date of initial search: March 2, 2015
Search updated: June 16, 2016

Supplemental Table 4.2 PubMed search strategy.

Search	Description	Items found
#1	"Aromatase Inhibitors"[Mesh] OR "Aromatase Inhibitors" [Pharmacological Action] OR "aromatase inhibitor" OR "aromatase inhibitors" [All Fields] OR "estrogen synthetase inhibitor" [All Fields] OR "anastrozole" [Supplementary Concept] OR "anastrozole" [All Fields] OR "letrozole" [Supplementary Concept] OR "letrozole" [All Fields] OR "Arimidex" [All Fields] OR "Femara" [All Fields] OR "exemestane" [Supplementary Concept] OR "exemestane" [All Fields] OR "Aromasin" [All Fields] OR "Tamoxifen"[Mesh] OR "Tamoxifen" [All Fields] OR "Selective Estrogen Receptor Modulators"[Mesh] OR "Selective Estrogen Receptor Modulator" [All Fields] OR "SERM" [All Fields] OR "SERMs" [All Fields]	34,310
#2	((((random*[Title/Abstract] OR placebo*[Title/Abstract] OR single blind*[Title/Abstract] OR double blind*[Title/Abstract] OR triple blind*[Title/Abstract] OR retraction of publication[Publication Type] OR retracted publication[Publication Type] OR randomized controlled trial[Publication Type])) NOT (((((("comment" OR "editorial" OR "meta-analysis" OR "practice-guideline" OR "review" OR "letter" OR "correspondence")) NOT randomized controlled trial[Publication Type])) OR (animals[MeSH Terms] NOT humans[MeSH Terms])))	780,002
#3	#1 AND #2 Filters: English	3,063

Date of search: March 2, 2015
Search updated: June 16, 2016

Supplemental Table 4.3 Cochrane Central Register of Controlled Trials (CENTRAL) search strategy.

Search	Description	Items found
#1	MeSH descriptor: [Aromatase Inhibitors] explode all trees	477
#2	MeSH descriptor: [Selective Estrogen Receptor Modulators] explode all trees	379
#3	aromatase inhibitor*	1,216
#4	anastrozole or letrozole or Arimidex or Femara or exemestane or Aromasin	1,778
#5	selective estrogen receptor modulator* or SERM* or estrogen synthetase inhibitor*	851
#6	MeSH descriptor: [Tamoxifen] explode all trees	1,974
#7	Tamoxifen	3,995
#8	#1 or #2 or #3 or #4 or #5 or #6 or #7 in RCTs	6,036

Date of search: March 2, 2015
Search updated: June 17, 2016

Supplemental Table 4.4 ClincialTrials.gov search strategy.

Search	Description	Items found
#1	Completed | Aromatase Inhibitor* OR estrogen synthetase inhibitor OR anastrozole OR letrozole OR Arimidex OR Femara OR exemestane OR Aromasin OR Tamoxifen OR Selective Estrogen Receptor Modulator* OR SERM* limited to completed RCTs	512

Date of search: March 2, 2015
Search updated: June 17, 2016

Supplemental Table 4.5 World Health Organization International Clinical Trials Registry Platform search strategy.

Search	Description	Items found
#1	Aromatase Inhibitor* OR estrogen synthetase inhibitor OR anastrozole OR letrozole OR Arimidex OR Femara OR exemestane OR Aromasin OR Tamoxifen OR Selective Estrogen Receptor Modulator* OR SERM*	1368

Date of search: March 2, 2015
Search updated: June 17, 2016

Supplemental Table 4.6 Cardiovascular and cerebrovascular events reported in randomized controlled trials included in the quantitative analysis.

Trial	Drug Dose (mg/day)	Year	Follow-up (median months)	Cardiovascular or Cerebrovascular Events Reported
ATAC[2]	T-20, A-1	2006	68	-Cardiovascular death, ischemic cardiovascular events, angina, other ischemic events (coronary artery disorder, myocardial infarction or ischemia), myocardial infarction, ischemic cerebrovascular events, cerebrovascular death
BIG 1-98[3]	L-2.5 T- 20	2011	74	-Cardiac event, ischemic heart disease, cardiac failure, other cardiovascular event, cerebrovascular accident/TIA
Abo-Touk N et al.[4]	L-2.5, T- 20	2010	41	-Cardiovascular events, cerebrovascular accidents
N-SAS BC03[5]	A-1, T-20	2010	42	-Heart disease
ITA[6]	A-1, T-20	2006	64	-Cardiovascular diseases, venous disorders
ARNO95[7]	A-1, T-20 or 30	2007	30	-Ischemic cardiovascular events, Ischemic cerebrovascular events
IES[8]	E-25 T-20 or 30	2012	91	-All cardiovascular (excluding hypertension and VTE), ischemic CVD, angina, other cardiovascular events
Paridaens RJ et al.[9]	E-25, T-20	2008	29	-Cardiac dysrhythmia, cardiac dysfunction
BIG 1-98[10]	L-2.5 T- 20	2009	76 71ǂ	-Any cardiac event, ischemic heart disease, cardiac failure, cardiovascular events excluding hypertension, CVA/TIA
TEAM[11]	E-25, T-20	2011	61	-Cardiac related death, cerebral related death, vascular related death, arrhythmia, cardiac failure, other cardiac disorders, myocardial ischemia or infarction, peripheral arterial disease, other vascular disorders
N-SAS BC04[12]	T-20, A-1, E-25	2012	12	-Cardiovascular disease
MA.17[13]	L-2.5	2005	30	-CVD (including thromboembolic events), MI, stroke/TIA, angina, other
MA.17R[14]	L-2.5	2016	75	-Cardiovascular events (including thromboembolic events), cardiovascular-associated mortality, myocardial infarction, angina, other cardiovascular event, serious cardiovascular events, stroke/TIA
ATLAS[15]	T-20	2013	91†	-Ischemic heart disease (ever hospitalized or died), heart disease-related mortality, stroke (ever hospitalized or died), stroke-related mortality
SITAM-01[16]	T-20	2003	52	-Cardiovascular death, cerebrovascular death, ischemic events, heart failure, other cardiovascular events, cerebrovascular events
NSABP-B14[17]	T-20	2001	81	-Ischemic heart disease related death, other heart disease related death, cerebrovascular disease-related death
UK Over 50s[18]	T-20	2011	120	-Cardiovascular events (fatal or nonfatal), cardiovascular deaths
Scottish[19]	T-20	1995	91	-MI hospitalization, other ischemic heart disease hospitalization, cerebrovascular disease hospitalization
NSABP-B14 phase I[20]	T-20	1997	107†	-Coronary heart disease related deaths: definite fatal MI, definite fatal coronary heart disease/possible MI, possible fatal coronary heart disease; definite coronary heart disease-related death, definite and possible coronary heart disease-related death
Cummings FJ et al.[21]	T-20	1993	120	- Acute MI-related mortality, cardiac arrest-related mortality, congestive heart failure-related mortality, cerebrovascular accident-related mortality

Abbreviations: A-Anastrozole, CVD-cardiovascular disease, E-Exemestane, L-Letrozole, MI: myocardial infarction, NA-Not available, NT-No Treatment, P-Placebo, T-Tamoxifen, TIA: Transient Ischemic attack, VTE: Venous Thromboembolism; Symbols: * median age, † mean follow-up, ǂ Sequential arm, arrow represents switching between endocrine therapy

Supplemental Table 4.7 Enumeration of cardiovascular and cerebrovascular events in randomized controlled trials included in the quantitative analysis.

Trial	Year	Median Follow-up	Trial Arm Size	Endpoints	Specific Endpoints	Patients with CVE
ATAC[2]	2006	68	A-3092, T-3094	Cardiovascular Cerebrovascular	-Ischemic cardiovascular -Ischemic cerebrovascular	A-127, T-104 A-62, T-88
BIG 1-98[3]	2011	74	L-2448, T-2447	Cardiovascular Cerebrovascular	-Cardiac events -Ischemic heart disease -CVA/TIA	L-169 , T-152 L-69, T-49 L-45. T-38
Abo-Touk N et al.[4]	2010	41	L-60, T-60	Cardiovascular Cerebrovascular	-Cardiovascular events -Cerebrovascular accidents	L-4, T-3 L-2, T-1
N-SAS BC03[5]	2010	42	A-347, T-349	Cardiovascular	-Heart disease	A-2, T-3
ITA[6]	2006	64	A-223, T-225	Cardiovascular	-Cardiovascular diseases	A-17, T-14
ARNO95[7]	2007	30	A-445, T-452	Cardiovascular Cerebrovascular	-Ischemic cardiovascular -Ischemic cerebrovascular	A-9, T-4 A-3. T-1
IES[8]	2012	91	E-2105, T-2036	Cardiovascular	-All cardiovascular events -Ischemic CVD	E-259. T-211 E-127, T-94
Paridaens RJ et al.[9]	2008	29	E-182, T-189	Cardiovascular	-Cardiac dysrhythmia -Cardiac dysfunction	E-13, T-5 E-9, T-7
BIG 1-98[10]	2009	76 71[*]	L-1534, T-1540 T→L-1540 L→T-1526	Cardiovascular Cerebrovascular	-Any cardiac event -Ischemic heart disease -CVIA/TIA	L-103, T-88, T→L-108, L→T-93 L-40, T-23, T→L-36, L→T-26 L-22, T-27, T→L-30, L→T-26
TEAM[11]	2011	61	E-4852 T→E-4814 E-4898 T→E-4868	Cardiovascular Cerebrovascular	-Arrhythmia -Cardiac failure -Other cardiac disorders -Myocardial infarction/ischemia -Peripheral arterial disease -Cerebral-related death	E-182, T→E-143 E-50, T→E-26 E-77. T→E- 73 E- 82, T→E-64 E-14, T→E-20 E-19, T-14
N-SAS BC04[12]	2012	12	E-55, A-55, T→E-56	Cardiovascular	-Cardiovascular disease	A-0, E-0, T-0
MA.17[13]	2005	30	L-2572, P-2577	Cardiovascular Cerebrovascular	-Myocardial infarction -Angina -Other -Stroke/TIA	L-9, P-11 L-39, P-42 L-100, P-95 L-17, P-15
MA.17R[14]	2016	75	L-959, P-954	Cardiovascular Cerebrovascular	-Myocardial infarction ǂ -Angina ǂ -Other ǂ -Stroke/TIA ǂ	L-7, P-13 L-15, P-19 L-74, P-62 L-23, P-20
ATLAS[15]	2013	91[†]	T-6454 NT-6440	Cardiovascular Cerebrovascular	-Ischemic heart disease (hospitalized or died) -Stroke (hospitalized or died)	T-127, NT-163 T-130, NT-119

Trial	Year	Median Follow-up	Trial Arm Size	Endpoints	Specific Endpoints	Patients with CVE
SITAM-01[16]	2003	52	T-943, NT-958	Cardiovascular	-Ischemic events	T-13, NT-8
					-Heart failure	T-4, NT-9
					-Other cardiovascular events	T-7, NT-4
				Cerebrovascular	-Cerebrovascular events	T-5, NT-10
NSABP-B14[17]	2001	81	T-583, P-569	Cardiovascular	-Ischemic heart disease-related death	T-6, P-2
					-Other heart disease-related death	T-0, P-1
				Cerebrovascular	-Cerebrovascular-disease-related death	T-4, P-0
UK Over 50s[18]	2011	120	T-1725, NT-1724	Cardiovascular	-Cardiovascular events (nonfatal or fatal)	T-302, NT-319
					-Cardiovascular deaths	T-108, NT-128
Scottish[19]	1995	91†	T-661, NT-651	Cardiovascular	-MI hospitalization	T-14, NT-23
					-Other Ischemic heart disease hospitalization	T-18, NT-24
				Cerebrovascular	-Cerebrovascular disease hospitalization	T-25, NT-20
NSABP-B14 (P-I)[20]	1997	107†	T-1435, P-1450	Cardiovascular	-Definite coronary heart disease-related death	T-8, P-12
Cummings FJ et al. [21]	1993	120	T-85, P-83	Cardiovascular	-Acute MI death	T-0, P-1
					-Cardiac arrest death	T-0, P-1
					-Congestive heart failure-related death	T-1, P-0
				Cerebrovascular	-Cerebrovascular accident-related death	T-0, P-1

Abbreviations: A-Anastrozole, CVD-cardiovascular disease, CVE-cardiovascular or cerebrovascular event, E-Exemestane, L-Letrozole, NT-No Treatment, P-Placebo, T-Tamoxifen, TIA-transient ischemic attack

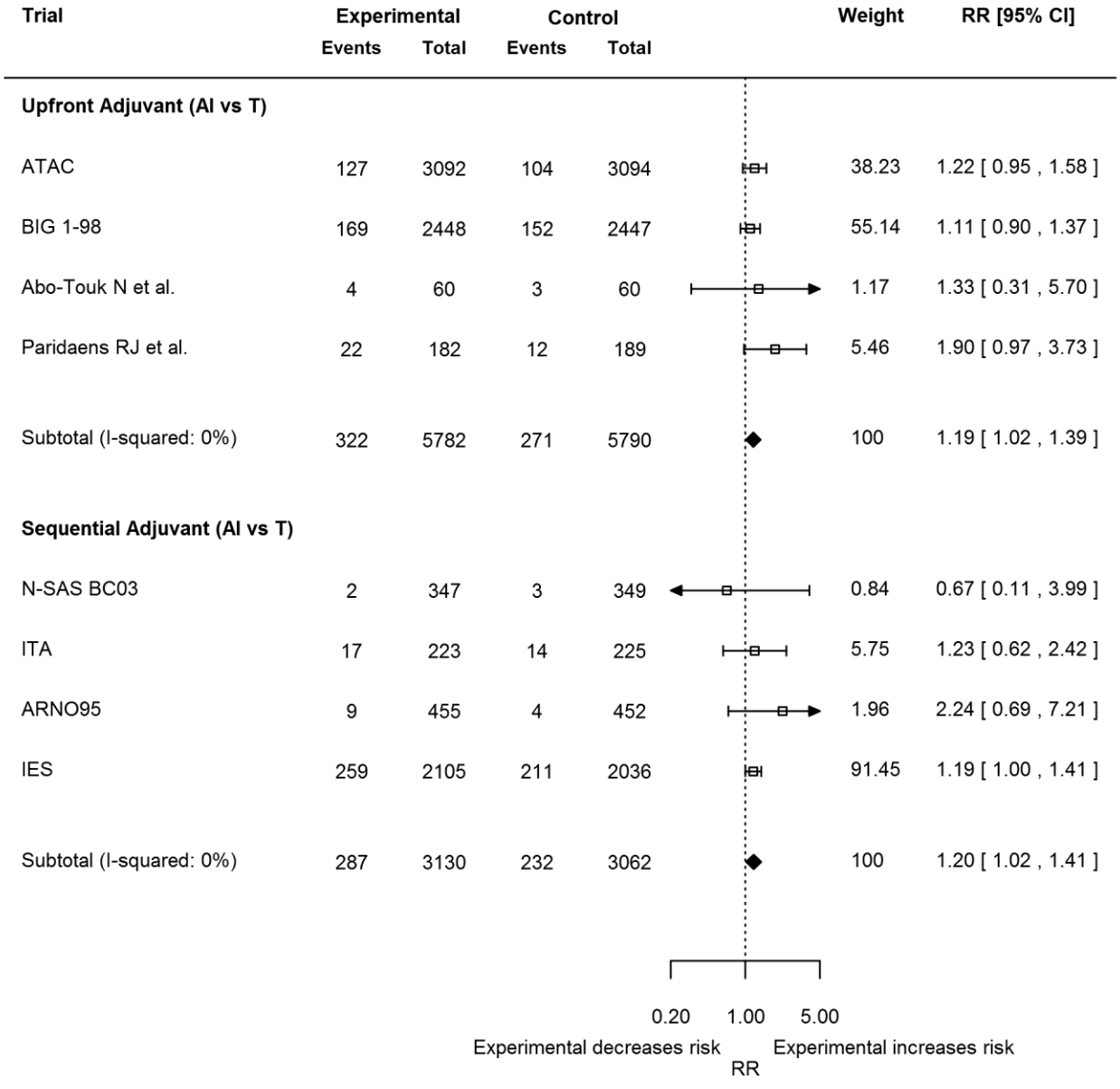
Symbols: * Sequential arm, †mean follow-up ‡ Data obtained with permission via personal communication with the Canadian Trials Group. Arrow represents switching between endocrine therapy.

Supplemental Table 4.8 Quality assessment of randomized controlled trials included in the quantitative analysis using Cochrane Collaboration tool for assessing risk of bias.[1]

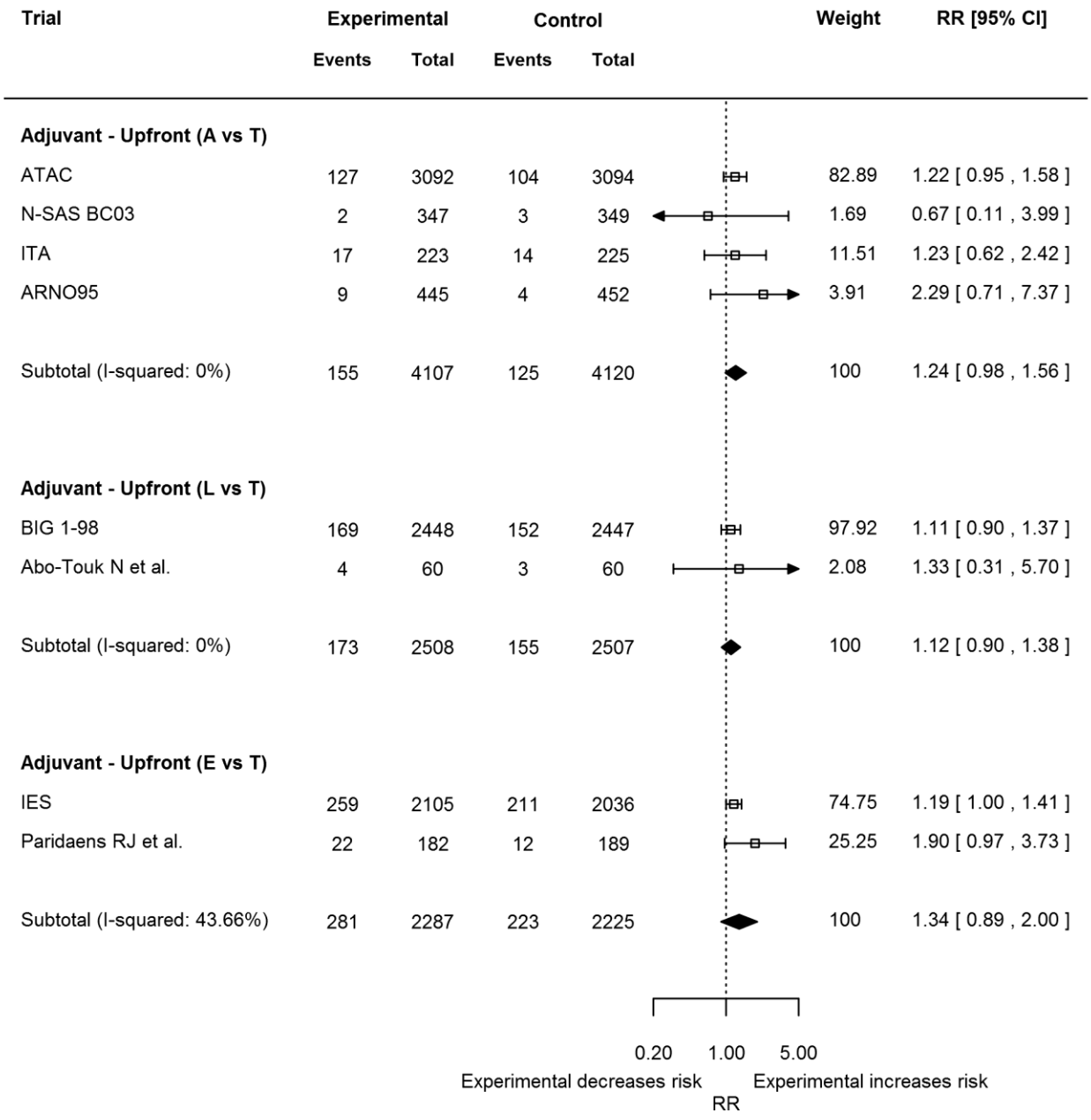
Trial Name	Follow-up (median months)	Random Sequence Generation	Allocation Concealment	Blinding of participants	Blinding of outcome	Incomplete Outcome	Selective Outcome	Other bias
ATAC[2]	68	-	-	-	-	-	-	-
BIG 1-98[3]	74	-	-	-	-	-	-	-
BIG 1-98[10]	76	-	-	-	-	-	-	-
N-SAS BC03[5]	42	?	?	+	+	-	-	-
ITA[6]	64	?	?	?	?	-	-	-
Abo-Touk N et al.[4]	41	-	?	?	?	-	-	-
IES[8]	91	-	-	-	-	-	-	-
ARNO95[7]	30	-	-	+	+	-	-	-
TEAM[11]	61	-	-	+	+	-	-	-
N-SAS BC04[12]	12	?	?	+	+	-	-	-
MA.17[13]	30	?	?	-	-	-	-	-
MA.17R[14]	75	?	?	-	-	-	-	-
ATLAS[15]	91	-	-	+	?	-	-	-
SITAM-01[16]	52	?	-	+	?	-	-	-
NSABP-B14[17, 22]	81	?	?	-	-	-	-	-
UK Over 50s[18, 23]	120	?	-	+	?	-	-	-
Scottish[19]	91	?	-	+	?	-	-	?
NSABP-B14 (phase I)	107	?	?	-	-	-	-	-
Cummings FJ et al.[21]	120	-	?	-	-	-	-	-
Paridaens RJ et al.[9]	29	?	-	+	+	-	-	-

Legend: low risk of bias (-), high risk of bias (+), unknown risk of bias (?)

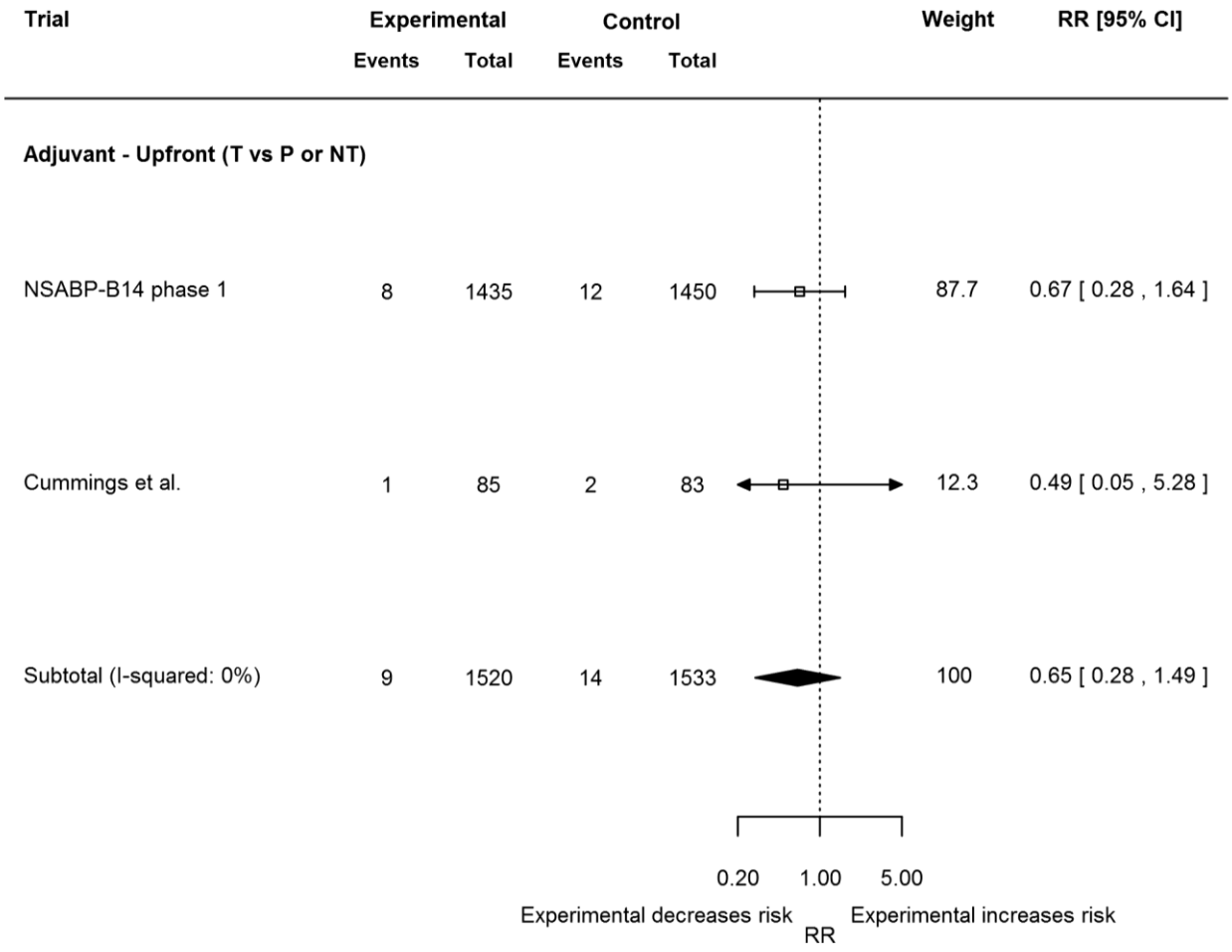
Supplemental Figure 4.1 Forest plot of relative risks of cardiovascular events in randomized controlled trials comparing aromatase inhibitor to tamoxifen when separating trials with no inclusion criteria for treatment with tamoxifen prior to randomization (upper panel) and trials including patients receiving 2-3 years of previous tamoxifen treatment (lower panel). Pooled relative risks and confidence intervals were obtained using DerSimonian and Laird random-effects models.



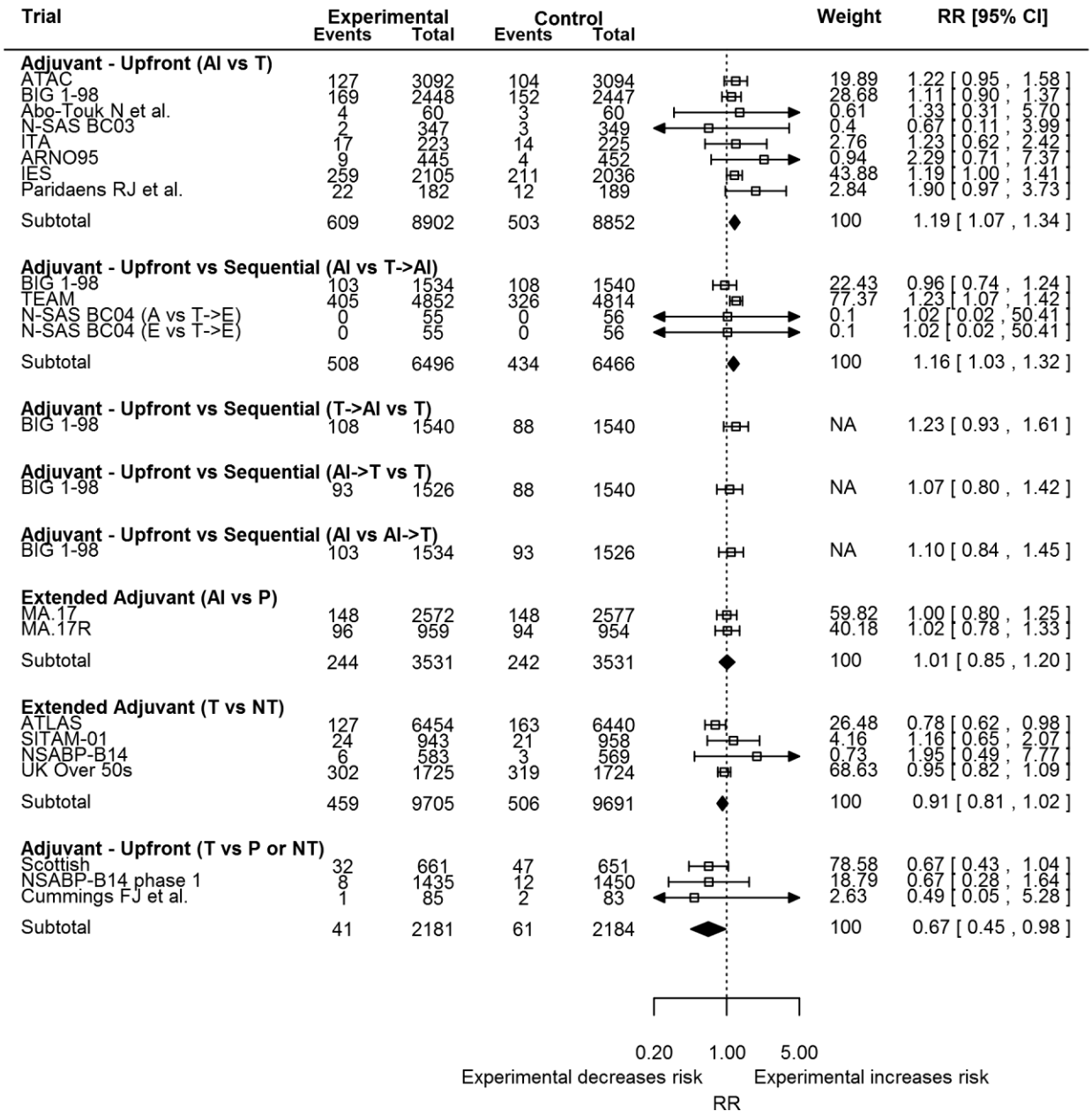
Supplemental Figure 4.2 Forest plot of relative risks of cardiovascular events in randomized controlled trials comparing AIs to tamoxifen in adjuvant setting stratified by drug molecule (A: anastrozole, L: letrozole, E: exemestane, T: tamoxifen). Pooled relative risks and confidence intervals were obtained using DerSimonian and Laird random-effects models.



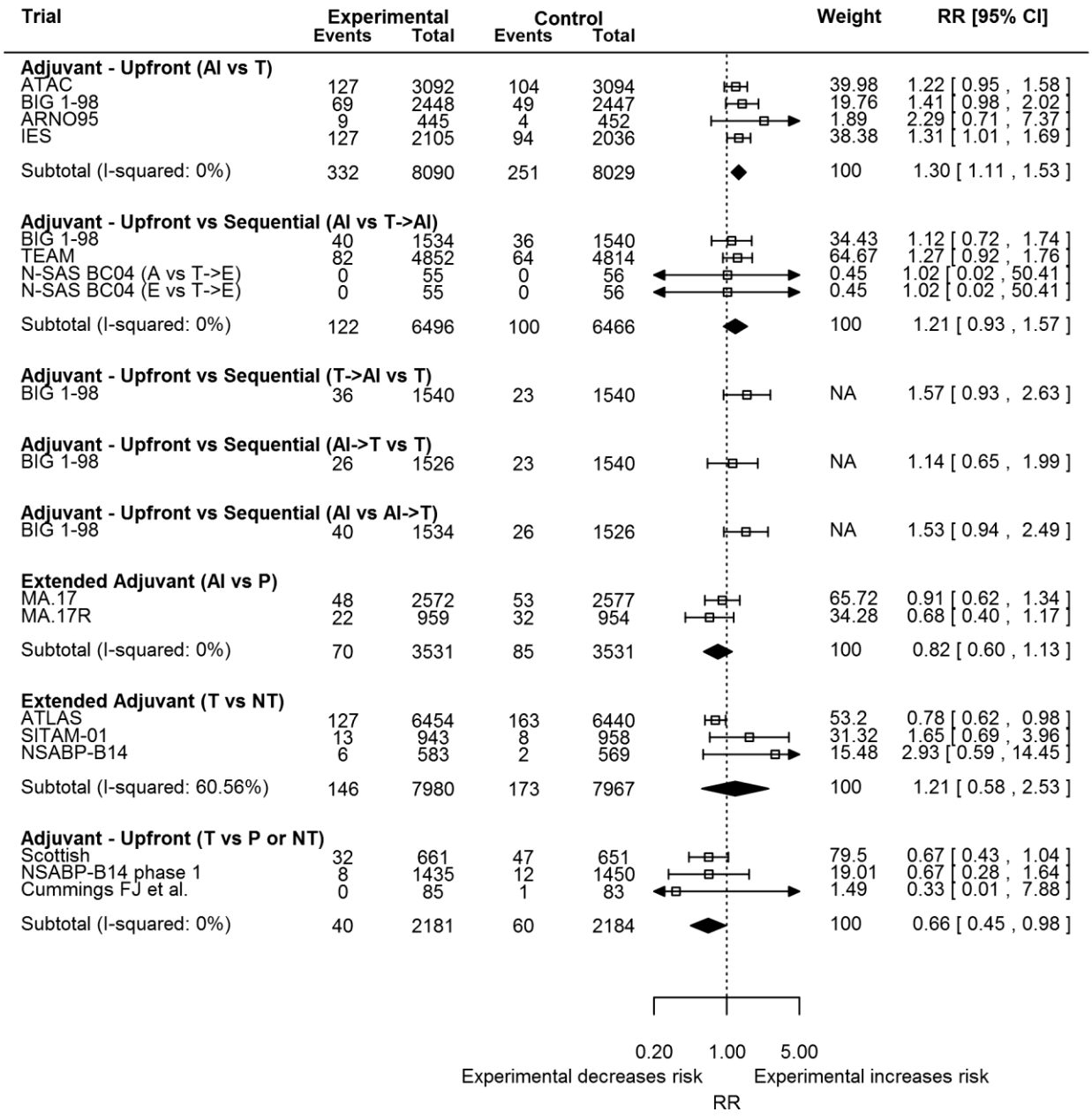
Supplemental Figure 4.3 Forest plot of relative risks of cardiovascular events in randomized controlled trials comparing tamoxifen to placebo or no-treatment when excluding Scottish trial. Pooled relative risks and confidence intervals were obtained using DerSimonian and Laird random-effects models.



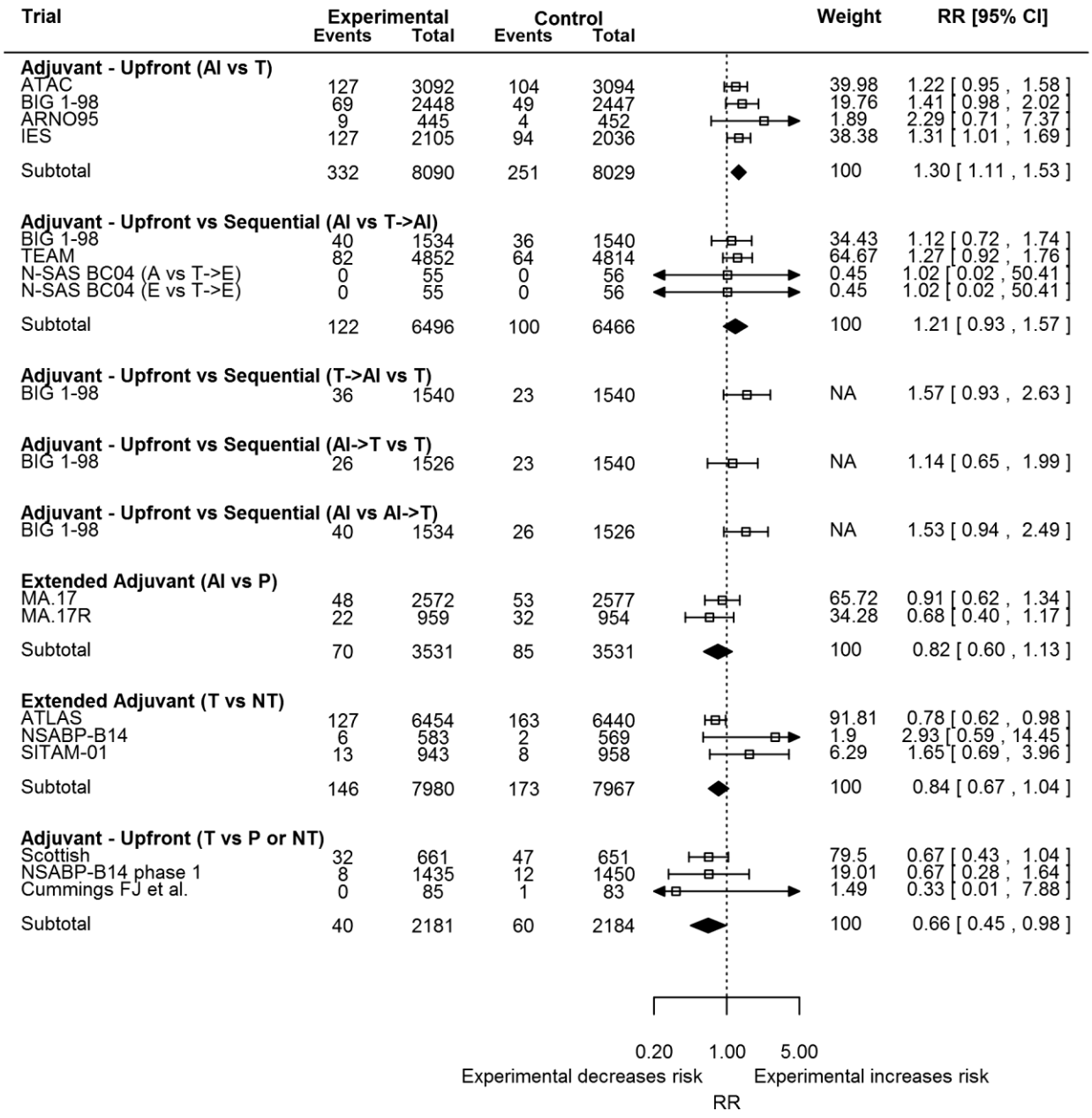
Supplemental Figure 4.4 Forest plot of relative risks of cardiovascular events by trial design. Pooled relative risks and confidence intervals were obtained using inverse-variance fixed-effects models.



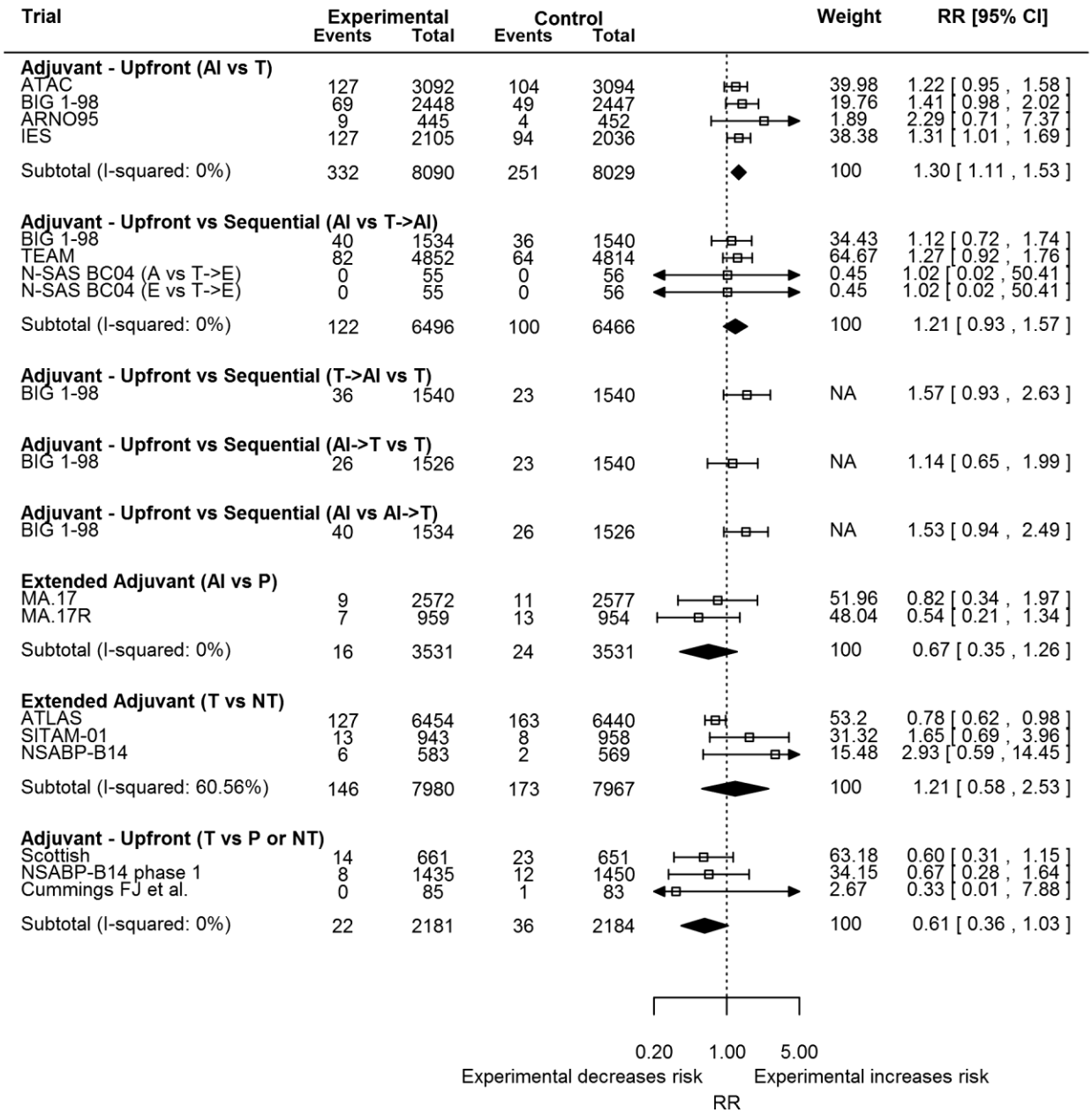
Supplemental Figure 4.5 Forest plot of relative risks of ischemic cardiovascular events by trial design. Pooled relative risks and confidence intervals were obtained using DerSimonian and Laird random-effects models.



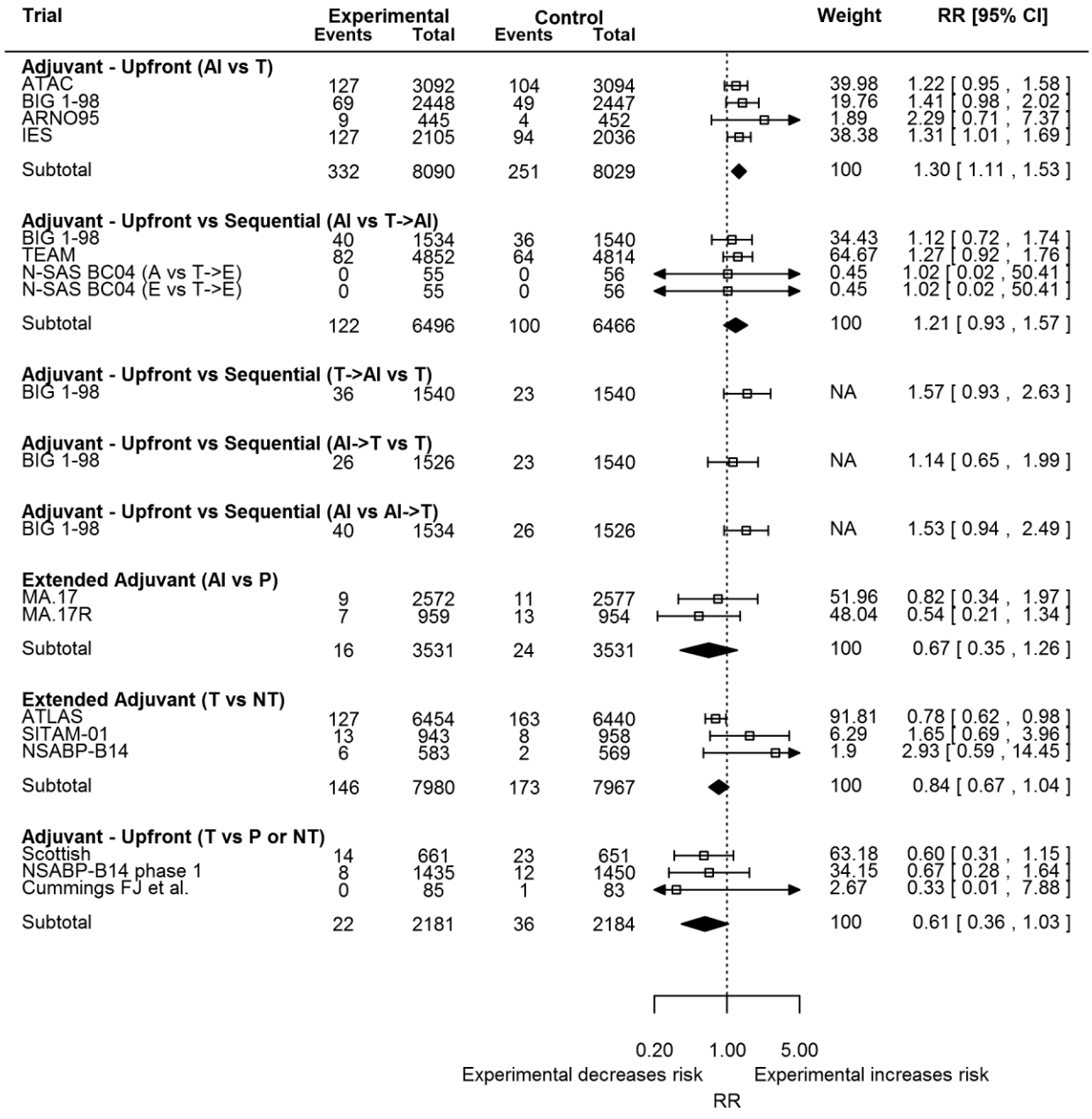
Supplemental Figure 4.6 Forest plot of relative risks of ischemic cardiovascular events by trial design. Pooled relative risks and confidence intervals were obtained using inverse-variance fixed-effects models.



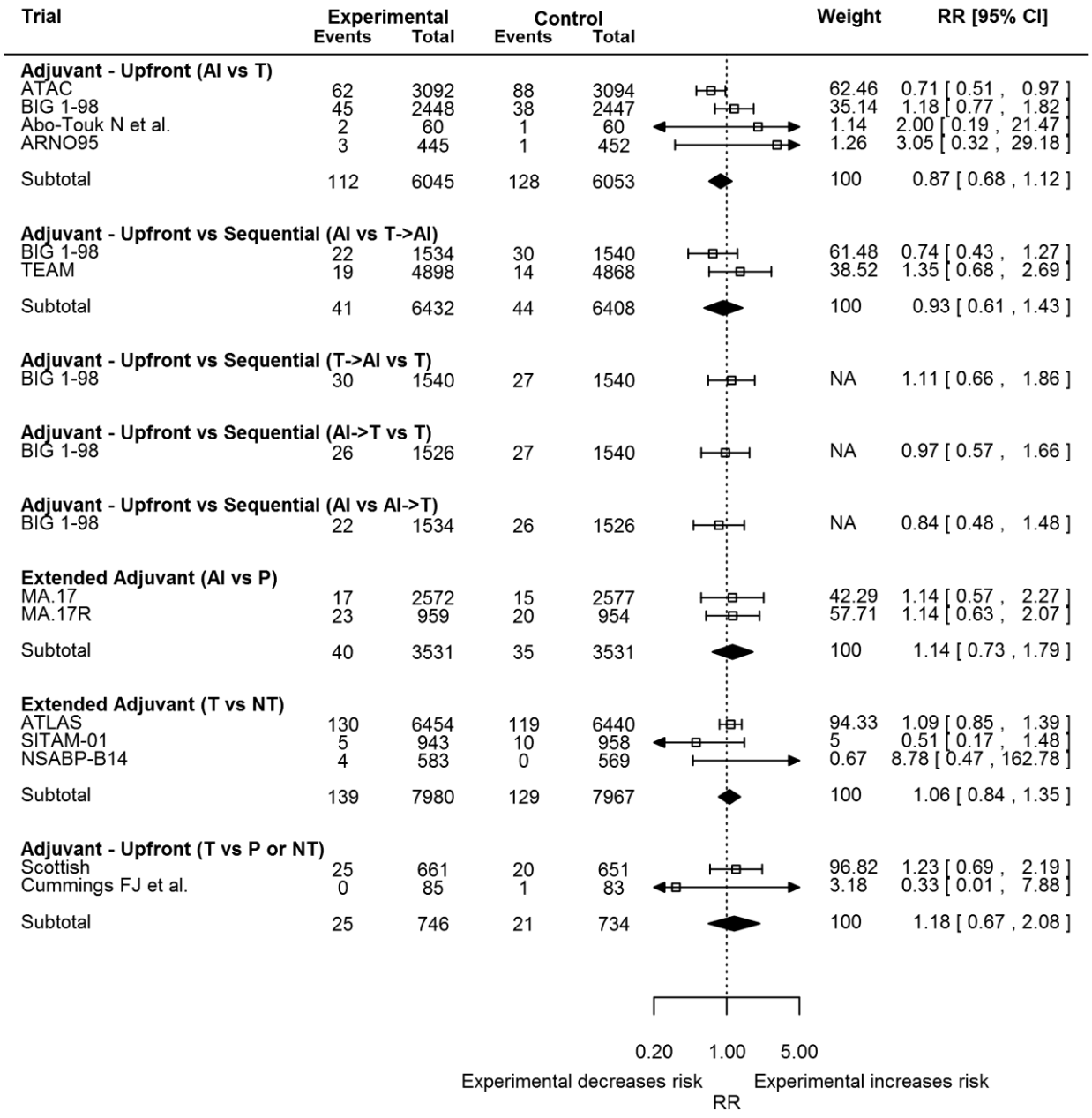
Supplemental Figure 4.7 Forest plot of relative risks of ischemic cardiovascular events by trial design when restricting outcome definition to myocardial infarction in trials reporting myocardial infarction and angina. Pooled relative risks and confidence intervals were obtained using DerSimonian and Laird random-effects models.



Supplemental Figure 4.8 Forest plot of relative risks of ischemic cardiovascular adverse events by trial design when restricting outcome definition to myocardial infarction in trials reporting myocardial infarction and angina. Pooled relative risks and confidence intervals were obtained using inverse-variance fixed-effects models.



Supplemental Figure 4.9 Forest plot of relative risks of cerebrovascular events of AIs and tamoxifen by trial design. Pooled relative risks and confidence intervals were obtained using inverse-variance fixed-effects models.



4.12 Supplemental References

1. Higgins JP, Altman DG, Gotzsche PC et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ 2011; 343: d5928.

2. Comprehensive side-effect profile of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: long-term safety analysis of the ATAC trial. Lancet Oncology 2006; 7: 633-643.

3. Colleoni M, Giobbie-Hurder A, Regan MM et al. Analyses adjusting for selective crossover show improved overall survival with adjuvant letrozole compared with tamoxifen in the BIG 1-98 study. Journal of Clinical Oncology 2011; 29: 1117-1124.

4. Abo-Touk NA, Sakr HA, Abd El-Lattef A. Switching to letrozole versus continued tamoxifen therapy in treatment of postmenopausal women with early breast cancer. J Egypt Natl Canc Inst 2010; 22: 79-85.

5. Aihara T, Takatsuka Y, Ohsumi S et al. Phase III randomized adjuvant study of tamoxifen alone versus sequential tamoxifen and anastrozole in Japanese postmenopausal women with hormone-responsive breast cancer: N-SAS BC03 study. Breast Cancer Research and Treatment 2010; 121: 379-387.

6. Boccardo F, Rubagotti A, Guglielmini P et al. Switching to anastrozole versus continued tamoxifen treatment of early breast cancer. Updated results of the Italian tamoxifen anastrozole (ITA) trial. Annals of Oncology 2006; 17: vii10-vii14.

7. Kaufmann M, Jonat W, Hilfrich J et al. Improved overall survival in postmenopausal women with early breast cancer after anastrozole initiated after treatment with tamoxifen compared with continued tamoxifen: The ARNO 95 study. Journal of Clinical Oncology 2007; 25: 2664-2670.

8. Bliss JM, Kilburn LS, Coleman RE et al. Disease-related outcomes with long-term follow-up: an updated analysis of the intergroup exemestane study. J Clin Oncol 2012; 30: 709-717.

9. Paridaens RJ, Dirix LY, Beex LV et al. Phase III study comparing exemestane with tamoxifen as first-line hormonal treatment of metastatic breast cancer in postmenopausal women: The European Organisation for Research and Treatment of Cancer Breast Cancer Cooperative Group. Journal of Clinical Oncology 2008; 26: 4883-4890.

10. Mouridsen H, Giobbie-Hurder A, Goldhirsch A et al. Letrozole therapy alone or in sequence with tamoxifen in women with breast cancer. New England Journal of Medicine 2009; 361: 766-776.

11. Van De Velde CJ, Rea D, Seynaeve C et al. Adjuvant tamoxifen and exemestane in early breast cancer (TEAM): A randomised phase 3 trial. The Lancet 2011; 377: 321-331.

12. Takei H, Ohsumi S, Shimozuma K et al. Health-related quality of life, psychological distress, and adverse events in postmenopausal women with breast cancer who receive tamoxifen,

exemestane, or anastrozole as adjuvant endocrine therapy: National Surgical Adjuvant Study of Breast Cancer 04 (N-SAS BC 04). Breast Cancer Research and Treatment 2012; 133: 227-236.

13. Goss PE, Ingle JN, Martino S et al. Randomized trial of letrozole following tamoxifen as extended adjuvant therapy in receptor-positive breast cancer: Updated findings from NCIC CTG MA.17. Journal of the National Cancer Institute 2005; 97: 1262-1271.

14. Goss PE, Ingle JN, Pritchard KI et al. Extending Aromatase-Inhibitor Adjuvant Therapy to 10 Years. N Engl J Med 2016.

15. Davies C, Pan H, Godwin J et al. Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial. The Lancet 2013; 381: 805-816.

16. Sacco M, Valentini M, Belfiglio M et al. Randomized trial of 2 versus 5 years of adjuvant tamoxifen for women aged 50 years or older with early breast cancer: Italian Interdisciplinary Group for Cancer Evaluation Study of Adjuvant Treatment in Breast Cancer 01. Journal of Clinical Oncology 2003; 21: 2276-2281.

17. Fisher B, Dignam J, Bryant J, Wolmark N. Five versus more than five years of tamoxifib for lymph node-nagative breast cancer: Updated findings from the national surgical adjuvant breast and bowel project B-14 Randomized trial. Journal of the National Cancer Institute 2001; 93: 684-690.

18. Hackshaw A, Roughton M, Forsyth S et al. Long-term benefits of 5 years of tamoxifen: 10-Year follow-up of a large randomized trial in women at least 50 years of age with early breast cancer. Journal of Clinical Oncology 2011; 29: 1657-1663.

19. McDonald CC, Alexander FE, Whyte BW et al. Cardiac and vascular morbidity in women receiving adjuvant tamoxifen for breast cancer in a randomised trial. The Scottish Cancer Trials Breast Group. Bmj 1995; 311: 977-980.

20. Costantino JP, Kuller LH, Ives DG et al. Coronary heart disease mortality and adjuvant tamoxifen therapy. Journal of the National Cancer Institute 1997; 89: 776-782.

21. Cummings FJ, Gray R, Tormey DC et al. Adjuvant tamoxifen versus placebo in elderly women with node-positive breast cancer: long-term follow-up and causes of death. J Clin Oncol 1993; 11: 29-35.

22. Fisher B, Dignam J, Bryant J et al. Five versus more than five years of tamoxifen therapy for breast cancer patients with negative lymph nodes and estrogen receptor-positive tumors. Journal of the National Cancer Institute 1996; 88: 1529-1542.

23. Preliminary results from the cancer research campaign trial evaluating tamoxifen duration in women aged fifty years or older with breast cancer. Current Trials working Party of the Cancer Research Campaign Breast Cancer Trials Group. J Natl Cancer Inst 1996; 88: 1834-1839.

Chapter 5. Manuscript 2-Aromatase Inhibitors and the Risk of Cardiovascular Outcomes in Post-Menopausal Women with Breast Cancer: A Population-Based Cohort Study

5.1 Preface

In Chapter 4, we observed an increased risk of cardiovascular outcomes in meta-analysis of RCTs comparing AIs directly with tamoxifen. However, this increased risk was not observed in RCTs comparing AIs with placebo in patients previously treated with five years of tamoxifen. In addition, tamoxifen was associated with a decreased risk of cardiovascular outcomes in comparison with placebo. There were limitations to assessing cardiovascular safety from data reported in RCTs. First, RCTs were designed to assess efficacy and not cardiovascular safety and cardiovascular outcomes were often reported as composite endpoints with heterogeneity in the outcome definition. Second, there was heterogeneity in regard to duration of follow-up, patient recruitment periods, and patient characteristics with majority of RCTs including a healthier patient population than those treated in clinical setting. Thus, the aim of this objective was to conduct an observational study in the setting of clinical practice to determine whether AIs, in comparison with tamoxifen, increase the risk of clinically relevant cardiovascular outcomes including MI, ischemic stroke, heart failure, and cardiovascular mortality in post-menopausal women with breast cancer. Four observational studies have been conducted on this topic and the results have been discordant due to methodological limitations and heterogeneity of the study populations.[94-97] The aim of this study was to address previous limitation of observational studies and comprehensively examine the risk of cardiovascular outcomes when comparing AIs with tamoxifen in treatment of post-menopausal women with breast cancer. This manuscript was published in *Circulation* (2020; 141(7): 549-559).

5.2 Title Page

Aromatase Inhibitors and the Risk of Cardiovascular Outcomes in Women with Breast Cancer: A Population-Based Cohort Study

Farzin Khosrow-Khavar MSc [1,2], Kristian B. Filion PhD [1,2,5], Nathaniel Bouganim MD [3,4], Samy Suissa PhD [1,2], Laurent Azoulay PhD [1,2,3]

[1] Centre for Clinical Epidemiology, Lady Davis Institute, Jewish General Hospital, Montreal, Canada

[2] Department of Epidemiology, Biostatistics, and Occupational Health, McGill University, Montreal, Canada

[3] Gerald Bronfman Department of Oncology, McGill University, Montreal, Canada

[4] Department of Oncology, Cedar Cancer Center, McGill University Health Center, Montreal, Canada

[5] Division of Clinical Epidemiology, Department of Medicine, McGill University, Montreal, Canada

Word count: 3,552

Running head: Aromatase inhibitors and cardiovascular outcomes

Correspondence:
Dr. Laurent Azoulay
Centre for Clinical Epidemiology, Lady Davis Institute, Jewish General Hospital
3755 Côte Sainte-Catherine, H-425.1, Montreal, Quebec, Canada, H3T 1E2
Tel: 514.340.8222 extension 28396; Fax: 514.340.7564
Email: laurent.azoulay@mcgill.ca
Twitter: @LaurentAzoulay0

5.3 CLINICAL PERSPECTIVE

What is New?

- Some randomized controlled trials have associated aromatase inhibitors with an increased risk of cardiovascular outcomes, but to date, the few real-world studies on the topic have generated conflicting results.
- In this population-based cohort study of 17,922 of women with breast cancer, use of aromatase inhibitors was associated with increased risks of heart failure and cardiovascular mortality, and trends towards increased risks of myocardial infarction and ischemic stroke, compared with tamoxifen.

What are Clinical Implications?

- In this population-based study, the use of aromatase inhibitors was associated with an increased risk of cardiovascular outcomes, compared with tamoxifen.
- This possible increased cardiovascular risk with aromatase inhibitors should be balanced with their favorable clinical profile, compared with tamoxifen.

5.4 ABSTRACT

Background: The association between aromatase inhibitors and cardiovascular outcomes among women with breast cancer is controversial. Given the discrepant findings from randomized controlled trials and observational studies, additional studies are needed to address this safety concern.

Methods: We conducted a population-based cohort study using the United Kingdom Clinical Practice Research Datalink linked to the Hospital Episode Statistics and Office for National Statistics databases. The study population consisted of women newly-diagnosed with breast cancer initiating hormonal therapy with aromatase inhibitors or tamoxifen between April 1, 1998 and February 29, 2016. Cox proportional hazards models using inverse probability of treatment and censoring weighting were used to estimate hazard ratios (HRs) with 95% confidence intervals (CIs) comparing new users of aromatase inhibitors with new users of tamoxifen for each of the study outcomes (myocardial infarction, ischemic stroke, heart failure, and cardiovascular mortality).

Results: The study population consisted of 23,525 patients newly-diagnosed with breast cancer, of whom 17,922 initiated treatment with either an aromatase inhibitor or tamoxifen (8,139 and 9,783 respectively). The use of aromatase inhibitors was associated with a significantly increased risk of heart failure (incidence rate: 5.4 vs 1.8 per 1,000 person-years; HR: 1.86; 95% CI: 1.14-3.03) and cardiovascular mortality (incidence rate: 9.5 vs 4.7 per 1,000 person-years; HR: 1.50; 95% CI: 1.11-2.04), compared with use of tamoxifen. Aromatase inhibitors were associated with elevated HRs, but with CIs including the null value, for myocardial infarction (incidence rate: 3.9 vs 1.8 per 1,000 person-years; HR: 1.37; 95% CI: 0.88-2.13) and ischemic stroke (incidence rate: 5.6 vs 3.2 per 1,000 person-years; HR: 1.19; 95% CI: 0.82-1.72).

Conclusions: In this population-based study, aromatase inhibitors were associated with increased risks of heart failure and cardiovascular mortality, when compared with tamoxifen. There were also trends towards increased risks, although non-significant, of myocardial infarction and ischemic stroke. The increased risk of cardiovascular events associated with aromatase inhibitors should be balanced with their favorable clinical benefits, when compared with tamoxifen.

Keywords: Aromatase inhibitors, tamoxifen, breast cancer, cardiovascular disease

Non-standard Abbreviations and Acronyms: AI, aromatase inhibitor; ASA, acetylsalicylic acid; ATAC, Anastrozole Tamoxifen Alone or in Combination; BIG 1-98, Breast International Group 1-98; CPRD, Clinical Practice Research Datalink; CI, Confidence Interval; ICD, International Classification of Diseases; HES, Hospital Episode Statistics; HR, Hazard Ratio; LDL, Low Density Lipoprotein; ONS, Office for National Statistics; RCT, Randomized Controlled Trial; UK, United Kingdom; US, United States

5.5 INTRODUCTION

Aromatase inhibitors (AIs) have become the preferred adjuvant treatment for post-menopausal women with estrogen-receptor positive breast cancer.[1] These drugs have been associated with favorable clinical outcomes, including decreased risks of all-cause and breast cancer-related mortality, compared with tamoxifen.[2] However, their safety has been a contentious issue, with some meta-analyses of randomized controlled trials (RCTs) reporting an increased risk of cardiovascular events, compared with tamoxifen.[3-5] However, the biological mechanism behind this possible association remains uncertain. While some RCTs associated the use of AIs with hypercholesterolemia,[6,7] others reported no effects on serum cholesterol levels.[8-12] Moreover, there is some evidence suggesting that tamoxifen may reduce cholesterol levels.[12-16]

To date, few observational studies have examined the cardiovascular effects of AIs.[17-20] In one study, AIs were associated with an increased risk of myocardial infarction,[17] while three other studies did not observe an association with this outcome.[18-20] With respect to other cardiovascular outcomes, only two studies investigated stroke and heart failure,[19,20] and none examined cardiovascular mortality. As a result, the response from regulatory agencies has also been mixed. While the US Food and Drug Administration imposed a label change to certain AIs (anastrozole) to include a possible increased risk of ischemic heart disease among women with established cardiovascular disease,[21] other agencies such as the European Medicine Agency have not indicated this concern in their product assessment.[22,23]

Given the increasing use of AIs[1] and continued concerns related to their cardiovascular safety,[24] we conducted a population-based cohort study to examine the association between these drugs and the risk of myocardial infarction, ischemic stroke, heart failure, and cardiovascular mortality among women with breast cancer.

5.6 METHODS

5.6.1 Data Sources

The analytic methods and study materials will be available to other researchers, upon request, for replication of the procedures and reproducing the results in this manuscript. This study was conducted by linking the United Kingdom (UK) Clinical Practice Research Datalink (CPRD) with the Hospital Episode Statistics (HES) and the Office for National Statistics (ONS) databases.

The CPRD includes information on medical diagnoses and procedures, lifestyle variables, anthropometric measurements, and prescriptions written by general practitioners.[25] The patient population enrolled in the CPRD has been shown to be representative of the UK population in terms of age, ethnicity, and body mass index.[25] Diagnoses have been shown to be well recorded in the CPRD.[26,27] These include validation studies reporting high concordance rates between breast cancer diagnoses recorded in the CPRD compared with the National Cancer Data Repository (96-97%)[28,29] and medical profile reviews (98%).[28-30] The HES repository includes information on all inpatient and outpatient hospital admissions, and includes primary and secondary diagnoses, as well as procedures.[31] Finally, the ONS database includes the electronic death certificates of all residents in the UK.[32]

The study protocol was approved by the Independent Scientific Advisory Committee of the CPRD (protocol 17_072RA) and by the Research Ethics Board of the Jewish General Hospital, Montreal, Canada.

5.6.2 Study Population

Using the CPRD, we identified a cohort of women, at least 50 years of age, with a first-ever diagnosis of breast cancer between April 1, 1998 and February 29, 2016. We excluded patients with less than one year of medical history before their first breast cancer diagnosis and those with evidence of metastatic disease (using diagnostic Read codes corresponding to secondary malignancy, recurrence, or metastases). Additionally, we excluded patients with prescriptions of AIs or tamoxifen before their breast cancer diagnosis to minimize the inclusion of prevalent users.

From this cohort, we employed a new-user, active comparator design where we identified patients newly-treated with either an AI (anastrozole, letrozole, exemestane) or tamoxifen after their breast cancer diagnosis. Cohort entry was defined by the date of the first prescription of either drug class during the study period. We then excluded patients prescribed an AI and tamoxifen on the same day, as well as patients prescribed more than one AI at cohort entry. Patients meeting the inclusion criteria were followed from cohort entry until an incident diagnosis of one of the study outcomes (defined in detail below), treatment discontinuation (defined in detail below), death, end of registration with the general practice, or end of the study period (February 29, 2016), whichever occurred first.

5.6.3 Exposure Definition

We used an *as-treated* exposure definition in which patients were followed while they were continuously exposed to the study drugs. Based on this exposure definition, patients were censored at discontinuation of initial treatment or at a switch between tamoxifen or AIs (or vice versa). Patients were considered continuously exposed if the duration of one prescription plus a

30-day grace period overlapped with the date of the next prescription of the same drug class. Thus, treatment discontinuation corresponded to the end of a 30-day grace period in the event of no overlapping subsequent prescription. The date of censoring due to a treatment switch was defined by the date of a switch between prescriptions from different drug classes (tamoxifen to AI, or vice versa).

5.6.4 Outcome Ascertainment

We considered the following four outcomes, which were assessed independently in the analyses, with separate follow-up durations determined for each: myocardial infarction, ischemic stroke, heart failure, and cardiovascular mortality (ICD-9 and ICD-10 codes provided in **Supplemental Table 1**). The HES repository was used to identify hospitalized events (in primary position), whereas the ONS was used to identify deaths for which a cardiovascular event was deemed to be the underlying cause of death. These outcomes have been shown to be well recorded in the HES, with myocardial infarction having a positive predictive value of 92%,[33] diagnoses of coronary heart disease having a specificity and negative predictive value of 96%,[34] and stroke having a perfect specificity and negative predictive value (100%).[34]

5.6.5 Potential Confounders

Overall, we considered 45 potential confounders assessed before or at cohort entry; these variables included lifestyle and anthropometric measures, comorbidities, prescriptions, and breast-cancer related variables. The variables measured at cohort entry included the following: age, body mass index (<25, 25-30, ≥30 kg/m^2, unknown), Townsend Deprivation Index, ethnicity (Caucasian, other, unknown), smoking status (current, past, never, unknown), and

alcohol-related disorders. We also included the following comorbidities measured at any time before cohort entry: myocardial infarction, stroke (ischemic or hemorrhagic) or transient ischemic attack, heart failure, peripheral vascular disease, venous thromboembolism, chronic obstructive pulmonary disease, chronic kidney disease, cancers (other than breast and non-melanoma skin cancer), and non-breast cancer surgeries in the year before cohort entry. We also considered use of the following prescription drugs measured in the year before cohort entry: anticoagulants, antidepressants, antidiabetic drugs, antihypertensive drugs, bisphosphonates, non-steroidal anti-inflammatory drugs, opioids, acetylsalicylic acid (ASA), non-ASA antiplatelets, statins, and hormone replacement therapy. Finally, the model included the following breast cancer-related variables measure between the breast cancer diagnosis date and cohort entry: receipt of chemotherapy, radiation therapy, breast cancer surgery, and time since the breast cancer diagnosis (defined as the time between the breast cancer diagnosis and cohort entry). Age and time since breast cancer diagnosis were modelled flexibly as restricted cubic splines with five interior knots.[35] We did not include calendar time in the model as it acted as an instrumental variable and generated unstable weights;[36] this is because of its strong association with the exposure and relatively weak association with the outcomes.

5.6.6 Statistical Analysis

Descriptive statistics (means and proportions) were used to summarize characteristics of each exposure group. Absolute standardized differences were used to compare characteristics of patients initiating treatment on AIs and tamoxifen respectively. Crude incidence rates for each outcome, with corresponding 95% confidence intervals (CIs) based on the Poisson distribution, were calculated for each exposure group. We used Cox proportional hazards models using

inverse probability of treatment and censoring weighting to estimate marginal hazard ratios (HRs) and 95% CIs using robust variance estimators for the outcomes of interest, comparing use of AIs with use of tamoxifen (details of this method are outlined in **Supplemental Methods 1**).[37] Weighted cumulative incidence curves, with duration of follow-up as the time axis, were generated for each of the four outcomes. In secondary analyses, we assessed effect measure modification by stratifying on the presence of cardiovascular disease before cohort entry (using Read and ICD-9 and ICD-10 diagnosis codes) and type of aromatase inhibitor (anastrozole and letrozole; analyses for exemestane were not performed due to the low number of exposed patients [n=47]).

Sensitivity Analyses

We conducted six sensitivity analyses to assess the robustness of our findings. First, we extended the grace period between consecutive prescriptions to 60 days. Second, we changed the outcome definition to hospitalized events recorded in primary and secondary position and deaths recorded in ONS. Third, we restricted the study population to patients aged at least 55 years to minimize the inclusion of premenopausal women. Fourth, to further assess residual confounding at baseline, we regenerated the inverse probability of treatment weights using the high-dimensional propensity score algorithm (described in detail in **Supplemental Method 2**).[38] Fifth, to account for residual confounding and informative censoring due to time-varying covariates, we constructed marginal structural models with inverse probability of treatment and censoring weights with covariates updated at monthly intervals (described in detail in **Supplemental Method 3**).[37] Finally, we assessed the impact of variables with missing information (i.e., body mass index, Townsend deprivation score, smoking status, and ethnicity) by conducting multiple

imputation and a complete case analysis. For the former, ten imputations were performed and the resulting datasets were analyzed using weighted Cox proportional hazard models with the results combined using Rubin's rule to compute standard errors.[39] All analyses were conducted with SAS version 9.4 (SAS Institute, Cary, NC) and R (R Foundation for Statistical Computing, Vienna, Austria).

5.7 RESULTS

Of the 23,525 patients newly-diagnosed with non-metastatic breast cancer during the study period, 17,922 (76.2%) were newly-treated with either an AI (n=8,139) or tamoxifen (n=9,783) **(Figure 1)**. The most commonly used AI was anastrozole (n=4700; 57.7%), followed by letrozole (n=3392; 41.7%) and exemestane (n=47; 0.6%).

The unweighted and weighted baseline characteristics of AI and tamoxifen users are shown in **Table 1**. Before weighting, AI users were older, had a higher body mass index, were more likely to have alcohol-related disorders, and to have smoked, compared with tamoxifen users. They were also more likely to have comorbidities and to have used prescription drugs. The proportion of missing data was low (0.1% for Townsend deprivation score, 3% for ethnicity, 3% for smoking status, and 10% for body mass index). The baseline characteristics were well balanced between the groups after weighting (**Table 1** and **Supplemental Tables 2-4**).

Table 2 presents the results of the primary analyses for each of the study outcomes. Overall, users of AI and tamoxifen generated 15,425 to 15,486 and 18,590 to 18,618 person-years of follow-up, respectively. The median durations of follow-up for AI and tamoxifen users were 1.3 and 1.4 years, respectively.

Myocardial Infarction

During the follow-up period, there were 61 myocardial infarction events among AI users compared with 34 events among tamoxifen users, generating incidence rates of 3.9 (95% CI: 3.0-5.1) versus 1.8 (95% CI: 1.3-2.6) per 1,000 person-years, respectively. This generated an elevated HR with a CI that included the null value (HR: 1.37, 95% CI: 0.88-2.13) (**Table 2**). In

secondary analyses, the cumulative incidence curves appeared to diverge after two years of use (**Figure 2**).

Ischemic Stroke

Overall, there were 86 ischemic stroke events among AI users compared with 59 cases among tamoxifen users. This yielded incidence rates of 5.6 (95% CI: 4.5-6.9) per 1,000 person-years for AI users versus 3.2 (95% CI: 2.4-4.1) per 1,000 person-years for tamoxifen users. This generated a slightly elevated HR with a CI that included the null value (HR: 1.19, 95% CI: 0.82-1.72). The cumulative incidence curves appeared to diverge after two years of use (**Figure 2**).

Heart Failure

There were 83 cases of heart failure among AI users compared with 33 cases among tamoxifen users, generating incidence rates of 5.4 (95% CI: 4.3-6.7) versus 1.8 (95% CI: 1.2-2.5) per 1,000 person-years. The use of AIs was associated with an 86% increased risk of heart failure, compared with use of tamoxifen (HR: 1.86, 95% CI: 1.14-3.03). The cumulative incidence curves diverged three months after treatment initiation (**Figure 2**).

Cardiovascular Mortality

Finally, there were 147 cardiovascular deaths among AI users compared with 87 events among tamoxifen users, generating incidence rates of 9.5 (95% CI: 8.0-11.2) versus 4.7 (95% CI: 3.7-5.8) per 1,000 person-years. The use of AIs was associated with a 50% increased risk of cardiovascular mortality, compared with use of tamoxifen (HR: 1.50, 95% CI: 1.11-2.04). The cumulative incidence curves diverged after two years of use (**Figure 2**).

Secondary Analyses

Overall, there were no significant differences between anastrozole and letrozole and risk of cardiovascular outcomes, although the number of events was low for these stratified analyses (**Figure 3**, **Supplemental Table 5**). Stratification by history of cardiovascular disease led to overlapping HRs that included the null, with exception of heart failure where the use of AIs was associated with a significant increased risk among patients without a history of cardiovascular disease (HR: 2.80, 95% CI: 1.29-6.08; **Figure 3**, **Supplemental Table 6**).

Sensitivity Analyses

The results of sensitivity analyses are summarized in **Supplemental Tables 7-13**. Lengthening the grace period and changing outcome definition to include hospitalized events recorded in both primary or secondary positions along with deaths recorded in ONS led to point estimates that were consistent with those observed in the primary analyses (**Supplemental Tables 7** and **8**, respectively). Similarly, restricting the patient population to those aged at least 55 years yielded point estimates that were consistent with those of the primary analyses (**Supplemental Table 9**). Likewise, generating treatment and censoring weights using high-dimensional propensity scores (investigator selected covariates and 200 additional covariates) led to similar findings (**Supplemental Table 10**), as did the marginal structural models assessing the potential impact of time-varying confounding, albeit with wider CIs (**Supplemental Table 11**). Finally, both multiple imputation and complete case analyses for variables with missing information led to results that were concordant with those of primary analyses (**Supplemental Tables 12** and **13**).

5.8 DISCUSSION

In this population-based study of women with breast cancer, initiation of an endocrine treatment with an AI was associated with an 86% increased risk of heart failure and a 50% increased risk of cardiovascular mortality. There was also a trend towards an increased risk of myocardial infarction and ischemic stroke. These findings remained consistent across several sensitivity analyses.

Overall, our results are consistent with those of three meta-analyses of RCTs which demonstrated that AIs are associated with an increased the risk of ischemic events (such as myocardial infarction), when compared with tamoxifen.[3-5] Furthermore, our heart failure finding corroborates the signal observed in the BIG 1-98 trial of letrozole, where a significant increased risk of severe heart failure was reported (letrozole: 26/3975 vs tamoxifen: 13/3988).[8] To date, the four observational studies that have examined the association between AIs and different cardiovascular outcomes have reported inconsistent findings.[17-20] In a study conducted using the Ontario health insurance databases, AIs were associated with doubling of the risk of myocardial infarction (HR: 2.02, 95% CI: 1.16-3.53), when compared with tamoxifen among women at least 65 years of age.[17] In contrast, a study using the Kaiser Permanente Health insurance database did not find an increased risk of cardiac ischemia (HR: 0.97, 95% CI: 0.78-1.22), stroke (HR: 0.97, 95% CI: 0.70-1.33) or a combined endpoint of heart failure and cardiomyopathy (HR: 1.10: 95% CI: 0.86-1.40) among women without a history of cardiovascular disease.[20] However, this study did find an association between AIs and other cardiovascular events defined as a composite endpoint of dysrhythmia, valvular dysfunction, and pericarditis (HR: 1.29, 95% CI: 1.11-1.50).[20] Similarly, a recent study using the SEER-Medicare database among women at least 67 years of age did not find an association between use of AIs and myocardial infarction, when compared

with tamoxifen (HR: 1.01, 95% CI: 0.72-1.42).[18] One study using HealthCore Integrated Research Databases found that among women at least 50 years of age, AIs were not associated with increased risks of myocardial infarction (HR: 0.90, 95% CI: 0.65-1.25) or ischemic stroke (HR: 0.71, 95% CI: 0.49-1.03), when compared to non-breast cancer women.[19]

Overall, the inconsistent findings across these studies may be due to heterogeneity in the study populations. This includes the inconsistent inclusion of patients with or without a history of cardiovascular disease and use of individual versus composite outcome definitions. In contrast, our study included patients with and without a history of cardiovascular disease and captured younger post-menopausal women diagnosed with breast cancer. Some of these previous studies had other limitations, including use of an intention-to-treat exposure definition which may lead to non-differential exposure misclassification and a dilution of the effect estimate,[17,20] informative censoring due to discontinuation and switching between treatments,[18] and potential confounding by indication.[19]

There are two hypothesized mechanisms that can explain an association between AIs and cardiovascular ischemic events. The first hypothesis involves a mechanism by which AIs increase the risk of cardiovascular events by increasing low-density lipoprotein (LDL) cholesterol levels.[3] In the Anastrozole Tamoxifen Alone or in Combination (ATAC) trial, the use of anastrozole was associated with increased LDL cholesterol levels, when compared with tamoxifen.[6,7] However, other RCTs have not observed important changes in cholesterol levels with anastrozole, letrozole, or exemestane.[8-10] Similarly, in extended adjuvant trials, there were no changes in LDL cholesterol or triglyceride levels when comparing AIs with placebo or no treatment;[9,11,12] there were also no increased risk of ischemic events.[5,40,41]

The second hypothesis involves a possible cardioprotective effect of tamoxifen. Indeed, tamoxifen has been shown to have favorable effects on serum lipid levels, with decreases of up to 39mg/dL for total cholesterol and 31mg/dL for LDL cholesterol when comparing baseline to three months of follow-up; this effect was shown to persist up to one year after treatment initiation.[10,13,16,42] Another study reported that reduction in total cholesterol occurred only during the treatment period and not after treatment discontinuation.[13] Thus, the increased risk of cardiovascular mortality observed with AIs in our study may be due, at least in part, to the cardioprotective effects of tamoxifen.[5,43] This hypothesis is supported by meta-analyses of RCTs which showed that compared with placebo or no treatment, tamoxifen is associated with a 34% decreased risk ischemic events, 26% decreased risk myocardial infarction, and a 45% decreased risk of fatal myocardial infarction.[5,44] With respect to the increased risk of heart failure observed with AIs, it is possible that this is due to tamoxifen's anti-inflammatory and anti-oxidant properties.[13-16] In addition, some studies suggest that tamoxifen may improve endothelial function by increasing flow-mediated dilation and decreasing carotid intima-media thickness.[45] However, a more recent study suggest that AIs may be associated with vascular injury and attenuated peripheral endothelial function.[46]

Our study has several strengths. To our knowledge, this is the largest observational study to have directly compared the risk of cardiovascular outcomes between AIs and tamoxifen among women with breast cancer. In addition, this study comprehensively examined the association between AIs and clinically-relevant endpoints, including myocardial infarction, ischemic stroke, heart failure, and cardiovascular mortality. Second, linkage to the HES and ONS databases likely minimized outcome misclassification.[33,34] Third, the new-user, active comparator design likely reduced confounding at the design stage, while eliminating prevalent-

user bias.[47] Fourth, our results remained consistent across sensitivity analyses meant to address different sources of bias. Finally, given the population-based nature of our study, our study population is likely to represent patients treated in the real-world setting.

Our study has some limitations. First, prescriptions in the CPRD represent those issued by general practitioners and thus misclassification of exposure is possible if patients did fully adhere with the treatment regimen or if they were treated by specialists. However, in our study, approximately 76% of the cohort initiated treatment with either an AI or tamoxifen, a finding that is consistent with the prevalence of hormone-receptor positive breast cancer reported in other studies.[48] In addition, general practitioners in the UK are extensively involved in the management and treatment of patients with breast cancer, which includes the administration of endocrine therapy to post-menopausal women with hormone-receptor positive breast cancer.[49,50] Second, residual confounding is possible given the observational nature of this study. However, the models considered a wide range of potential confounders, ranging from demographic, lifestyle (e.g. smoking), anthropometric (e.g. body mass index), comorbidities, cardiovascular history, prescription drugs, and breast-cancer related variables (including previous breast surgery, chemotherapy and radiation therapy as proxies for breast cancer severity). Furthermore, the decision to initiate treatment with either an AI versus tamoxifen is typically influenced by hormone-receptor status and not cardiovascular risk profile.[49,50] Third, the results for myocardial infarction and ischemic stroke generated wider CIs that included the null value, which may be due to the lower number of exposed events. Similarly, some of our secondary analyses had limited statistical power, such as those assessing the association with specific AIs and stratification by history of cardiovascular disease. Thus, further large studies are required to

corroborate our findings and investigate the risk of cardiovascular outcomes by AI type and history of cardiovascular disease.

In summary, in this population-based study, use of AIs was associated with 86% increased risk of heart failure and a 50% increased risk of cardiovascular mortality, when compared with use of tamoxifen. There was also a trend towards an increased risk of myocardial infarction and ischemic stroke. The increased risk of cardiovascular events associated with AIs should be balanced with their favorable clinical benefits when compared with tamoxifen.

5.9 ACKNOWLEDGEMENTS

Farzin Khosrow-Khavar is the recipient of a doctoral award from the Fonds de recherche du Québec – Santé (FRQS). Dr. Kristian Filion holds a Chercheur-Boursier Junior 2 award from the FRQS and is the recipient of a William Dawson Scholar award from McGill University. Dr. Samy Suissa is the recipient of a James McGill award from McGill University. Dr. Laurent Azoulay holds a Senior Chercheur-Boursier career award from the FRQS and is the recipient of a William Dawson Scholar award from McGill University. We would like to acknowledge Hui Yin for her assistance in conducting sensitivity analyses for this study.

5.10 FUNDING

This study was funded by a Foundation Scheme Grant from the Canadian Institutes of Health Research (FDN-14328). The funding source had no influence on the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.
McGill University.

5.11 CONFLICTS OF INTEREST DISCLOSURE

NB served as a consultant from Amgen, Novartis, and Roche. SS has received research funding, participated in advisory board meetings or as a speaker for AstraZeneca, Boehringer-Ingelheim, Novartis, Pfizer and Merck. LA has received consulting fees from Janssen for work unrelated to this study. All other authors have no conflicts to disclose and have no financial relationships with any organizations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

5.12 REFERENCES

1. Kelly E, Lu CY, Albertini S, Vitry A. Longitudinal trends in utilization of endocrine therapies for breast cancer: an international comparison. *J Clin Pharm Ther.* 2015;40(1):76-82.

2. Early Breast Cancer Trialists' Collaborative G, Dowsett M, Forbes JF, et al. Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. *Lancet.* 2015;386(10001):1341-1352.

3. Amir E, Seruga B, Niraula S, Carlsson L, Ocana A. Toxicity of adjuvant endocrine therapy in postmenopausal breast cancer patients: a systematic review and meta-analysis. *JNatlCancer Inst.* 2011;103(17):1299-1309.

4. Cuppone F, Bria E, Verma S, et al. Do adjuvant aromatase inhibitors increase the cardiovascular risk in postmenopausal women with early breast cancer? Meta-analysis of randomized trials. *Cancer.* 2008;112(2):260-267.

5. Khosrow-Khavar F, Filion KB, Al-Qurashi S, et al. Cardiotoxicity of Aromatase Inhibitors and Tamoxifen in Post-Menopausal Women with Breast Cancer: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Ann Oncol.* 2017;28(3):487-496.

6. Bundred NJ. The effects of aromatase inhibitors on lipids and thrombosis. *Br J Cancer.* 2005;93 Suppl 1:S23-27.

7. Buzdar A, Howell A, Cuzick J, et al. Comprehensive side-effect profile of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: long-term safety analysis of the ATAC trial. *Lancet Oncol.* 2006;7(8):633-643.

8. Mouridsen H, Keshaviah A, Coates AS, et al. Cardiovascular adverse events during adjuvant endocrine therapy for early breast cancer using letrozole or tamoxifen: safety analysis of BIG 1-98 trial. *J Clin Oncol.* 2007;25(36):5715-5722.

9. Markopoulos C, Chrissochou M, Michailidou A, et al. Effect of exemestane on the lipidemic profile of post-menopausal operable breast cancer patients following 5-7 years of adjuvant tamoxifen: preliminary results of the ATENA substudy. *Anticancer Drugs.* 2005;16(8):879-883.

10. Hozumi Y, Suemasu K, Takei H, et al. The effect of exemestane, anastrozole, and tamoxifen on lipid profiles in Japanese postmenopausal early breast cancer patients: final results of National Surgical Adjuvant Study BC 04, the TEAM Japan sub-study. *Ann Oncol.* 2011;22(8):1777-1782.

11. Wasan KM, Goss PE, Pritchard PH, et al. The influence of letrozole on serum lipid concentrations in postmenopausal women with primary breast cancer who have completed 5 years of adjuvant tamoxifen (NCIC CTG MA.17L). *Ann Oncol.* 2005;16(5):707-715.

12. Younus M, Kissner M, Reich L, Wallis N. Putting the cardiovascular safety of aromatase inhibitors in patients with early breast cancer into perspective: a systematic review of the literature. *Drug Saf.* 2011;34(12):1125-1149.

13. Dewar JA, Horobin JM, Preece PE, Tavendale R, Tunstall-Pedoe H, Wood RA. Long term effects of tamoxifen on blood lipid values in breast cancer. *BMJ.* 1992;305(6847):225-226.

14. Grainger DJ, Schofield PM. Tamoxifen for the prevention of myocardial infarction in humans: preclinical and early clinical evidence. *Circulation.* 2005;112(19):3018-3024.

15. Guetta V, Lush RM, Figg WD, Waclawiw MA, Cannon RO, 3rd. Effects of the antiestrogen tamoxifen on low-density lipoprotein concentrations and oxidation in postmenopausal women. *Am J Cardiol.* 1995;76(14):1072-1073.

16. Love RR, Wiebe DA, Feyzi JM, Newcomb PA, Chappell RJ. Effects of tamoxifen on cardiovascular risk factors in postmenopausal women after 5 years of treatment. *J Natl Cancer Inst.* 1994;86(20):1534-1539.

17. Abdel-Qadir H, Amir E, Fischer HD, et al. The risk of myocardial infarction with aromatase inhibitors relative to tamoxifen in post-menopausal women with early stage breast cancer. *Eur J Cancer.* 2016;68:11-21.

18. Kamaraju S, Shi Y, Smith E, Nattinger AB, Laud P, Neuner J. Are aromatase inhibitors associated with higher myocardial infarction risk in breast cancer patients? A Medicare population-based study. *Clin Cardiol.* 2018;42(1):93-100.

19. Ligibel JA, James OMA, Fisher M, Daniel GW, Winer EP, Keating NL. Risk of myocardial infarction, stroke, and fracture in a cohort of community-based breast cancer patients. *Breast Cancer ResTreat.* 2012;131(2):589-597.

20. Haque R, Shi J, Schottinger JE, et al. Cardiovascular Disease After Aromatase Inhibitor Use. *JAMA Oncol.* 2016;2(12):1590-1597.

21. U.S. Food and Drug Administration. Arimidex (anastrozole) Tablet Label. 2011; https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/020541s026lbl.pdf.

22. European Medicines Agency. Arimidex-Article 30 referral-Annex III. 2011; https://www.ema.europa.eu/en/medicines/human/referrals/arimidex.

23. European Medicines Agency. Femara-Article 30 referral-Assessment Report. 2012; https://www.ema.europa.eu/en/documents/referral/femara-article-30-referral-assessment-report_en.pdf

24. Foglietta J, Inno A, de Iuliis F, et al. Cardiotoxicity of Aromatase Inhibitors in Breast Cancer Patients. *Clin Breast Cancer.* 2016;17(1):11-17.

25. Herrett E, Gallagher AM, Bhaskaran K, et al. Data Resource Profile: Clinical Practice Research Datalink (CPRD). *Int J Epidemiol.* 2015;44(3):827-836.

26. Herrett E, Thomas SL, Schoonen WM, Smeeth L, Hall AJ. Validation and validity of diagnoses in the General Practice Research Database: a systematic review. *Br J Clin Pharmacol.* 2010;69(1):4-14.

27. Herrett EL, Thomas SL, Smeeth L. Validity of diagnoses in the general practice research database. *Br J Gen Pract.* 2011;61(588):438-439.

28. Arhi CS, Bottle A, Burns EM, et al. Comparison of cancer diagnosis recording between the Clinical Practice Research Datalink, Cancer Registry and Hospital Episodes Statistics. *Cancer Epidemiol.* 2018;57:148-157.

29. Boggon R, van Staa TP, Chapman M, Gallagher AM, Hammad TA, Richards MA. Cancer recording and mortality in the General Practice Research Database and linked cancer registries. *Pharmacoepidemiol Drug Saf.* 2013;22(2):168-175.

30. Margulis AV, Fortuny J, Kaye JA, et al. Validation of Cancer Cases Using Primary Care, Cancer Registry, and Hospitalization Data in the United Kingdom. *Epidemiology.* 2018;29(2):308-313.

31. Hospital Episode Statistics. https://digital.nhs.uk/data-and-information/data-tools-and-services/data-services/hospital-episode-statistics.

32. Office for National Statistics. https://www.ons.gov.uk/.

33. Herrett E, Shah AD, Boggon R, et al. Completeness and diagnostic validity of recording acute myocardial infarction events in primary care, hospital care, disease registry, and national mortality records: cohort study. *BMJ.* 2013;346:f2350.

34. Kivimaki M, Batty GD, Singh-Manoux A, Britton A, Brunner EJ, Shipley MJ. Validity of Cardiovascular Disease Event Ascertainment Using Linkage to UK Hospital Records. *Epidemiology.* 2017;28(5):735-739.

35. Durrleman S, Simon R. Flexible regression models with cubic splines. *Stat Med.* 1989;8(5):551-561.

36. Wyss R, Girman CJ, LoCasale RJ, Brookhart AM, Sturmer T. Variable selection for propensity score models when estimating treatment effects on multiple outcomes: a simulation study. *Pharmacoepidemiol Drug Saf.* 2013;22(1):77-85.

37. Hernan MA, Brumback B, Robins JM. Marginal structural models to estimate the causal effect of zidovudine on the survival of HIV-positive men. *Epidemiology.* 2000;11(5):561-570.

38. Schneeweiss S, Rassen JA, Glynn RJ, Avorn J, Mogun H, Brookhart MA. High-dimensional propensity score adjustment in studies of treatment effects using health care claims data. *Epidemiology.* 2009;20(4):512-522.

39. Rubin D. *Multiple Imputation for Nonresponse in Surveys.* New York: John Wiley & Sons; 1987.

40. Goss PE, Ingle JN, Martino S, et al. Randomized trial of letrozole following tamoxifen as extended adjuvant therapy in receptor-positive breast cancer: updated findings from NCIC CTG MA.17. *JNatlCancer Inst.* 2005;97(17):1262-1271.

41. Goss PE, Ingle JN, Pritchard KI, et al. Extending Aromatase-Inhibitor Adjuvant Therapy to 10 Years. *N Engl J Med.* 2016;375(3):209-219.

42. Sahebkar A, Serban MC, Penson P, et al. The Effects of Tamoxifen on Plasma Lipoprotein(a) Concentrations: Systematic Review and Meta-Analysis. *Drugs.* 2017;77(11):1187-1197.

43. Matthews A, Stanway S, Farmer RE, et al. Long term adjuvant endocrine therapy and risk of cardiovascular disease in female breast cancer survivors: systematic review. *BMJ.* 2018;363:k3845.

44. Braithwaite RS, Chlebowski RT, Lau J, George S, Hess R, Col NF. Meta-analysis of vascular and neoplastic events associated with tamoxifen. *J Gen Intern Med.* 2003;18(11):937-947.

45. Stamatelopoulos KS, Lekakis JP, Poulakaki NA, et al. Tamoxifen improves endothelial function and reduces carotid intima-media thickness in postmenopausal women. *Am Heart J.* 2004;147(6):1093-1099.

46. Maor R, Sara JDS, Wanous AA, et al. Attenuated peripheral endothelial function among women treated with aromatase inhibitors for breast cancer. Coron Artery Dis. 2018;29(8):687-693.

47. Lund JL, Richardson DB, Sturmer T. The active comparator, new user study design in pharmacoepidemiology: historical foundations and contemporary application. *Curr Epidemiol Rep.* 2015;2(4):221-228.

48. Kohler BA, Sherman RL, Howlader N, et al. Annual Report to the Nation on the Status of Cancer, 1975-2011, Featuring Incidence of Breast Cancer Subtypes by Race/Ethnicity, Poverty, and State. J Natl Cancer Inst. 2015;107(6):djv048.

49. National Institute for Clinical Excellence. Improving Outcomes in Breast Cancer. 2002; www.nice.org.uk/guidance/csg1/resources/improving-outcomes-in-breast-cancer-update-pdf-773371117.

50. National Institute for Health and Care Excellence. Early and locally advanced breast cancer: diagnosis and management. 2018; https://www.nice.org.uk/guidance/ng101/chapter/Recommendations#endocrine-therapy.

5.13 FIGURE LEGENDS

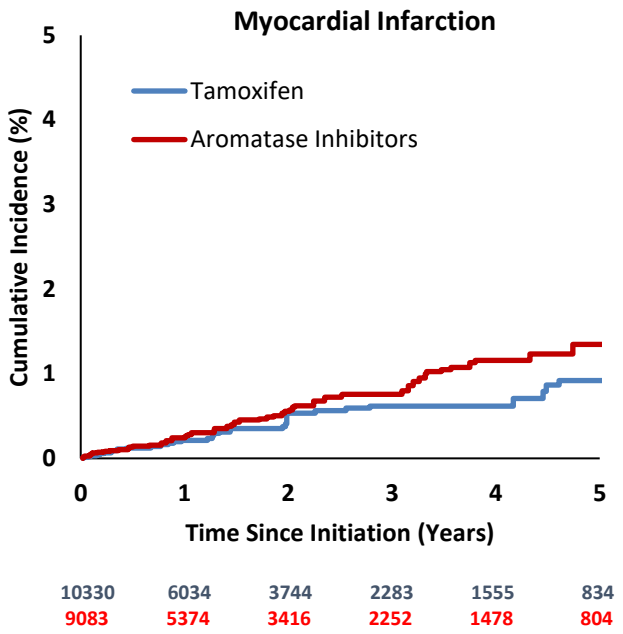
Figure 5.1: Flow chart of patients included in the study population

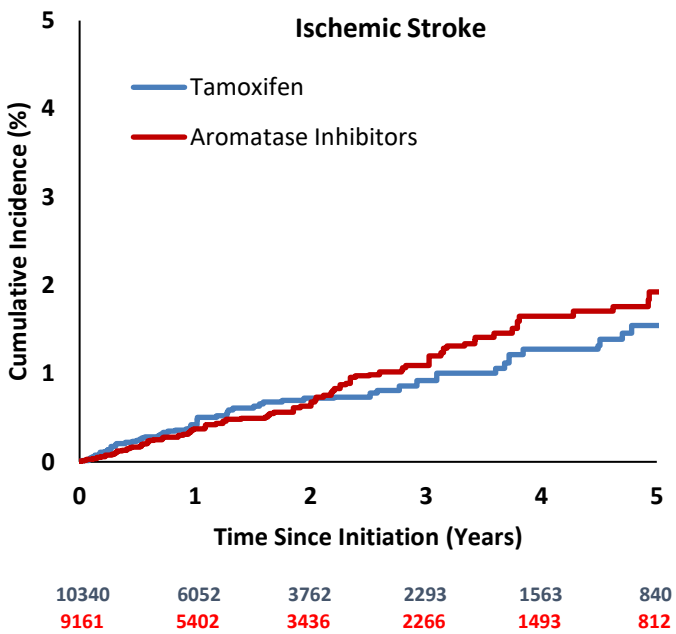
Figure 5.2: Cumulative incidence plots of cardiovascular outcomes according to use of aromatase inhibitors and tamoxifen

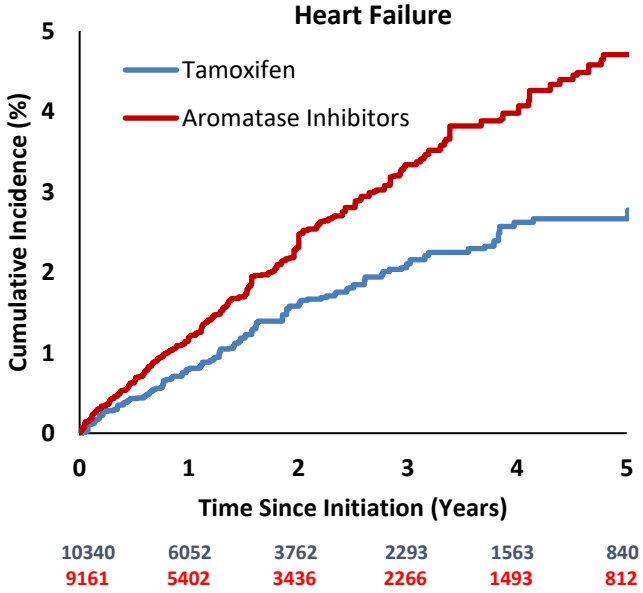
Figure 5.3: Secondary analyses by type of aromatase inhibitor and history of cardiovascular disease

Figure 5.1 Flow chart of patients included in the study population

Figure 5.2 Cumulative incidence plot of cardiovascular outcomes







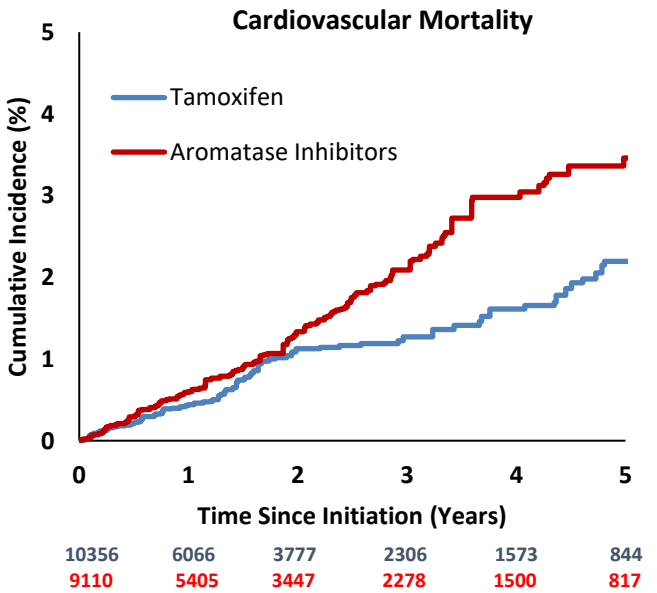


Figure 5.3 Secondary analyses by type of aromatase inhibitor and history of cardiovascular disease

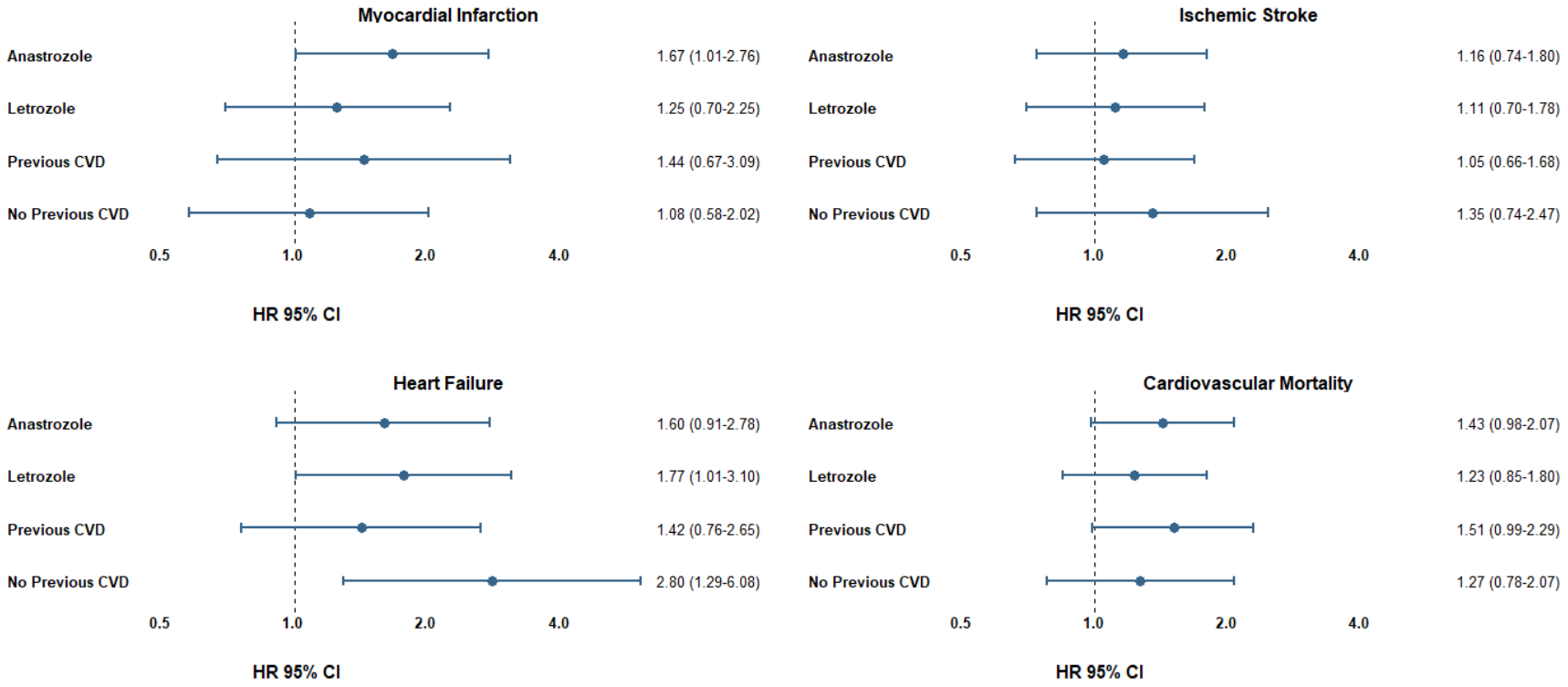


Table 5.1 Baseline characteristics of the study population before and after weighting

Characteristic	Before Weighting			After Weighting*		
	AIs (n=8,139)	Tamoxifen (n=9,783)	Sd. Diff.	AIs	Tamoxifen	Sd. Diff.
Age, mean (SD)	70.8 (11.2)	66.2 (11.5)	0.41	68.1 (11.4)	67.3 (10.9)	0.07
Body mass index (n, %)						
<25 kg/m^2	2,574 (31.6)	3,581 (36.6)	0.11	36.1	36.7	0.01
25-30 kg/m^2	2,630 (32.3)	2,887 (29.5)	0.06	29.6	30.1	0.01
≥30 kg/m^2	2,265 (27.8)	2,091 (21.4)	0.15	22.2	21.5	0.02
Unknown	670 (8.2)	1,224 (12.5)	0.14	12.1	11.7	0.01
Townsend deprivation score (n, %)						
Quintile 1	2,057 (25.3)	2,570 (26.3)	0.02	25.0	25.8	0.02
Quintile 2	2,075 (25.5)	2,595 (26.5)	0.02	27.0	27.7	0.02
Quintile 3	1,814 (22.3)	2,117 (21.6)	0.02	21.1	20.7	0.01
Quintile 4	1,418 (17.4)	1,665 (17.0)	0.01	17.7	16.9	0.02
Quintile 5	768 (9.4)	831 (8.5)	0.03	9.1	8.7	0.01
Unknown	7 (0.1)	5 (0.1)	0.01	0.1	0.2	0.02
Ethnicity (n, %)						
Caucasian	7,696 (94.6)	9,184 (93.9)	0.03	94.5	95.0	0.02
Other	230 (2.8)	223 (2.3)	0.03	2.6	2.5	0.01
Unknown	213 (2.6)	376 (3.8)	0.07	3.0	2.6	0.02
Smoking status (n, %)						
Current	1,130 (13.9)	1,528 (15.6)	0.05	15.7	15.3	0.01
Past	2,925 (35.9)	2,540 (26.0)	0.22	28.3	27.1	0.02
Never	3,974 (48.8)	5,201 (53.2)	0.09	51.5	52.7	0.02
Unknown	110 (1.4)	514 (5.3)	0.22	4.5	4.9	0.02

Characteristic	Before Weighting			After Weighting*		
	AIs (n=8,139)	Tamoxifen (n=9,783)	Sd. Diff.	AIs	Tamoxifen	Sd. Diff.
Comorbidities (n, %)						
Alcohol-related disorders	682 (8.4)	480 (4.9)	0.14	5.5	4.9	0.02
Myocardial infarction	277 (3.4)	167 (1.7)	0.11	2.6	2.2	0.02
Stroke or transient ischemic attack	503 (6.2)	286 (2.9)	0.16	4.6	4.1	0.03
Heart failure	313 (3.8)	229 (2.3)	0.09	2.8	2.4	0.03
Peripheral vascular disease	231 (2.8)	160 (1.6)	0.08	2.3	2.2	0.01
Venous thromboembolism	839 (10.3)	457 (4.7)	0.22	7.5	7.5	0.00
Chronic obstructive pulmonary disease	493 (6.1)	310 (3.2)	0.14	4.3	3.5	0.03
Chronic kidney disease	1127 (13.8)	391 (4.0)	0.35	7.0	5.8	0.04
Other cancers	905 (11.1)	660 (6.7)	0.15	8.9	8.0	0.03
Non-breast cancer surgery	2,096 (25.8)	2,244 (22.9)	0.07	25.3	25.5	0.01
Anticoagulants (n, %)						
Vitamin K antagonists	561 (6.9)	186 (1.9)	0.25	4.1	4.0	0.01
Direct oral anticoagulants	22 (0.3)	S†	0.07	0.1	0.0	0.01
Heparin	189 (2.3)	47 (0.5)	0.16	1.0	1.0	0.00
Antidepressants (n, %)						
Selective serotonin reuptake inhibitors	887 (10.9)	848 (8.7)	0.08	9.4	8.6	0.03
Serotonin and noradrenaline reuptake	92 (1.1)	100 (1.0)	0.01	1.5	1.4	0.00
Tricyclic antidepressants	854 (10.5)	934 (9.5)	0.03	10.7	10.4	0.01
Other	131 (1.6)	98 (1.0)	0.05	1.0	0.8	0.01
Antidiabetic drugs (n, %)						
Metformin	509 (6.3)	340 (3.5)	0.13	4.6	4.1	0.02
Sulfonylureas	280 (3.4)	227 (2.3)	0.07	2.9	2.8	0.01
Thiazolidinediones	68 (0.8)	48 (0.5)	0.04	0.9	1.0	0.00
Incretin-based drugs	51 (0.6)	13 (0.1)	0.08	0.2	0.2	0.01
Insulin	175 (2.2)	96 (1.0)	0.09	1.6	1.5	0.01
Other	7 (0.1)	7 (0.1)	0.01	0.1	0.0	0.01

Characteristic	Before Weighting			After Weighting*		
	AIs (n=8,139)	Tamoxifen (n=9,783)	Sd. Diff.	AIs	Tamoxifen	Sd. Diff.
Antihypertensive drugs (n, %)						
Diuretics	2,578 (31.7)	2,547 (26.0)	0.12	29.5	28.9	0.01
Beta-blockers	1,663 (20.4)	1,618 (16.5)	0.10	18.6	19.1	0.01
Calcium channel blockers	1,756 (21.6)	1,350 (13.8)	0.20	16.6	16.0	0.02
Angiotensin converting enzyme inhibitors	1,704 (20.9)	1,268 (13.0)	0.21	15.4	14.6	0.02
Angiotensin II receptor blockers	891 (10.9)	577 (5.9)	0.18	7.7	7.3	0.01
Other	532 (6.5)	396 (4.0)	0.11	5.5	5.1	0.02
Other drugs (n, %)						
Bisphosphonates	524 (6.4)	406 (4.2)	0.10	5.1	4.3	0.04
Non-steroidal anti-inflammatory drugs	1,117 (13.7)	1,675 (17.1)	0.09	18.3	19.0	0.02
Opioids	2,680 (32.9)	2,555 (26.1)	0.15	30.0	28.5	0.03
Acetylsalicylic acid	1,584 (19.5)	1,276 (13.0)	0.17	17.5	16.6	0.03
Non-ASA antiplatelets	287 (3.5)	121 (1.2)	0.15	2.3	2.1	0.02
Statins	2,361 (29.0)	1,370 (14.0)	0.37	19.9	18.7	0.03
Hormone replacement therapy	548 (6.7)	1,605 (16.4)	0.31	17.1	19.7	0.08
Breast-cancer related variables (n, %)						
Chemotherapy	1,424 (17.5)	1,060 (10.8)	0.19	12.4	12.4	0.00
Radiation therapy	391 (4.8)	433 (4.4)	0.02	4.5	4.5	0.01
Breast cancer surgery	5,702 (70.1)	7,959 (81.4)	0.27	74.4	76.5	0.05
Time since diagnosis, months, mean (SD)	4.1 (9.3)	3.0 (4.8)	0.15	3.3 (6.9)	3.3 (6.9)	0.00

Abbreviations: AI, aromatase inhibitor; S, suppressed; SD, standard deviation; Sd. Diff., standardized difference (absolute).

* Baseline characteristics are displayed in study population weighted for inverse probability of treatment and censoring weights with myocardial infarction as the outcome. Similar characteristics were observed with ischemic stroke, heart failure, and cardiovascular mortality as the outcome. Numbers correspond to percentage of patients.

† Cells with less than five observations are not displayed, as per the confidentiality policies of the Clinical Practice Research Datalink.

Table 5.2 The risk of myocardial infarction, ischemic stroke, heart failure, and cardiovascular mortality with the use of aromatase inhibitors versus tamoxifen in women with breast cancer

Outcome	Exposure *	Events	Person-years	Incidence rate † (95% CI)	Weighted HR (95% CI)
Myocardial Infarction	Tamoxifen	34	18,590	1.8 (1.3-2.6)	1.00 [Reference]
	Aromatase inhibitors	61	15,449	3.9 (3.0-5.1)	1.37 (0.88-2.13)
Ischemic Stroke	Tamoxifen	59	18,594	3.2 (2.4-4.1)	1.00 [Reference]
	Aromatase inhibitors	86	15,440	5.6 (4.5-6.9)	1.19 (0.82-1.72)
Heart Failure	Tamoxifen	33	18,603	1.8 (1.2-2.5)	1.00 [Reference]
	Aromatase inhibitors	83	15,425	5.4 (4.3-6.7)	1.86 (1.14-3.03)
Cardiovascular Mortality	Tamoxifen	87	18,618	4.7 (3.7-5.8)	1.00 [Reference]
	Aromatase Inhibitors	147	15,486	9.5 (8.0-11.2)	1.50 (1.11-2.04)

* The aromatase inhibitor (n=8,139) and tamoxifen (n=9,783) exposure groups were weighted by inverse probability of treatment and censoring weights.
† Per 1,000 person-years.

5.14 Supplementary Methods

Supplemental Method 5.14.1 Inverse Probability of Treatment and Censoring Weights

Inverse probability of treatment weights were constructed using a logistic regression model to determine the probability of receiving an AI versus tamoxifen, conditional on variables measured at or before cohort entry.[1, 2] The model included demographic and lifestyle variables, including age, body mass index (<25, 25-30, ≥30 kg/m^2, unknown), Townsend Deprivation Index, ethnicity (Caucasian, other, unknown), smoking status (current, past, never, unknown), and alcohol-related disorders. The model also included the following comorbidities measured at any time before cohort entry: myocardial infarction, stroke or transient ischemic attack, heart failure, peripheral vascular disease, venous thromboembolism, chronic obstructive pulmonary disease, chronic kidney disease, cancers other than breast cancer (other than non-melanoma skin cancer), and non-breast cancer surgeries in the year prior to cohort entry. We also included the following prescription drugs measured in the year before cohort entry: anticoagulants, antidepressants, antidiabetic drugs, antihypertensive drugs, bisphosphonates, non-steroidal anti-inflammatory drugs, opioids, acetylsalicylic acid (ASA), non-ASA antiplatelets, statins, and hormone replacement therapy. Finally, the model included breast cancer-related variables, including receipt of chemotherapy, radiation therapy, breast cancer surgery, and time since the breast cancer diagnosis (defined as the time between the first breast cancer diagnosis and cohort entry). Age and time since breast cancer diagnosis were modelled flexibly as restricted cubic splines with five interior knots.[3] The inverse probability of treatment weight was stabilized using the probability of treatment received as the numerator.

We also generated inverse probability of censoring weights to minimize potential informative censoring from treatment termination (discontinuation or switching) and death.

Thus, we fitted two logistic regression models to estimate the probability of not being censored due to treatment discontinuation and death. The models included the same variables as those used to generated inverse probability of treatment weights and the exposure. The censoring weights were stabilized by the probability of not being censored conditional on treatment received. The three stabilized weights were multiplied to generate a final set of weights. We used standardized differences to assess model specification and covariate baseline after weighting.

Supplemental Method 5.14.2 High Dimensional Propensity Scores

To further minimize residual confounding, the propensity score model for inverse probability of treatment weights included both investigator selected covariates and covariates empirically selected by the high-dimensional propensity scores (HDPS) algorithm.4 This is a seven-step algorithm that empirically selects variables from different data dimensions. The covariates identification steps include identifying data dimensions, empirically identifying candidate covariates based on prevalence and recurrence of codes, and prioritizing covariates based on their potential for controlling confounding not conditional on exposure and other covariates. The HdPS selected covariates were then combined with investigator selected covariates to estimate the propensity score for exposure and the inverse probability of treatment weights. For this study, the HDPS empirically selected 200 variables measured at cohort entry from seven data dimensions including drug prescriptions, procedures in Clinical Practice Research Datalink (CPRD), diagnoses in CPRD, disease history in CPRD, administrative information in CPRD, procedure in Hospital Episode Statistics (HES), and diagnoses in HES. The HdPS specified IPTW was multiplied by two stabilized IPCW weights (for treatment switch/discontinuation and non-cardiovascular mortality as competing risk). The final weights were truncated at 0.5 and 99.5 percentile of the distribution of the final weight.

Supplemental Method 5.14.3 Marginal Structural Models

The inverse probability of treatment weights for the marginal structural model were estimated using a logistic regression model to determine the probability of observed treatment conditional on baseline covariates.[2, 5] All continuous variables were modelled flexibly using restricted cubic splines to minimize bias by model misspecification from linearity assumption. Inverse probability of censoring weights (IPCW) were generated independently for the probability of not being censored due discontinuation or switch and probability of not experiencing mortality due to non-cardiovascular causes. IPCW was estimated using the probability of not being censored as the independent variable in the logistic model conditional on time-varying covariates, lagged treatment history, and follow-up time. The IPCW was time-updated in monthly intervals. The product of IPTW and IPCW were used as weights to estimate the parameters of a marginal Cox proportional hazard model (using pooled logistic regression) that included the exposure and the weights. Robust variance estimators were included in the model to account for weighting which can induce within subject correlation.[2, 5]

Supplemental Table 5.1 ICD-9 and ICD-10 Diagnostic codes for cardiovascular outcomes

Study Outcome	**ICD-9 diagnosis codes**	**ICD-10 diagnosis codes**
Myocardial infarction	410x	I21x
Ischemic stroke	433x, 434x, 436x	I63x, I64x
Congestive Heart Failure	428x	I50x
Cardiovascular Mortality	390x-398x, 401x-405x, 410x-417x, 420x-429x (excluding 427.5), 430x-438x, 440x-447x	I00x-I77x excluding I46.9

Supplemental Table 5.2 Baseline characteristics of women with breast cancer initiating treatment with aromatase inhibitors or tamoxifen in the weighted study population with ischemic stroke as the outcome

Characteristic	Aromatase Inhibitors	Tamoxifen	Standardized difference
Age, mean (SD)	67.9 (11.4)	67.2 (10.8)	0.07
Body Mass Index, %			
<25	36.2	36.7	0.01
25-30	29.6	30.2	0.01
≥30	22.3	21.6	0.02
Unknown	11.9	11.5	0.02
Townsend deprivation score, %			
Quintile 1	25.0	25.7	0.02
Quintile 2	27.1	27.8	0.02
Quintile 3	21.0	20.6	0.01
Quintile 4	17.8	17.0	0.02
Quintile 5	9.0	8.7	0.01
Unknown	0.1	0.2	0.02
Ethnicity, %			
Caucasian	94.6	95.0	0.02
Other	2.6	2.4	0.01
Unknown	2.9	2.5	0.02
Smoking status, %			
Current	15.9	15.7	0.01
Past	28.3	27.2	0.02
Never	51.4	52.5	0.02
Unknown	4.3	4.6	0.02
Co-morbidities, %			
Alcohol-related disorders	5.5	4.9	0.02
Myocardial infarction	2.5	2.2	0.02
Stroke or transient ischemic attack	4.3	3.8	0.03
Heart failure	2.8	2.3	0.03
Peripheral vascular disease	2.3	2.2	0.01
Venous thromboembolism	7.5	7.5	0.00
Chronic obstructive pulmonary disease	4.2	3.5	0.03
Chronic kidney disease	6.9	5.7	0.04
Other cancers	9.0	8.1	0.03
Non-breast cancer surgery	25.3	25.3	0.00

Characteristic	Aromatase Inhibitors	Tamoxifen	Standardized difference
Anticoagulants, %			
Vitamin K antagonists	4.1	4.0	0.01
Direct oral anticoagulants	0.1	0.0	0.01
Heparin	1.0	1.0	0.00
Antidepressants, %			
Selective serotonin reuptake inhibitors	9.4	8.6	0.03
Serotonin and noradrenaline reuptake inhibitors	1.5	1.4	0.00
Tricyclic antidepressants	10.8	10.4	0.01
Other	0.9	0.8	0.01
Antidiabetic drugs, %			
Metformin	4.5	4.0	0.02
Sulfonylureas	2.9	2.8	0.01
Thiazolidinediones	0.9	0.9	0.00
Incretin-based drugs	0.2	0.2	0.01
Insulin	1.6	1.5	0.01
Other	0.1	0.0	0.01
Antihypertensive drugs, %			
Diuretics	29.3	28.8	0.01
Beta-blockers	18.7	19.3	0.02
Calcium channel blockers	16.7	16.1	0.01
Angiotensin converting enzyme inhibitors	15.3	14.6	0.02
Angiotensin II receptor blockers	7.7	7.4	0.01
Other	5.4	5.0	0.02
Other drugs, %			
Bisphosphonates	5.0	4.2	0.04
Non-steroidal anti-inflammatory drugs	18.5	19.2	0.02
Opioids	30.2	28.6	0.04
Acetylsalicylic acid (ASA)	17.4	16.6	0.02
Non-ASA Antiplatelets	2.3	2.0	0.02
Statins	19.8	18.7	0.03
Hormone replacement therapy	17.3	19.7	0.08
Breast-cancer related variables, %			
Chemotherapy	12.5	12.5	0.00
Radiation therapy	4.5	4.4	0.01
Breast cancer surgery	74.9	76.9	0.05
Time since diagnosis, months, mean (SD)	3.3 (6.9)	3.3 (6.8)	0.00

Supplemental Table 5.3 Baseline characteristics of women with breast cancer initiating treatment with aromatase inhibitors or tamoxifen in the weighted study population with heart failure as the outcome

Characteristic	Aromatase Inhibitors	Tamoxifen	Standardized difference
Age, mean (SD)	68.0 (11.4)	67.2 (10.8)	0.07
Body Mass Index, %			
<25	36.2	36.7	0.01
25-30	29.6	30.2	0.01
≥30	22.2	21.5	0.02
Unknown	12.0	11.6	0.01
Townsend deprivation score, %			
Quintile 1	25.3	25.9	0.02
Quintile 2	27.1	27.8	0.02
Quintile 3	20.9	20.5	0.01
Quintile 4	17.7	16.9	0.02
Quintile 5	9.1	8.7	0.01
Unknown	0.1	0.2	0.02
Ethnicity, %			
Caucasian	94.5	95.0	0.02
Other	2.6	2.4	0.01
Unknown	2.9	2.5	0.02
Smoking status, %			
Current	15.9	15.6	0.01
Past	28.2	27.1	0.03
Never	51.5	52.6	0.02
Unknown	4.4	4.8	0.02
Co-morbidities, %			
Alcohol-related disorders	5.5	4.9	0.02
Myocardial infarction	2.5	2.2	0.02
Stroke or transient ischemic attack	4.5	4.0	0.03
Heart failure	2.7	2.2	0.03
Peripheral vascular disease	2.3	2.2	0.01
Venous thromboembolism	7.5	7.5	0.00
Chronic obstructive pulmonary disease	4.2	3.6	0.03
Chronic kidney disease	7.0	5.8	0.04
Other cancers	9.0	8.1	0.03
Non-breast cancer surgery	25.2	25.2	0.00

Characteristic	Aromatase Inhibitors	Tamoxifen	Standardized difference
Anticoagulants, %			
Vitamin K antagonists	4.0	3.9	0.00
Direct oral anticoagulants	0.1	0.0	0.01
Heparin	1.0	1.1	0.00
Antidepressants, %			
Selective serotonin reuptake inhibitors	9.3	8.5	0.03
Serotonin and noradrenaline reuptake inhibitors	1.5	1.4	0.01
Tricyclic antidepressants	10.8	10.4	0.01
Other	1.0	0.8	0.01
Antidiabetic drugs, %			
Metformin	4.5	4.1	0.02
Sulfonylureas	2.9	2.7	0.01
Thiazolidinediones	0.9	0.9	0.00
Incretin-based drugs	0.2	0.2	0.01
Insulins	1.6	1.4	0.01
Other	0.1	0.0	0.01
Antihypertensive drugs, %			
Diuretics	29.2	28.7	0.01
Beta-blockers	18.8	19.4	0.02
Calcium channel blockers	16.7	16.2	0.01
Angiotensin converting enzyme inhibitors	15.2	14.5	0.02
Angiotensin II receptor blockers	7.7	7.4	0.01
Other	5.3	4.9	0.02
Other drugs, %			
Bisphosphonates	5.1	4.3	0.04
Non-steroidal anti-inflammatory drugs	18.4	19.1	0.02
Opioids	30.1	28.5	0.04
Acetylsalicylic acid (ASA)	17.5	16.7	0.02
Non-ASA antiplatelets	2.3	2.0	0.02
Statins	19.9	18.8	0.03
Hormone replacement therapy	17.3	19.8	0.08
Breast-cancer related variables, %			
Chemotherapy	12.4	12.4	0.00
Radiation therapy	4.5	4.5	0.00
Breast cancer surgery	74.7	76.8	0.05
Time since diagnosis, months, mean (SD)	3.3 (6.9)	3.3 (6.8)	0.01

Supplemental Table 5.4 Baseline characteristics of women with breast cancer initiating treatment with aromatase inhibitors or tamoxifen in the weighted study population with cardiovascular mortality as the outcome

Characteristic	Aromatase Inhibitors	Tamoxifen	Standardized difference
Age, mean (SD)	67.8	67.1	0.06
Body Mass Index, %			
<25	36.1	36.6	0.01
25-30	29.8	30.4	0.01
≥30	22.4	21.6	0.02
Unknown	11.7	11.4	0.01
Townsend deprivation score, %			
Quintile 1	25.1	25.8	0.02
Quintile 2	27.1	27.8	0.01
Quintile 3	20.9	20.6	0.01
Quintile 4	17.7	16.9	0.02
Quintile 5	9.1	8.7	0.01
Unknown	0.1	0.2	0.02
Ethnicity, %			
Caucasian	94.7	95.2	0.02
Other	2.6	2.4	0.01
Unknown	2.8	2.4	0.02
Smoking status, %			
Current	15.9	15.5	0.01
Past	28.3	27.2	0.02
Never	51.5	52.6	0.02
Unknown	4.4	4.7	0.02
Co-morbidities, %			
Alcohol-related disorders	5.5	4.9	0.02
Myocardial infarction	2.5	2.2	0.02
Stroke or transient ischemic attack	4.3	3.9	0.02
Heart failure	2.6	2.2	0.02
Peripheral vascular disease	2.3	2.3	0.00
Venous thromboembolism	7.5	7.5	0.00
Chronic obstructive pulmonary disease	4.2	3.5	0.03
Chronic kidney disease	6.8	5.7	0.04
Other cancers	8.9	8.1	0.03
Non-breast cancer surgery	25.3	25.4	0.00

Characteristic	Aromatase Inhibitors	Tamoxifen	Standardized difference
Anticoagulants, %			
Vitamin K antagonists	4.0	4.0	0.00
Direct oral anticoagulants	0.1	0.0	0.01
Heparin	1.0	1.0	0.00
Antidepressants, %			
Selective serotonin reuptake inhibitors	9.4	8.6	0.03
Serotonin and noradrenaline reuptake inhibitors	1.5	1.4	0.01
Tricyclic antidepressants	10.8	10.4	0.01
Other	1.0	0.8	0.01
Antidiabetic drugs, %			
Metformin	4.6	4.1	0.02
Sulfonylureas	2.9	2.8	0.00
Thiazolidinediones	0.9	0.9	0.00
Incretin-based drugs	0.2	0.2	0.01
Insulin	1.6	1.5	0.01
Other	0.1	0.0	0.01
Antihypertensive drugs, %			
Diuretics	29.3	29.0	0.01
Beta-blockers	18.8	19.5	0.02
Calcium channel blockers	16.7	16.2	0.01
Angiotensin converting enzyme inhibitors	15.2	14.6	0.02
Angiotensin II receptor blockers	7.7	7.5	0.01
Other	5.4	5.1	0.01
Other drugs, %			
Bisphosphonates	5.0	4.2	0.04
Non-steroidal anti-inflammatory drugs	18.5	19.2	0.02
Opioids	30.0	28.5	0.04
Acetylsalicylic acid (ASA)	17.4	16.8	0.02
Non-ASA antiplatelets	2.3	2.1	0.01
Statins	19.9	18.9	0.02
Hormone replacement therapy	17.4	19.8	0.08
Breast-cancer related variables, %			
Chemotherapy	12.5	12.4	0.00
Radiation therapy	4.5	4.4	0.00
Breast Cancer Surgery	75.2	76.9	0.04
Time since diagnosis, months, mean (SD)	3.3 (6.9)	3.3 (6.8)	0.00

Supplemental Table 5.5 The risk of myocardial infarction, ischemic stroke, heart failure, and cardiovascular mortality with the use of aromatase inhibitors versus tamoxifen in women with breast cancer stratified by type of aromatase inhibitor

Outcome	Exposure *	Events	Person-years	Incidence rate † (95% CI)	Weighted HR (95% CI) ‡
Myocardial Infarction	Tamoxifen	34	18,591	1.8 (1.3-2.6)	1.00 [Reference]
	Anastrozole	37	9,342	4.0 (2.8-5.5)	1.67 (1.01-2.76)
	Letrozole	23	5,229	4.4 (2.8-6.6)	1.25 (0.70-2.25)
Ischemic Stroke	Tamoxifen	59	18,594	3.2 (2.4-4.1)	1.00 [Reference]
	Anastrozole	41	9,337	4.4 (3.2-6.0)	1.16 (0.74-1.80)
	Letrozole	40	5,222	7.7 (5.5-10.4)	1.11 (0.70-1.78)
Heart Failure	Tamoxifen	33	18,603	1.8 (1.2-2.5)	1.00 [Reference]
	Anastrozole	40	9,324	4.3 (3.1-5.8)	1.60 (0.91-2.78)
	Letrozole	38	5,221	7.3 (5.2-10.0)	1.77 (1.01-3.10)
Cardiovascular Mortality	Tamoxifen	87	18,618	4.7 (3.7-5.8)	1.00 [Reference]
	Anastrozole	70	9,361	7.5 (5.8-9.4)	1.43 (0.98-2.07)
	Letrozole	65	5,243	12.4 (9.6-15.8)	1.23 (0.85-1.80)

* The aromatase inhibitor (n=8,139) and tamoxifen (n=9,783) exposure groups were weighted by inverse probability of treatment and censoring weights

† Per 1,000 person-years

‡ Results for exemestane are not provided given the low number of events

Supplemental Table 5.6 The risk of myocardial infarction, ischemic stroke, heart failure, and cardiovascular mortality with the use of aromatase inhibitors versus tamoxifen in women with breast cancer stratified by history of previous cardiovascular disease

Outcome	Previous Cardiovascular Disease	Exposure *	Events	Person-years	Incidence rate † (95% CI)	Weighted HR (95% CI)
Myocardial Infarction	Yes	Tamoxifen	14	3,226	4.3 (2.4-7.3)	1.00 [Reference]
		Aromatase inhibitors	38	4,431	8.6 (6.1-11.8)	1.44 (0.67-3.09)
	No	Tamoxifen	20	15,365	1.3 (0.8-2.0)	1.00 [Reference]
		Aromatase inhibitors	23	11,019	2.1 (1.3-3.1)	1.08 (0.58-2.02)
Ischemic Stroke	Yes	Tamoxifen	36	3,222	11.2 (7.8-15.5)	1.00 [Reference]
		Aromatase inhibitors	58	4,420	13.1 (10.0-17.0)	1.05 (0.66-1.68)
	No	Tamoxifen	23	15,372	1.5 (0.9-2.2)	1.00 [Reference]
		Aromatase inhibitors	28	11,020	2.5 (1.7-3.7)	1.35 (0.74-2.47)
Heart Failure	Yes	Tamoxifen	22	3,233	6.8 (4.3-10.3)	1.00 [Reference]
		Aromatase inhibitors	58	4,406	13.2 (10.0-17.0)	1.42 (0.76-2.65)
	No	Tamoxifen	11	15,369	0.7 (0.4-1.3)	1.00 [Reference]
		Aromatase inhibitors	25	11,018	2.3 (1.5-3.3)	2.80 (1.29-6.08)
Cardiovascular Mortality	Yes	Tamoxifen	47	3,240	14.5 (10.7-19.3)	1.00 [Reference]
		Aromatase Inhibitors	100	4,451	22.5 (18.3-27.3)	1.51 (0.99-2.29)
	No	Tamoxifen	40	15,378	2.6 (1.9-3.5)	1.00 [Reference]
		Aromatase Inhibitors	47	11,035	4.3 (3.1-5.7)	1.27 (0.78-2.07)

* The aromatase inhibitor (n=8,139) and tamoxifen (n=9,783) exposure groups were weighted by inverse probability of treatment and censoring weights

† Per 1,000 person-years

Supplemental Table 5.7 The risk of myocardial infarction, ischemic stroke, heart failure, and cardiovascular mortality with the use of aromatase inhibitors versus tamoxifen in women with breast cancer using 60-day grace period as exposure definition

Outcome	Exposure *	Events	Person-years	Incidence rate † (95% CI)	Weighted HR (95% CI)
Myocardial Infarction	Tamoxifen	47	24,971	1.9 (1.4-2.5)	1.00 [Reference]
	Aromatase inhibitors	74	19,345	3.8 (3.0-4.8)	1.30 (0.88-1.93)
Ischemic Stroke	Tamoxifen	84	24,979	3.4 (2.7-4.2)	1.00 [Reference]
	Aromatase inhibitors	121	19,333	6.3 (5.2-7.5)	1.33 (0.97-1.82)
Heart Failure	Tamoxifen	50	24,998	2.0 (1.5-2.6)	1.00 [Reference]
	Aromatase inhibitors	115	19,301	6.0 (4.9-7.2)	1.89 (1.29-2.77)
Cardiovascular Mortality	Tamoxifen	131	25,034	5.2 (4.4-6.2)	1.00 [Reference]
	Aromatase Inhibitors	205	19,420	10.6 (9.2-12.1)	1.41 (1.09-1.83)

* The aromatase inhibitor (n=8,139) and tamoxifen (n=9,783) exposure groups were weighted by inverse probability of treatment and censoring weights

† Per 1,000 person-years

Supplemental Table 5.8 The risk of myocardial infarction, ischemic stroke, and heart failure with the use of aromatase inhibitors versus tamoxifen in women with breast cancer when changing outcome definition to hospitalized events recorded in primary and secondary position and fatal cardiovascular outcomes

Outcome	Exposure *	Events	Person-years	Incidence rate † (95% CI)	Weighted HR (95% CI)
Myocardial Infarction	Tamoxifen	41	18,727	2.2 (1.6-3.0)	1.00 [Reference]
	Aromatase inhibitors	78	15,892	4.9 (3.9-6.1)	1.35 (0.90-2.03)
Ischemic Stroke	Tamoxifen	65	18,593	3.5 (2.7-4.5)	1.00 [Reference]
	Aromatase inhibitors	98	15,430	6.4 (5.2-7.7)	1.24 (0.87-1.77)
Heart Failure	Tamoxifen	111	18,540	6.0 (4.9-7.2)	1.00 [Reference]
	Aromatase inhibitors	255	15,311	16.7 (14.7-18.8)	1.60 (1.21-2.11)

* The aromatase inhibitor (n=8,139) and tamoxifen (n=9,783) exposure groups were weighted by inverse probability of treatment and censoring weights

† Per 1,000 person-years

Supplemental Table 5.9 The risk of myocardial infarction, ischemic stroke, heart failure, and cardiovascular mortality with the use of aromatase inhibitors versus tamoxifen in women with breast cancer and at least 55 years of age

Outcome	Exposure *	Events	Person-years	Incidence rate † (95% CI)	Weighted HR (95% CI)
Myocardial Infarction	Tamoxifen	33	14,992	2.2 (1.5-3.1)	1.00 [Reference]
	Aromatase inhibitors	60	14,370	4.2 (3.2-5.4)	1.42 (0.91-2.24)
Ischemic Stroke	Tamoxifen	58	14,991	3.9 (2.9-5.0)	1.00 [Reference]
	Aromatase inhibitors	86	14,359	6.0 (4.8-7.4)	1.22 (0.84-1.77)
Heart Failure	Tamoxifen	32	15,000	2.1 (1.5-3.0)	1.00 [Reference]
	Aromatase inhibitors	83	14,343	5.8 (4.6-7.2)	1.86 (1.12-3.07)
Cardiovascular Mortality	Tamoxifen	87	15,015	5.8 (4.6-7.2)	1.00 [Reference]
	Aromatase Inhibitors	147	14,405	10.2 (8.6-12.0)	1.52 (1.12-2.07)

* The aromatase inhibitor (n=8,139) and tamoxifen (n=9,783) exposure groups were weighted by inverse probability of treatment and censoring weights

† Per 1,000 person-years

Supplemental Table 5.10 The risk of myocardial infarction, ischemic stroke, heart failure, and cardiovascular mortality with the use of aromatase inhibitors versus tamoxifen in women with breast cancer when using inverse probability of treatment weighting specified using high dimensional propensity scores and inverse probability of censoring weights for mortality as competing risk and treatment discontinuation or switch

Outcome	Exposure *	Events	Person-years	Incidence rate † (95% CI)	Weighted HR (95% CI)
Myocardial Infarction	Tamoxifen	34	18,590	1.8 (1.3-2.6)	1.00 [Reference]
	Aromatase inhibitors	61	15,449	3.9 (3.0-5.1)	1.54 (0.99-2.40)
Ischemic Stroke	Tamoxifen	59	18,594	3.2 (2.4-4.1)	1.00 [Reference]
	Aromatase inhibitors	86	15,440	5.6 (4.5-6.9)	1.42 (1.01-2.00)
Heart Failure	Tamoxifen	33	18,603	1.8 (1.2-2.5)	1.00 [Reference]
	Aromatase inhibitors	83	15,425	5.4 (4.3-6.7)	1.74 (1.19-2.56)
Cardiovascular Mortality	Tamoxifen	87	18,618	4.7 (3.7-5.8)	1.00 [Reference]
	Aromatase Inhibitors	147	15,486	9.5 (8.0-11.2)	1.47 (1.13-1.92)

* The aromatase inhibitor (n=8,139) and tamoxifen (n=9,783) exposure groups were weighted by inverse probability of treatment and censoring weights
† Per 1,000 person-years

Supplemental Table 5.11 The risk of myocardial infarction, ischemic stroke, heart failure, and cardiovascular mortality with the use of aromatase inhibitors versus tamoxifen in women with breast cancer when using marginal structural models

Outcome	Exposure *	Events	Person-months	Incidence rate † (95% CI)	Weighted HR (95% CI)
Myocardial Infarction	Tamoxifen	34	18,590	1.8 (1.3-2.6)	1.00 [Reference]
	Aromatase inhibitors	61	15,449	3.9 (3.0-5.1)	1.55 (0.93-2.59)
Ischemic Stroke	Tamoxifen	59	18,594	3.2 (2.4-4.1)	1.00 [Reference]
	Aromatase inhibitors	86	15,440	5.6 (4.5-6.9)	1.01 (0.69-1.49)
Heart Failure	Tamoxifen	33	18,603	1.8 (1.2-2.5)	1.00 [Reference]
	Aromatase inhibitors	83	15,425	5.4 (4.3-6.7)	1.57 (0.94-2.62)
Cardiovascular Mortality	Tamoxifen	87	18,618	4.7 (3.7-5.8)	1.00 [Reference]
	Aromatase Inhibitors	147	15,486	9.5 (8.0-11.2)	1.32 (0.98-1.77)

* The aromatase inhibitor (n=8,139) and tamoxifen (n=9,783) exposure groups were weighted by inverse probability of treatment and censoring weights
† Per 1,000 person-years

Supplemental Table 5.12 The risk of myocardial infarction, ischemic stroke, heart failure, and cardiovascular mortality with the use of aromatase inhibitors versus tamoxifen in women with breast cancer using multiple imputation for variables with missing information (body mass index, Townsend deprivation score, ethnicity, and smoking status)

Outcome	Exposure *	Events	Person-years	Incidence rate † (95% CI)	Weighted HR (95% CI)
Myocardial Infarction	Tamoxifen	34	18,590	1.8 (1.3-2.6)	1.00 [Reference]
	Aromatase inhibitors	61	15,449	3.9 (3.0-5.1)	1.41 (0.90-2.19)
Ischemic Stroke	Tamoxifen	59	18,594	3.2 (2.4-4.1)	1.00 [Reference]
	Aromatase inhibitors	86	15,440	5.6 (4.5-6.9)	1.16 (0.80-1.67)
Heart Failure	Tamoxifen	33	18,603	1.8 (1.2-2.5)	1.00 [Reference]
	Aromatase inhibitors	83	15,425	5.4 (4.3-6.7)	1.75 (1.09-2.83)
Cardiovascular Mortality	Tamoxifen	87	18,618	4.7 (3.7-5.8)	1.00 [Reference]
	Aromatase Inhibitors	147	15,486	9.5 (8.0-11.2)	1.34 (1.00-1.81)

* The aromatase inhibitor (n=8,139) and tamoxifen (n=9,783) exposure groups were weighted by inverse probability of treatment and censoring weights
† Per 1,000 person-years

Supplemental Table 5.13 The risk of myocardial infarction, ischemic stroke, heart failure, and cardiovascular mortality with the use of aromatase inhibitors versus tamoxifen in women with breast cancer when using complete case analysis

Outcome	Exposure *	Events	Person-months	Incidence rate † (95% CI)	Weighted HR (95% CI)
Myocardial Infarction	Tamoxifen	28	15,668	1.8 (1.2-2.6)	1.00 [Reference]
	Aromatase inhibitors	54	13,910	3.9 (2.9-5.1)	1.42 (0.87-2.31)
Ischemic Stroke	Tamoxifen	41	15,574	2.6 (1.9-3.6)	1.00 [Reference]
	Aromatase inhibitors	73	13,902	5.3 (4.1-6.6)	1.12 (0.74-1.69)
Heart Failure	Tamoxifen	22	15,581	1.4 (0.9-2.1)	1.00 [Reference]
	Aromatase inhibitors	76	13,890	5.5 (4.3-6.8)	1.88 (1.07-3.29)
Cardiovascular Mortality	Tamoxifen	50	15,593	3.2 (2.4-4.2)	1.00 [Reference]
	Aromatase Inhibitors	116	13,945	8.3 (6.9-10.0)	1.47 (1.01-2.14)

* The aromatase inhibitor (n=8,139) and tamoxifen (n=9,783) exposure groups were weighted by inverse probability of treatment and censoring weights

† Per 1,000 person-years

5.15 List of Supplemental References

1. Cole SR and Hernan MA. Constructing inverse probability weights for marginal structural models. Am J Epidemiol. 2008;168:656-664.

2. Hernán MA, Brumback B and Robins JM. Marginal Structural Models to Estimate the Joint Causal Effect of Nonrandomized Treatments. Journal of the American Statistical Association. 2001;96:440-448.

3. Durrleman S and Simon R. Flexible regression models with cubic splines. Stat Med. 1989;8:551-561.

4. Schneeweiss S, Rassen JA, Glynn RJ, Avorn J, Mogun H and Brookhart MA. High-dimensional propensity score adjustment in studies of treatment effects using health care claims data. Epidemiology. 2009;20:512-522.

5. Hernan MA, Brumback B and Robins JM. Marginal structural models to estimate the causal effect of zidovudine on the survival of HIV-positive men. Epidemiology. 2000;11:561-570.

Chapter 6. Manuscript 3-Cardiotoxic Effects of Sequential Aromatase Inhibitors Use in Women with Breast Cancer

6.1 Preface

In Chapter 5, upfront treatment with AIs in comparison with upfront treatment with tamoxifen was associated with 86% increased risk of heart failure and a 50% increased risk of cardiovascular mortality and with a trend towards an increased risk of MI and ischemic stroke. Current guidelines in North America and Europe recommend treatment of post-menopausal women with breast cancer with either upfront treatment with AIs or sequential treatment strategy with tamoxifen and AIs in the adjuvant setting.[9-11] These recommendations are based on results from RCTs showing similar efficacy of AIs in both upfront and sequential settings. Thus, it is imperative to also examine the cardiovascular safety of AIs in the sequential setting which may be an important consideration when deciding on the optimal treatment strategy for patients with estrogen-receptor positive breast cancer. Thus, the objective of this study was to ascertain whether switching to AIs, in comparison to continuing treatment with tamoxifen, is associated with increased risk of cardiovascular outcomes (MI, ischemic stroke, heart failure, and cardiovascular-associated mortality) in post-menopausal women with breast cancer. This manuscript has been accepted for publication to the *American Journal of Epidemiology*.

6.2 Title Page

Cardiotoxic Effects of Sequential Aromatase Inhibitors Use in Women with Breast Cancer

Farzin Khosrow-Khavar, Nathaniel Bouganim, Kristian B. Filion, Samy Suissa, Laurent Azoulay

Correspondence: Dr. Laurent Azoulay, Centre for Clinical Epidemiology, Lady Davis Institute, Jewish General Hospital **,** 3755 Côte Sainte-Catherine, H-425.1, Montreal, Quebec, Canada, H3T 1E2; Tel: 514.340.8222 extension 28396; Fax: 514.340.7564; Email: laurent.azoulay@mcgill.ca

Author Affiliations: Centre for Clinical Epidemiology, Lady Davis Institute, Jewish General Hospital, Montreal, Canada (Farzin Khosrow-Khavar, Kristian B. Filion, Samy Suissa, Laurent Azoulay); Department of Epidemiology, Biostatistics, and Occupational Health, McGill University, Montreal, Canada (Farzin Khosrow-Khavar, Kristian B. Filion, Samy Suissa, Laurent Azoulay); Gerald Bronfman Department of Oncology, McGill University, Montreal, Canada (Nathaniel Bouganim, Laurent Azoulay); Department of Oncology, Cedar Cancer Center, McGill University Health Center, Montreal, Canada (Nathaniel Bouganim); Division of Clinical Epidemiology, Department of Medicine, McGill University, Montreal, Canada (Kristian B. Filion, Samy Suissa).

Acknowledgements: This study was supported by a Foundation Scheme Grant from the Canadian Institutes of Health Research (grant FDN-333744). Farzin Khosrow-Khavar is the recipient of a doctoral award from the Fonds de recherche du Québec – Santé (FRQS). Kristian B. Filion holds a Chercheur-Boursier Junior 2 award from the FRQS and is the recipient of a William Dawson Scholar award from McGill University. Samy Suissa is the recipient of the James McGill Chair. Laurent Azoulay holds a Senior Chercheur-Boursier career award from the FRQS and is the recipient of a William Dawson Scholar award from McGill University.

Conflict of Interest: Nathaniel Bouganim served as a consultant from Amgen, Novartis, and Roche. Samy Suissa has received research funding, participated in advisory board meetings or as a speaker for AstraZeneca, Boehringer-Ingelheim, Novartis, Pfizer and Merck. Laurent Azoulay has received consulting fees from Janssen for work unrelated to this study. All other authors have no conflicts to disclose and have no financial relationships with any organizations that might have an interest in the submitted work and no other relationships or activities that could appear to have influenced the submitted work.

Word count: 3,209

6.3 ABSTRACT

The association between aromatase inhibitors and cardiovascular outcomes is controversial. While some observational studies have assessed their cardiovascular safety as up-front treatments, their cardiotoxic effects as sequential treatments with tamoxifen remains unknown. Thus, we conducted a population-based cohort study using the United Kingdom Clinical Practice Research Datalink linked to the Hospital Episode Statistics and Office for National Statistics databases. A prevalent new-user design was used to propensity score match, in a 1:2 ratio, patients switching from tamoxifen to aromatase inhibitors to patients continuing tamoxifen between 1998 and 2016. Cox proportional hazards models were used to estimate hazard ratios (HRs) with 95% confidence intervals (CIs) for the study outcomes (myocardial infarction, ischemic stroke, heart failure, and cardiovascular mortality). Overall, 1,962 patients switching to aromatase inhibitors were matched to 3,874 patients continuing tamoxifen. Compared with tamoxifen, aromatase inhibitors were associated with an increased risk of myocardial infarction (HR=2.08; 95% CI: 1.02, 4.27). The hazard ratio was elevated with ischemic stroke (HR=1.58; 95% CI: 0.85, 2.93), heart failure (HR=1.69; 95% CI: 0.79, 3.62), but not cardiovascular mortality (HR=0.87; 95% CI: 0.49, 1.54), with the CIs including the null. The elevated HRs observed for the cardiovascular outcomes should be corroborated in future large observational studies.

Keywords: Breast Cancer, Aromatase Inhibitors, Tamoxifen, Endocrine Therapy, Cardiovascular Disease

Abbreviations: AI**,** Aromatase inhibitor; ASA, Acetylsalicylic acid; CI, Confidence Interval; CPRD, Clinical Practice Research Datalink; HES, Hospital Episode Statistics; HR, Hazard Ratio; ICD, International Classification of Disease; LDL, Low-Density Lipoprotein; ONS, Office for National Statistics; RCT, Randomized Controlled Trial; RR, Relative Risk; UK, United Kingdom

6.4 INTRODUCTION

Aromatase inhibitors (AIs) used in either the upfront or sequential setting with tamoxifen have become the mainstay treatment for post-menopausal women with breast cancer.[1] Indeed, compared with tamoxifen, AIs have been associated with improved efficacy in both upfront and sequential settings,[2] with the latter strategy employed in up to 35% of patients.[3-5] When compared with upfront tamoxifen treatment, a sequential treatment strategy with tamoxifen followed by AIs is associated with improved efficacy, while reducing the incidence of musculoskeletal symptoms typically associated with upfront AI treatment.[6]

Despite their clinical benefits, there is evidence from some randomized controlled trials (RCTs) and observational studies that upfront treatment with AIs may increase the risk of cardiovascular outcomes, when compared with tamoxifen.[7-9] As a result, regulatory agencies such as the US Food and Drug Administration have identified ischemic heart disease as a potential safety concern.[10] The biological mechanism for this association remains unclear as some RCTs have implicated the use of AIs with hypercholesterolemia,[11,12] while others have reported no effect of these drugs on serum cholesterol levels.[13-15] Conversely, studies have shown that tamoxifen may have cardioprotective effects through reduction in cholesterol levels.[15-19]

While upfront and sequential AI treatment have been shown to have similar efficacy,[6] there is uncertainty as to the choice of the treatment strategy. Thus, it is imperative to fully assess the cardiovascular safety of AIs in the sequential setting when deciding on the optimal treatment strategy for patients with estrogen-receptor positive breast cancer. To date, few RCTs assessing the sequential treatment strategy have reported on cardiovascular outcomes. Overall, these RCTs reported that sequential treatment with AIs was associated with an increased risk of cardiovascular outcomes, when compared with tamoxifen.[7] However, these RCTs were designed

to assess efficacy and not cardiovascular safety and used heterogeneous composite definitions for the cardiovascular outcomes. To our knowledge, no observational studies have examined the cardiovascular effects of sequential treatment with AIs, compared with tamoxifen. Thus, to address this question, we conducted a population-based cohort study to determine whether use of AIs in sequential treatment with tamoxifen, is associated with an increased risk of cardiovascular outcomes when compared with upfront tamoxifen treatment among women with breast cancer.

6.5 METHODS

6.5.1 Data Sources

We conducted a population-based matched cohort study by using the United Kingdom (UK) Clinical Practice Research Datalink (CPRD) linked with the Hospital Episode Statistics (HES) and the Office for National Statistics (ONS) databases.[20] The CPRD is a primary-care based database which captures anonymous information on medical diagnoses, procedures, lifestyle variables (such as smoking), anthropometric measurements (including body mass index) and prescriptions written by general practitioners.[20] The CPRD captures over 4 million active patients in the United Kingdom[20] and has been shown to be representative of UK population in regards to key characteristics such as age, ethnicity, and body mass index.[20] Clinical diagnoses and procedures are classified according to the Read code classification system whereas prescriptions are classified according to the UK Pricing Authority Dictionary.[20] Overall, diagnoses have been shown to be valid in the CPRD.[21,22] The diagnosis of breast cancer in CPRD has been shown to be concordant with the National Cancer Data Repository (96-97%) [23,24] and medical profile reviews (98%).[23-25]

The HES is a repository which captures all inpatient and outpatient hospital admissions. Primary and secondary diagnoses are recorded in the HES using the International Classification of Disease 10th revision [ICD-10] codes) and procedures are recorded using the Office of Population Censuses and Surveys classification of interventions and procedures (4th revision).[26] Last, the ONS database includes the electronic death certificates of all residents in the UK and includes primary and secondary causes of death recorded using ICD-9 and ICD-10 codes.[26] Approximately 75% of practices in England have been linked to HES and ONS databases since April 1, 1997 with linkage restricted to English practices that have provided consent.[20] The study protocol was approved by the Independent Scientific Advisory Committee of the CPRD

(protocol 17_072RA) and by the Research Ethics Board of the Jewish General Hospital, Montreal, Canada.

6.5.2 Study Population

Cohort of Women with Breast Cancer

We first identified a cohort of women, at least 50 years of age, who were newly-diagnosed with breast cancer between April 1, 1998 and February 29, 2016 (**Web Figure 1**). We excluded patients with less than one year of medical history, those with metastatic disease, and those with prescriptions of AIs or tamoxifen before their breast cancer diagnosis.

Prevalent New-User Design

Using the cohort defined above, we used a prevalent new-user design to match and compare patients switching from tamoxifen to AIs with patients continuing tamoxifen treatment (**Web Figure 2**).[27] In this approach, we divided the time since the first tamoxifen prescription into 30-days intervals. We then identified patients switching to AIs and patients receiving a prescription for tamoxifen in each of these intervals which corresponds to the treatment decision point.[28] Thus, cohort entry was determined by the date of a first AI prescription for switchers and the date of a tamoxifen prescription for patients continuing their treatment during a given interval. We then excluded patients diagnosed with metastatic disease at any time before cohort entry. A schematic of this approach is illustrated in **Web Figure 3.**

Time-Conditional Propensity Scores

Time-conditional propensity scores were generated to estimate the predicted probability of switching to AIs versus continuing tamoxifen during each interval using conditional logistic regression.[29] The propensity score model included the following variables measured at cohort entry: age, body mass index, Townsend Deprivation Index,[30] ethnicity, smoking status, and alcohol-related disorders. The model also included the following comorbidities, all measured at any time before cohort entry: myocardial infarction, stroke or transient ischemic attack, heart failure, peripheral vascular disease, venous thromboembolism, chronic obstructive pulmonary disease, chronic kidney disease, and cancer (other than non-melanoma skin cancer). The model considered non-breast cancer surgeries and the following prescription drugs (all measured in the year before cohort entry): anticoagulants, antidepressants, antidiabetic drugs, antihypertensive drugs, bisphosphonates, non-steroidal anti-inflammatory drugs, opioids, acetylsalicylic acid (ASA), non-ASA antiplatelets, statins, and hormone replacement therapy. Finally, breast cancer-related variables included receipt of chemotherapy, radiation therapy, breast cancer surgery, and time between the first breast cancer diagnosis and cohort entry. Calendar time was not included in the model as it was strongly associated with the exposure and had a relatively weak association with the outcomes. This variable acted as an instrumental variable, and was thus excluded from the propensity score model to minimize bias.[31]

Starting with the first interval, each patient switching from tamoxifen to an AI was matched to two patients (to obtain best balance of bias reduction and precision) with a tamoxifen prescription within the same 30-day interval on duration of tamoxifen treatment and propensity score using nearest neighbor matching without replacement with a caliper of 0.2 standard

deviation of the logit of the propensity score.[32] Tamoxifen users could contribute to the AI group, but only after the time of their switch.

6.5.3 Exposure Ascertainment

We used an *as-treated* exposure definition whereby patients were followed while continuously exposed to AIs or tamoxifen. Patients were considered continuously exposed if a prescription plus a 30-day grace period overlapped with the date of the next prescription of a drug from the same class. Thus, patients were followed until a study outcome (defined below, with separate follow-up for each outcome), treatment discontinuation (end of a 30-day grace period or date of a switch between prescriptions from different drug classes), non-cardiovascular death, end of registration with the general practice, or end of the study period (February 29, 2016).

6.5.4 Cardiovascular Outcomes

Separate analyses were conducted for each of the following cardiovascular outcomes: myocardial infarction, ischemic stroke, heart failure, and cardiovascular mortality **(Web Table 1)**. Myocardial infarction, stroke, and heart failure were defined using HES (primary or secondary diagnosis) or ONS (underlying cause of death) and cardiovascular death was defined using ONS. HES has been shown to have high a 92% positive predictive for myocardial infarction,[33] 96% specificity and negative predictive value for coronary heart disease, and perfect specificity and negative predictive value for stroke.[34]

6.5.5 Statistical Analysis

We calculated incidence rates and corresponding 95% confidence intervals (CIs) based on the Poisson distribution for each exposure group. The Kaplan-Meier method was used to plot cumulative incidence curves for each exposure group. Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% CIs for each outcome, comparing use of AIs with tamoxifen. In a secondary analysis, we examined the association with the composite of major adverse cardiovascular events (MACE), including non-fatal MI, non-fatal stroke, and cardiovascular mortality. We also assessed the risk of cardiovascular outcomes by duration of use and flexibly modelled the hazard by duration of use using restricted cubic splines with three interior knots at tertiles of follow-up time. We also assessed the hazard of MACE by previous duration of tamoxifen use.

Sensitivity Analyses

We conducted four sensitivity analyses to assess the robustness of the analyses. First, we extended the grace period between consecutive prescriptions to 60 days. Second, we conducted an analysis using inverse probability of censoring weighting with separate weights for mortality, discontinuation, and switch between treatments as competing risks. Third, we lagged the exposures by 90 days to account for potential delayed effects on the cardiovascular outcomes. Fourth, we adjusted for calendar time in the outcome model to account for temporal trends in the management of breast cancer and cardiovascular disease during the study period. All analyses were conducted with SAS version 9.4 (SAS Institute, Cary, NC) and R (R Foundation for Statistical Computing, Vienna, Austria).

6.6 RESULTS

Overall, there were 23,525 patients with non-metastatic breast cancer, of whom 9,783 initiated treatment on tamoxifen (**Web Figure 1 and 2**). These patients generated 231,988 intervals during the study period (**Web Figure 2)**. Overall, there were 2,145 intervals where patients switched from tamoxifen to AIs and 150,673 intervals where patients received repeat prescription of tamoxifen. A total of 1,962 patients who switched to AIs were propensity score-matched to 3,874 patients continuing tamoxifen (**Web Figure 2**). Overall, a lower proportion of the study population received AIs between 1998 and 2002 (8.5% vs 17.9%), whereas a higher proportion of patients received AIs after 2002 (**Web Table 3**). Approximately 5% of patients were censored due to end of the study period, 9% due to loss to follow-up, 19% due to treatment switch, and 62% were censored due to treatment discontinuation.

In the unmatched population, AI users were generally similar to tamoxifen users, with exception of venous thromboembolism, non-breast cancer malignancies, use of vitamin K antagonists, chemotherapy, and breast cancer surgery that had higher prevalences in the former group (**Web Table 1**). After propensity score matching, all characteristics were well balanced between groups (**Table 1**). Depending on the outcome, AI users generated between 3,820 to 3,843 person-years of follow-up, whereas tamoxifen users generated between 7,120 and 7,134 person-years of follow-up. The median follow-up for AI and tamoxifen users was 1.5 years.

Compared with continuing tamoxifen, switching to AIs was associated with a doubling of the risk of myocardial infarction (incidence rates 4.7; 95% CI: 2.8, 7.5) versus (2.0; 95% CI: 1.1, 3.3) per 1,000 person-years, respectively; HR=2.08; 95% CI: 1.02, 4.27) (**Table 2**). With respect to ischemic stroke, the use of AIs was associated with an elevated HR with a CI that included the null value, when compared with continuing tamoxifen (incidence rates 5.0 (95% CI: 3.0, 7.7) versus 3.1 (95% CI: 1.9, 4.7) per 1,000 person-years, respectively; HR=1.58; 95% CI: 0.85,

2.93) (**Table 2).** Overall, the use of AIs generated a higher incidence rate of heart failure, compared with continuing tamoxifen (3.4 (95% CI: 1.8, 5.8) versus 2.0 (95% CI: 1.1, 3.3) per 1,000 person-years, respectively). This generated an elevated HR, but with a CI that included the null value (HR=1.69; 95% CI: 0.79, 3.62) when comparing use of AIs to continuing tamoxifen (**Table 2**). Finally, the use of AIs was not associated with an increased risk of cardiovascular mortality, compared with continuous tamoxifen use (incidence rates 4.9 (95% CI: 3.0, 7.7) versus 5.0 (95% CI: 3.5, 7.0) per 1,000 person-years, respectively; HR=0.87, 95% CI: 0.49, 1.54) (**Table 2**). The cumulative incidence curves for MI and ischemic stroke diverged starting three years after switching to AIs while the curves diverged after two years for heart failure (**Figure 2**), albeit with fewer patients remaining at risk with long-term use. For cardiovascular mortality, cumulative incidence curves overlapped during the follow-up period **(Figure 2).**

Secondary Analyses

The hazard ratio for MACE was elevated though non-significant with switching to AIs, compared with continuing tamoxifen (**Web Table 4**; HR=1.47; 95% CI: 0.98, 2.18). There were no systematic differences in the HR when comparing AIs to continuing tamoxifen by duration of previous tamoxifen use (**Web Table 5**), albeit the event rate was low in some strata. When modelling the HR with restricted cubic splines (**Web Figure 4**), the hazards for myocardial infarction and heart failure increased with duration of use, whereas for ischemic stroke there was an initially elevated HR that declined over time. For cardiovascular mortality, the HR remained around the null value. The HR remained elevated for MACE by time on tamoxifen treatment or duration of previous tamoxifen use **(Web Figure 5)**.

Sensitivity Analyses

Sensitivity analysis using inverse probability of censoring weighting led to results that were consistent with those of the primary analyses **(Web Table 6)**. In contrast, extending the grace period for each prescription to 60 days **(Web Table 7)** led to dilution of the association towards the null. Imposing a 90-day exposure lag period led to effect estimates that were consistent with the primary analysis, albeit with slightly wider confidence intervals due to lower number of events (**Web Table 8**). Similarly, adjusting for calendar time in the outcome model led to results that were consistent with the primary analysis (**Web Table 9**).

6.7 DISCUSSION

In this population-based cohort study, treatment with AIs in the sequential setting with tamoxifen, when compared with continuing tamoxifen, was associated with a doubling of risk of myocardial infarction. The HRs were also elevated albeit non-significant for ischemic stroke and heart failure, while no association with cardiovascular mortality. These results remained consistent in secondary and sensitivity analyses.

The results of this study are consistent with previous meta-analyses of RCTs, where AIs were associated with an increased the risk of ischemic events, compared with tamoxifen in the upfront setting.[7,35,36] They also corroborate the signal for severe heart failure observed in the Breast Cancer International Group (BIG) 1-98 trial (letrozole: 0.65% vs tamoxifen: 0.33%).[13] To date, however, few RCTs have assessed the cardiovascular safety of AIs in the sequential setting with tamoxifen. In a meta-analysis of RCTs comparing AIs in sequential treatment with tamoxifen, compared with upfront tamoxifen treatment, AIs were associated with a 16% increased risk of cardiovascular outcomes (relative risk (RR)=1.16, 95% CI: 1.03, 1.32) with an elevated RR for ischemic cardiovascular outcomes (RR=1.21, 95% CI: 0.93, 1.57).[7] In the BIG 1-98 trial, there were imbalances in cardiovascular outcomes and ischemic heart disease in the sequential AI arm versus upfront tamoxifen (7.0% vs 5.7% and 2.3% vs 1.5%) after 71 and 76 months, respectively.[37] Similarly, in RCTs which randomized patients to AIs or continued tamoxifen after two to three years of tamoxifen treatment, there was a 20% increased risk of cardiovascular outcomes associated with AIs (RR=1.20, 95% CI: 1.02, 1.41).[7] However, this association was not observed in RCTs comparing AIs to placebo or no treatment after five years of tamoxifen treatment.[7] Overall, these RCTs were designed to assess efficacy and not cardiovascular safety and used a heterogeneous composite outcome definition.[7,8]

To date, four observational studies have compared the risk of cardiovascular outcomes between AIs and tamoxifen.[9,38-40] In one study, the use of AIs was associated with a doubling of the risk in myocardial infarction,[9] while other studies did not find an association with ischemic cardiovascular outcomes.[38-40] However, none of these studies specifically examined the association between AIs and cardiovascular outcomes in the sequential setting with tamoxifen. Overall, patients treated with upfront AIs had more comorbidities and history of cardiovascular disease in comparison to patients treated with upfront tamoxifen. However, in the present study, patients who switched to AIs were similar to patients on continuous tamoxifen treatment.

There is some evidence that an increased risk of cardiovascular events with AIs may be due to their effects on lipid levels. Indeed, in RCTs comparing AIs to tamoxifen, the use of anastrozole and letrozole was associated with an increased risk of hypercholesterolemia.[41-43] However, it remains unclear whether this increased risk is due to the lipid-lowering effects of tamoxifen or unfavorable effects of AIs. In RCTs, tamoxifen has been shown to decrease LDL and total cholesterol levels between 25-39mg/dL within three months to one year of initiation of tamoxifen treatment, with effects persisting to five years when on tamoxifen treatment.[15,16,44,45] These results are consistent with a meta-analysis of RCTs that showed that tamoxifen decreased the risks of ischemic heart disease by 34%, non-fatal myocardial infarction by 26%, and fatal myocardial infarction by 45%.[7,46] Evidence from one trial suggests that there may be a rebound effect where lipid levels return to baseline levels after discontinuation of treatment for five years with tamoxifen.[47]

This study has several strengths. First, to our knowledge, it is the first study to specifically examine the association between AIs and cardiovascular outcomes in the sequential setting. Second, the cardiovascular outcomes in this study were defined using HES and ONS,

which have been shown to have high specificity.[33,34] Third, we applied a rigorous study design where patients who switched to AIs were matched to patients on tamoxifen on duration of previous tamoxifen use and time-conditional propensity scores. Finally, we observed consistent results in secondary and sensitivity analyses.

This study has some limitations. As the CPRD records prescriptions issued by general practitioners, exposure misclassification is possible. However, 76% of patients in the study population initiated treatment on either tamoxifen or AIs, concordant with prevalence of hormone receptor positive breast cancer.[48-50] In addition, general practitioners in UK are involved in routine management and treatment of patients with breast cancer.[51,52] However, patients' non-adherence to treatment could have led to non-differential exposure misclassification and underestimation of the effect estimates. Second, given the observational nature of this study, residual confounding is possible. Reassuringly, the exposure groups were already similar in the unmatched population, indicating that the reason for switching was not motivated by comorbidity. In addition, we achieved near perfect balance when matching the exposure groups on time-conditional propensity score. Finally, some secondary analyses were underpowered due to fewer exposed events, and it was not possible to assess the risk of cardiovascular outcomes by specific AIs.

6.8 CONCLUSIONS

In this population-based study, AIs in sequential setting were associated with a doubling of the risk of myocardial infarction, when compared with continuous tamoxifen in women with breast cancer. The hazard ratio was also elevated although non-significant for ischemic stroke and heart failure, while no association with cardiovascular mortality. Overall, additional large

observational studies are needed to corroborate these findings in the sequential setting among patients with breast cancer.

6.9 REFERENCES

1. Kelly E, Lu CY, Albertini S, Vitry A. Longitudinal trends in utilization of endocrine therapies for breast cancer: an international comparison. *J Clin Pharm Ther.* 2015;40(1):76-82.

2. Early Breast Cancer Trialists' Collaborative G, Dowsett M, Forbes JF, et al. Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. *Lancet.* 2015;386(10001):1341-1352.

3. Bosco-Levy P, Jove J, Robinson P, Moore N, Fourrier-Reglat A, Bezin J. Persistence to 5-year hormonal breast cancer therapy: a French national population-based study. *Br J Cancer.* 2016.

4. Hadji P, Ziller V, Kyvernitakis J, et al. Persistence in patients with breast cancer treated with tamoxifen or aromatase inhibitors: a retrospective database analysis. *Breast Cancer Res Treat.* 2013;138(1):185-191.

5. Huiart L, Dell'Aniello S, Suissa S. Use of tamoxifen and aromatase inhibitors in a large population-based cohort of women with breast cancer. *Br J Cancer.* 2011;104(10):1558-1563.

6. van de Velde CJ, Verma S, van Nes JG, Masterman C, Pritchard KI. Switching from tamoxifen to aromatase inhibitors for adjuvant endocrine therapy in postmenopausal patients with early breast cancer. *Cancer treatment reviews.* 2010;36(1):54-62.

7. Khosrow-Khavar F, Filion KB, Al-Qurashi S, et al. Cardiotoxicity of Aromatase Inhibitors and Tamoxifen in Post-Menopausal Women with Breast Cancer: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Ann Oncol.* 2017;28(3):487-496.

8. Matthews A, Stanway S, Farmer RE, et al. Long term adjuvant endocrine therapy and risk of cardiovascular disease in female breast cancer survivors: systematic review. *BMJ.* 2018;363:k3845.

9. Abdel-Qadir H, Amir E, Fischer HD, et al. The risk of myocardial infarction with aromatase inhibitors relative to tamoxifen in post-menopausal women with early stage breast cancer. *Eur J Cancer.* 2016;68:11-21.

10. U.S. Food and Drug Administration. Arimidex (anastrozole) Tablet Label. 2011; https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/020541s026lbl.pdf.

11. Bundred NJ. The effects of aromatase inhibitors on lipids and thrombosis. *Br J Cancer.* 2005;93 Suppl 1:S23-27.

12. Buzdar A, Howell A, Cuzick J, et al. Comprehensive side-effect profile of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: long-term safety analysis of the ATAC trial. *Lancet Oncol.* 2006;7(8):633-643.

13. Mouridsen H, Keshaviah A, Coates AS, et al. Cardiovascular adverse events during adjuvant endocrine therapy for early breast cancer using letrozole or tamoxifen: safety analysis of BIG 1-98 trial. *J Clin Oncol.* 2007;25(36):5715-5722.

14. Hozumi Y, Suemasu K, Takei H, et al. The effect of exemestane, anastrozole, and tamoxifen on lipid profiles in Japanese postmenopausal early breast cancer patients: final results of National Surgical Adjuvant Study BC 04, the TEAM Japan sub-study. *Ann Oncol.* 2011;22(8):1777-1782.

15. Younus M, Kissner M, Reich L, Wallis N. Putting the cardiovascular safety of aromatase inhibitors in patients with early breast cancer into perspective: a systematic review of the literature. *Drug Saf.* 2011;34(12):1125-1149.

16. Dewar JA, Horobin JM, Preece PE, Tavendale R, Tunstall-Pedoe H, Wood RA. Long term effects of tamoxifen on blood lipid values in breast cancer. *BMJ.* 1992;305(6847):225-226.

17. Grainger DJ, Schofield PM. Tamoxifen for the prevention of myocardial infarction in humans: preclinical and early clinical evidence. *Circulation.* 2005;112(19):3018-3024.

18. Guetta V, Lush RM, Figg WD, Waclawiw MA, Cannon RO, 3rd. Effects of the antiestrogen tamoxifen on low-density lipoprotein concentrations and oxidation in postmenopausal women. *Am J Cardiol.* 1995;76(14):1072-1073.

19. Love RR, Wiebe DA, Feyzi JM, Newcomb PA, Chappell RJ. Effects of tamoxifen on cardiovascular risk factors in postmenopausal women after 5 years of treatment. *J Natl Cancer Inst.* 1994;86(20):1534-1539.

20. Herrett E, Gallagher AM, Bhaskaran K, et al. Data Resource Profile: Clinical Practice Research Datalink (CPRD). *Int J Epidemiol.* 2015;44(3):827-836.

21. Herrett E, Thomas SL, Schoonen WM, Smeeth L, Hall AJ. Validation and validity of diagnoses in the General Practice Research Database: a systematic review. *Br J Clin Pharmacol.* 2010;69(1):4-14.

22. Herrett EL, Thomas SL, Smeeth L. Validity of diagnoses in the general practice research database. *Br J Gen Pract.* 2011;61(588):438-439.

23. Arhi CS, Bottle A, Burns EM, et al. Comparison of cancer diagnosis recording between the Clinical Practice Research Datalink, Cancer Registry and Hospital Episodes Statistics. *Cancer Epidemiol.* 2018;57:148-157.

24. Boggon R, van Staa TP, Chapman M, Gallagher AM, Hammad TA, Richards MA. Cancer recording and mortality in the General Practice Research Database and linked cancer registries. *Pharmacoepidemiol Drug Saf.* 2013;22(2):168-175.

25. Margulis AV, Fortuny J, Kaye JA, et al. Validation of Cancer Cases Using Primary Care, Cancer Registry, and Hospitalization Data in the United Kingdom. *Epidemiology.* 2018;29(2):308-313.

26. Hospital Episode Statistics. https://digital.nhs.uk/data-and-information/data-tools-and-services/data-services/hospital-episode-statistics.

27. Ross ME, Kreider AR, Huang YS, Matone M, Rubin DM, Localio AR. Propensity Score Methods for Analyzing Observational Data Like Randomized Experiments: Challenges and Solutions for Rare Outcomes and Exposures. *Am J Epidemiol.* 2015;181(12):989-995.

28. Brookhart MA. Counterpoint: the treatment decision design. *Am J Epidemiol.* 2015;182(10):840-845.

29. Suissa S, Moodie EE, Dell'Aniello S. Prevalent new-user cohort designs for comparative drug effect studies by time-conditional propensity scores. *Pharmacoepidemiol Drug Saf.* 2017;26(4):459-468.

30. National Centre for Research Methods. Geographical Referencing Learning Resources.

31. Wyss R, Girman CJ, LoCasale RJ, Brookhart AM, Sturmer T. Variable selection for propensity score models when estimating treatment effects on multiple outcomes: a simulation study. *Pharmacoepidemiol Drug Saf.* 2013;22(1):77-85.

32. Austin PC. An Introduction to Propensity Score Methods for Reducing the Effects of Confounding in Observational Studies. *Multivariate Behav Res.* 2011;46(3):399-424.

33. Herrett E, Shah AD, Boggon R, et al. Completeness and diagnostic validity of recording acute myocardial infarction events in primary care, hospital care, disease registry, and national mortality records: cohort study. *BMJ.* 2013;346:f2350.

34. Kivimaki M, Batty GD, Singh-Manoux A, Britton A, Brunner EJ, Shipley MJ. Validity of Cardiovascular Disease Event Ascertainment Using Linkage to UK Hospital Records. *Epidemiology.* 2017;28(5):735-739.

35. Amir E, Seruga B, Niraula S, Carlsson L, Ocana A. Toxicity of adjuvant endocrine therapy in postmenopausal breast cancer patients: a systematic review and meta-analysis. *JNatlCancer Inst.* 2011;103(17):1299-1309.

36. Cuppone F, Bria E, Verma S, et al. Do adjuvant aromatase inhibitors increase the cardiovascular risk in postmenopausal women with early breast cancer? Meta-analysis of randomized trials. *Cancer.* 2008;112(2):260-267.

37. B. I. G. 1-98 Collaborative Group, Mouridsen H, Giobbie-Hurder A, et al. Letrozole therapy alone or in sequence with tamoxifen in women with breast cancer. *N Engl J Med.* 2009;361(8):766-776.

38. Haque R, Shi J, Schottinger JE, et al. Cardiovascular Disease After Aromatase Inhibitor Use. *JAMA Oncol.* 2016;2(12):1590-1597.

39. Kamaraju S, Shi Y, Smith E, Nattinger AB, Laud P, Neuner J. Are aromatase inhibitors associated with higher myocardial infarction risk in breast cancer patients? A Medicare population-based study. *Clin Cardiol.* 2018;42(1):93-100.

40. Ligibel JA, James OMA, Fisher M, Daniel GW, Winer EP, Keating NL. Risk of myocardial infarction, stroke, and fracture in a cohort of community-based breast cancer patients. *Breast Cancer ResTreat.* 2012;131(2):589-597.

41. Baum M, Buzdar A, Cuzick J, et al. Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early-stage breast cancer: results of the ATAC (Arimidex, Tamoxifen Alone or in Combination) trial efficacy and safety update analyses. *Cancer.* 2003;98(9):1802-1810.

42. Thurlimann B, Keshaviah A, Coates AS, et al. A comparison of letrozole and tamoxifen in postmenopausal women with early breast cancer. *New England Journal of Medicine.* 2005;353(26):2747-2757.

43. Boccardo F, Rubagotti A, Guglielmini P, et al. Switching to anastrozole versus continued tamoxifen treatment of early breast cancer. Updated results of the Italian tamoxifen anastrozole (ITA) trial. *Ann Oncol.* 2006;17 Suppl 7:vii10-14.

44. Love RR, Newcomb PA, Wiebe DA, et al. Effects of tamoxifen therapy on lipid and lipoprotein levels in postmenopausal patients with node-negative breast cancer. *J Natl Cancer Inst.* 1990;82(16):1327-1332.

45. Sawada S, Sato K, Kusuhara M, et al. Effect of anastrozole and tamoxifen on lipid metabolism in Japanese postmenopausal women with early breast cancer. *Acta oncologica.* 2005;44(2):134-141.

46. Braithwaite RS, Chlebowski RT, Lau J, George S, Hess R, Col NF. Meta-analysis of vascular and neoplastic events associated with tamoxifen. *J Gen Intern Med.* 2003;18(11):937-947.

47. Markopoulos C, Chrissochou M, Antonopoulou Z, et al. Duration of tamoxifen effect on lipidemic profile of postmenopausal breast cancer patients following deprivation of treatment. *Oncology.* 2006;70(4):301-305.

48. Kohler BA, Sherman RL, Howlader N, et al. Annual Report to the Nation on the Status of Cancer, 1975-2011, Featuring Incidence of Breast Cancer Subtypes by Race/Ethnicity, Poverty, and State. *J Natl Cancer Inst.* 2015;107(6):djv048.

49. Lumachi F, Santeufemia DA, Basso SM. Current medical treatment of estrogen receptor-positive breast cancer. *World J Biol Chem.* 2015;6(3):231-239.

50. Khosrow-Khavar F, Yin H, Barkun A, Bouganim N, Azoulay L. Aromatase inhibitors and the risk of colorectal cancer in postmenopausal women with breast cancer. *Ann Oncol.* 2018;29(3):744-748.

51. National Institute for Clinical Excellence. Improving Outcomes in Breast Cancer. 2002; www.nice.org.uk/guidance/csg1/resources/improving-outcomes-in-breast-cancer-update-pdf-773371117.

52. National Institute for Health and Care Excellence. Early and locally advanced breast cancer: diagnosis and management. 2018; https://www.nice.org.uk/guidance/ng101/chapter/Recommendations#endocrine-therapy.

Table 6.1 Baseline Characteristics of Women with Breast Cancer After Matching on Propensity Score

Characteristic	Aromatase Inhibitors (n=1962)		Tamoxifen (n=3874)		Standardized Difference
	No	%	No	%	
Age, mean (SD)	68.2 (10.7)		67.7 (11.1)		0.04
Body mass index, n (%)					
<25 kg/m^2	732	37.3	1,486	38.4	0.02
25-30 kg/m^2	633	32.3	1,287	33.2	0.02
≥30 kg/m^2	440	22.4	796	20.6	0.05
Unknown	157	8.0	305	7.9	0.00
Townsend deprivation score, n (%)					
Quintile 1	527	26.9	1,045	27.0	0.00
Quintile 2	536	27.3	1,066	27.5	0.00
Quintile 3	413	21.1	799	20.6	0.01
Quintile 4	332	16.9	669	17.3	0.01
Quintile 5	154	7.9	295	7.6	0.01
Ethnicity, n (%)					
Caucasian	1,869	95.3	3,685	95.1	0.01
Other	38	1.9	88	2.3	0.02
Unknown	55	2.8	101	2.6	0.01
Smoking status, n (%)					
Current	284	14.5	553	14.3	0.01
Past	476	24.3	940	24.3	0.00
Never	1,132	57.7	2,256	58.2	0.01
Unknown	70	3.6	125	3.2	0.02
Comorbidities, n (%)					
Alcohol-related disorders	119	6.1	217	5.6	0.02
Myocardial infarction	41	2.1	75	1.9	0.01
Stroke or transient ischemic attack	74	3.8	136	3.5	0.01
Heart failure	67	3.4	126	3.3	0.01
Peripheral vascular disease	43	2.2	85	2.2	0.00
Venous thromboembolism	166	8.5	314	8.1	0.01
Chronic obstructive pulmonary disease	91	4.6	162	4.2	0.02
Chronic kidney disease	123	6.3	212	5.5	0.03
Other cancers	467	23.8	954	24.6	0.02
Non-breast cancer surgery	480	24.5	910	23.5	0.02

Characteristic	Aromatase Inhibitors (n=1962)		Tamoxifen (n=3874)		Standardized Difference
	No	%	No	%	
Anticoagulants, n (%)					
Vitamin K antagonists	81	4.1	141	3.6	0.03
Direct oral anticoagulants	§	§	§	§	0.00
Heparin	17	0.9	28	0.7	0.02
Antidepressants, n (%)					
Selective serotonin reuptake inhibitors	208	10.6	371	9.6	0.03
Serotonin and noradrenaline reuptake	48	2.5	81	2.1	0.02
Tricyclic antidepressants	217	11.1	425	11.0	0.00
Other	22	1.1	34	0.9	0.02
Antidiabetic drugs, n (%)					
Metformin	90	4.6	169	4.4	0.01
Sulfonylureas	52	2.7	108	2.8	0.01
Thiazolidinediones	12	0.6	25	0.7	0.00
Incretin-based drugs	§	§	§	§	0.01
Insulin	25	1.3	37	1.0	0.03
Other	§	§	§	§	0.00
Antihypertensive drugs, n (%)					
Diuretics	556	28.3	1,022	26.4	0.04
Beta-blockers	380	19.4	682	17.6	0.05
Calcium channel blockers	305	15.6	578	14.9	0.02
Angiotensin converting enzyme inhibitors	308	15.7	575	14.8	0.02
Angiotensin II receptor blockers	138	7.0	250	6.5	0.02
Other	109	5.6	199	5.1	0.02
Other drugs, n (%)					
Bisphosphonates	93	4.7	176	4.5	0.01
Non-steroidal anti-inflammatory drugs	338	17.2	667	17.2	0.00
Opioids	541	27.6	1,030	26.6	0.02
Acetylsalicylic acid	315	16.1	581	15.0	0.03
Non-ASA antiplatelets	30	1.5	72	1.9	0.03
Statins	350	17.8	669	17.3	0.02
Hormone replacement therapy	119	6.1	235	6.1	0.00
Breast-cancer related variables, n (%)					
Chemotherapy	281	14.3	537	13.9	0.01
Radiation therapy	379	19.3	760	19.6	0.01
Breast cancer surgery	1,639	83.5	3,282	84.7	0.03
Time since diagnosis, months, mean (SD)	19.5	13.5	19.2	13.6	0.02
Duration of previous tamoxifen use, months, mean (SD)	16.4	13.0	16.1	12.8	0.02

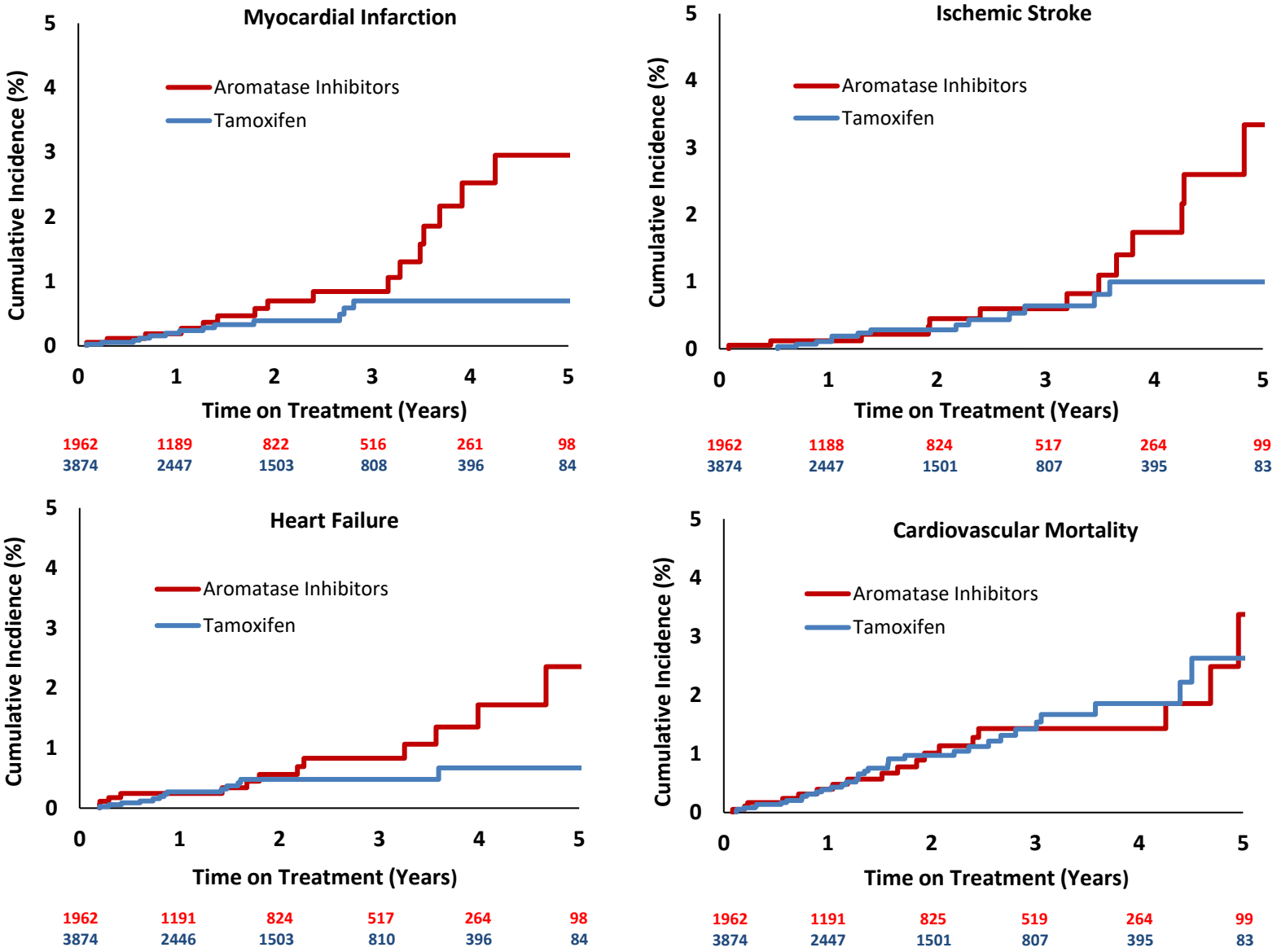
§Cells with less than five observations are not displayed, as per the confidentiality policies of the Clinical Practice Research Datalink

Table 6.2 The Risk of Myocardial Infarction, Ischemic Stroke, Heart Failure, and Cardiovascular Mortality When Comparing Aromatase Inhibitors Switchers Versus Continuing Tamoxifen in Women with Breast Cancer

Outcome	Exposure	Events	Person-years	Incidence rate [a]		HR [b]	
				IR	95% CI	HR	95% CI
Myocardial Infarction	Tamoxifen	14	7,126	2.0	1.1, 3.3	1.00	Referent
	Aromatase inhibitors	18	3,820	4.7	2.8, 7.5	2.08	1.02, 4.27
Ischemic Stroke	Tamoxifen	22	7,120	3.1	1.9, 4.7	1.00	Referent
	Aromatase inhibitors	19	3,831	5.0	3.0, 7.7	1.58	0.85, 2.93
Heart Failure	Tamoxifen	14	7,128	2.0	1.1, 3.3	1.00	Referent
	Aromatase inhibitors	13	3,835	3.4	1.8, 5.8	1.69	0.79, 3.62
Cardiovascular Mortality	Tamoxifen	36	7,134	5.0	3.5, 7.0	1.00	Referent
	Aromatase Inhibitors	19	3,843	4.9	3.0, 7.7	0.87	0.49, 1.54

a. Per 1,000 person-years
b. Hazard ratio obtained from matched population

Figure 6.1 Cumulative Incidence of Myocardial Infarction, Ischemic Stroke, Heart Failure, and Cardiovascular Mortality When Comparing Aromatase Inhibitors Switchers Versus Continuing Tamoxifen in Women with Breast Cancer.



Web Table 6.1 ICD-9 and ICD-10 Diagnostic Codes for Cardiovascular Outcomes

Study Outcome	**ICD-9 diagnosis codes**	**ICD-10 diagnosis codes**
Myocardial infarction	410x	I21x
Ischemic stroke	433x, 434x, 436x	I63x, I64x
Congestive Heart Failure	428x	I50x
Cardiovascular Mortality	390x-398x, 401x-405x, 410x-417x, 420x-429x (excluding 427.5), 430x-438x, 440x-447x	I00x-I77x excluding I46.9

Web Table 6.2 Baseline Characteristics of Women with Breast Cancer Treated with Aromatase Inhibitors or Tamoxifen Before Matching

Characteristic	Aromatase Inhibitors (n=2,145)		Tamoxifen (n=150,673)		Standardized Difference
	No	%	No	%	
Age, mean (SD)	68.2 (10.7)		68.5 (11.1)		0.03
Body mass index, n (%)					
<25 kg/m^2	795	37.1	55,648	36.9	0.00
25-30 kg/m^2	707	33.0	47,264	31.4	0.03
≥30 kg/m^2	474	22.1	34,456	22.9	0.02
Unknown	169	7.9	13,305	8.8	0.03
Townsend deprivation score, n (%)					
Quintile 1	569	26.5	38,877	25.8	0.02
Quintile 2	598	27.9	40,516	26.9	0.02
Quintile 3	446	20.8	33,354	22.1	0.03
Quintile 4	365	17.0	25,541	17.0	0.00
Quintile 5	167	7.8	12,358	8.2	0.02
Unknown	0	0.0	27	0.0	0.02
Ethnicity, n (%)					
Caucasian	2,040	95.1	142,506 (95)	94.6	0.02
Other	47	2.2	2,758	1.8	0.03
Unknown	58	2.7	5,409	3.6	0.05
Smoking status, n (%)					
Current	312	14.6	20,863	13.9	0.02
Past	537	25.0	33,679	22.4	0.06
Never	1,224	57.1	88,367	58.7	0.03
Unknown	72	3.4	7,764	5.2	0.09
Comorbidities, n (%)					
Alcohol-related disorders	124	5.8	8,668	5.8	0.00
Myocardial infarction	42	2.0	2,652	1.8	0.01
Stroke or transient ischemic attack	77	3.6	4,865	3.2	0.02
Heart failure	74	3.5	4,367	2.9	0.03
Peripheral vascular disease	50	2.3	2,787	1.9	0.03
Venous thromboembolism	208	9.7	9,928	6.6	0.11
Chronic obstructive pulmonary disease	104	4.9	6,632	4.4	0.02
Chronic kidney disease	140	6.5	10,319	6.9	0.01
Other cancers	574	26.8	31,291	20.8	0.14
Non-breast cancer surgery	530	24.7	34,286	22.8	0.05

Characteristic	Aromatase Inhibitors (n=2,145)		Tamoxifen (n=150,673)		Standardized Difference
	No	%	No	%	
Anticoagulants, n (%)					
Vitamin K antagonists	98	4.6	3,557	2.4	0.12
Direct oral anticoagulants	§	§	14	0.0	0.02
Heparin	21	1.0	544	0.4	0.08
Antidepressants, n (%)					
Selective serotonin reuptake inhibitors	228	10.6	14,598	9.7	0.03
Serotonin and noradrenaline reuptake	52	2.4	2,199	1.5	0.07
Tricyclic antidepressants	247	11.5	14,568	9.7	0.06
Other	23	1.1	1,535	1.0	0.01
Antidiabetic drugs, n (%)					
Metformin	104	4.9	7,102	4.7	0.01
Sulfonylureas	60	2.8	4,281	2.8	0.00
Thiazolidinediones	18	0.8	808	0.5	0.04
Incretin-based drugs	§	§	418	0.3	0.04
Insulin	26	1.2	1,861	1.2	0.00
Other	§	§	93	0.1	0.01
Antihypertensive drugs, n (%)					
Diuretics	600	28.0	44,380	29.5	0.03
Beta-blockers	409	19.1	27,963	18.6	0.01
Calcium channel blockers	348	16.2	23,758	15.8	0.01
Angiotensin converting enzyme inhibitors	343	16.0	24,035	16.0	0.00
Angiotensin II receptor blockers	156	7.3	10,800	7.2	0.00
Other	129	6.0	7,452	5.0	0.05
Other drugs, n (%)					
Bisphosphonates	106	4.9	7,670	5.1	0.01
Non-steroidal anti-inflammatory drugs	365	17.0	24,156	16.0	0.03
Opioids	599	27.9	37,909	25.2	0.06
Acetylsalicylic acid	344	16.0	22,933	15.2	0.02
Non-ASA antiplatelets	36	1.7	2,272	1.5	0.01
Statins	396	18.5	25,651	17.0	0.04
Hormone replacement therapy	120	5.6	6,979	4.6	0.04
Breast-cancer related variables, n (%)					
Chemotherapy	330	15.4	16,390	10.9	0.13
Radiation therapy	414	19.3	28,561	19.0	0.01
Breast cancer surgery	1,777	82.8	131,641 (87)	87.4	0.13
Time since diagnosis, months, mean (SD)	22.2 (16.2)		22.5 (17.3)		0.02
Duration of previous tamoxifen use, months, mean (SD)	19.0 (15.6)		19.7 (16.9)		0.04

§Cells with less than five observations are not displayed, as per the confidentiality policies of the Clinical Practice Research Datalink

Web Table 6.3 Cohort Entry Year of Patients Who Switch to Aromatase Inhibitors and those Who Continue on Tamoxifen

Year of Cohort Entry	Aromatase Inhibitors (n=1,962)	Tamoxifen (n=3,874)
1998-2002	167 (8.5)	693 (17.9)
2003-2007	963 (49.1)	1665 (43.0)
2008-2012	677 (34.5)	1098 (28.3)
2013-2016	155 (7.9)	418 (10.8)

Web Table 6.4 The Risk of Major Adverse Cardiovascular Events (MACE) a When Comparing Aromatase Inhibitors Switchers Versus Continuing Tamoxifen in Women with Breast Cancer

Outcome	Exposure	Events	Person-years	Incidence rate [b]		HR [c]	
				IR	95% CI	HR	95% CI
MACE	Tamoxifen	55	7,114	7.7	5.8, 10.1	1.00	Referent
	Aromatase inhibitors	46	3,809	12.1	8.8, 16.1	1.47	0.98, 2.18

a. MACE includes nonfatal myocardial infarction, nonfatal stroke, and cardiovascular mortality
b. Per 1,000 person-years
c. Hazard ratio obtained from matched population

Web Table 6.5 The Risk of Myocardial Infarction, Ischemic Stroke, Heart Failure, and Cardiovascular Mortality When Comparing Aromatase Inhibitors Switchers versus Continuing Tamoxifen in Women with Breast Cancer When Stratifying by History of Previous Tamoxifen Use

Outcome	Duration of Previous Tamoxifen Use	Exposure	Events	Person-years	Incidence rate [a]		HR [b]	
					IR	95% CI	HR	95% CI
Myocardial Infarction	≤1year	Tamoxifen	9	3,613	2.5	1.1, 4.7	1.00	Referent
		Aromatase inhibitors	11	1,876	5.9	2.9, 10.5	2.00	0.81, 4.93
	>1year	Tamoxifen	5	3,513	1.4	0.5, 3.3	1.00	Referent
		Aromatase inhibitors	7	1,944	3.6	1.5, 7.4	2.29	0.72, 7.27
Ischemic Stroke	≤1year	Tamoxifen	13	3,614	3.6	1.9, 6.2	1.00	Referent
		Aromatase inhibitors	11	1,874	5.9	2.9, 10.5	1.46	0.65, 3.29
	>1year	Tamoxifen	9	3,507	2.6	1.2, 4.9	1.00	Referent
		Aromatase inhibitors	8	1,957	4.1	1.8, 8.1	1.45	0.56, 3.77
Heart Failure	≤1year	Tamoxifen	5	3,616	1.4	0.5, 3.2	1.00	Referent
		Aromatase inhibitors	9	1.878	4.8	2.2, 9.1	3.16	1.05, 9.51
	>1year	Tamoxifen	9	3,512	2.6	1.2, 4.9	1.00	Referent
		Aromatase inhibitors	§	§	2.0	0.6, 5.2	0.73	0.22, 2.38
Cardiovascular Mortality	≤1year	Tamoxifen	22	3,616	6.1	3.8, 9.2	1.00	Referent
		Aromatase Inhibitors	11	1,878	5.9	2.9, 10.5	0.83	0.40, 1.76
	>1year	Tamoxifen	14	3,512	4.0	2.2, 6.7	1.00	Referent
		Aromatase inhibitors	8	1,958	4.1	1.8, 8.1	0.96	0.40, 2.29

a. Per 1,000 person-years

b. Hazard ratio obtained from matched population

§Cells with less than five observations are not displayed, as per the confidentiality policies of the Clinical Practice Research Datalink

Web Table 6.6 The Risk of Myocardial Infarction, Ischemic Stroke, Heart Failure, and Cardiovascular Mortality When Comparing Aromatase Inhibitors Switchers versus Continuing Tamoxifen in Women with Breast Cancer Using Inverse Probability of Censoring Weighting for Discontinuation, Switch, and Mortality

Outcome	Exposure	Events	Person-years	Incidence rate [a]		HR [b]	
				IR	95% CI	HR	95% CI
Myocardial Infarction	Tamoxifen	14	7,126	2.0	1.1, 3.3	1.00	Referent
	Aromatase inhibitors	18	3,820	4.7	2.8, 7.5	1.95	0.91, 4.17
Ischemic Stroke	Tamoxifen	22	7,120	3.1	1.9, 4.7	1.00	Referent
	Aromatase inhibitors	19	3,831	5.0	3.0, 7.7	1.82	0.94, 3.52
Heart Failure	Tamoxifen	14	7,128	2.0	1.1, 3.3	1.00	Referent
	Aromatase inhibitors	13	3,835	3.4	1.8, 5.8	1.77	0.77, 4.07
Cardiovascular Mortality	Tamoxifen	36	7,134	5.0	3.5, 7.0	1.00	Referent
	Aromatase Inhibitors	19	3,843	4.9	3.0, 7.7	0.98	0.52, 1.84

a. Per 1,000 person-years

b. Hazard ratio obtained from matched population

Web Table 6.7 The Risk of Myocardial Infarction, Ischemic Stroke, Heart Failure, and Cardiovascular Mortality When Comparing Aromatase Inhibitors Switchers Versus Continuing Tamoxifen in Women with Breast Cancer Using 60-day Grace Period

Outcome	Exposure	Events	Person-years	Incidence rate [a]		HR [b]	
				IR	95% CI	HR	95% CI
Myocardial Infarction	Tamoxifen	32	15,091	2.1	1.5, 3.0	1.00	Referent
	Aromatase inhibitors	26	7,742	3.4	2.2, 4.9	1.59	0.95, 2.68
Ischemic Stroke	Tamoxifen	56	15,075	3.7	2.8, 4.8	1.00	Referent
	Aromatase inhibitors	30	7,748	3.9	2.6, 5.5	1.07	0.68, 1.66
Heart Failure	Tamoxifen	39	15,069	2.6	1.8, 3.5	1.00	Referent
	Aromatase inhibitors	31	7,766	4.0	2.7, 5.7	1.54	0.96, 2.48
Cardiovascular Mortality	Tamoxifen	90	15,144	5.9	4.8, 7.3	1.00	Referent
	Aromatase Inhibitors	35	7,791	4.5	3.1, 6.3	0.77	0.52, 1.13

a. Per 1,000 person-years
b. Hazard ratio obtained from matched population

Web Table 6.8 The Risk of Myocardial Infarction, Ischemic Stroke, Heart Failure, and Cardiovascular Mortality When Comparing Aromatase Inhibitors Switchers Versus Continuing Tamoxifen in Women with Breast Cancer Using 90-day Exposure Lag

Outcome	Exposure	Events	Person-years	Incidence rate [a]		HR [b]	
				IR	95% CI	HR	95% CI
Myocardial Infarction	Tamoxifen	12	6,199	1.9	0.8, 3.0	1.00	Referent
	Aromatase inhibitors	17	3,371	5.0	2.6, 7.4	2.24	1.05, 4.80
Ischemic Stroke	Tamoxifen	20	6,195	3.2	1.8, 4.6	1.00	Referent
	Aromatase inhibitors	15	3,388	4.4	2.2, 6.7	1.34	0.68, 2.63
Heart Failure	Tamoxifen	14	6,198	2.3	1.1, 3.4	1.00	Referent
	Aromatase inhibitors	11	3,387	3.2	1.3, 5.2	1.41	0.63, 3.13
Cardiovascular Mortality	Tamoxifen	33	6,203	5.3	3.5, 7.1	1.00	Referent
	Aromatase Inhibitors	16	3,394	4.7	2.4, 7.0	0.78	0.41, 1.43

a. Per 1,000 person-years

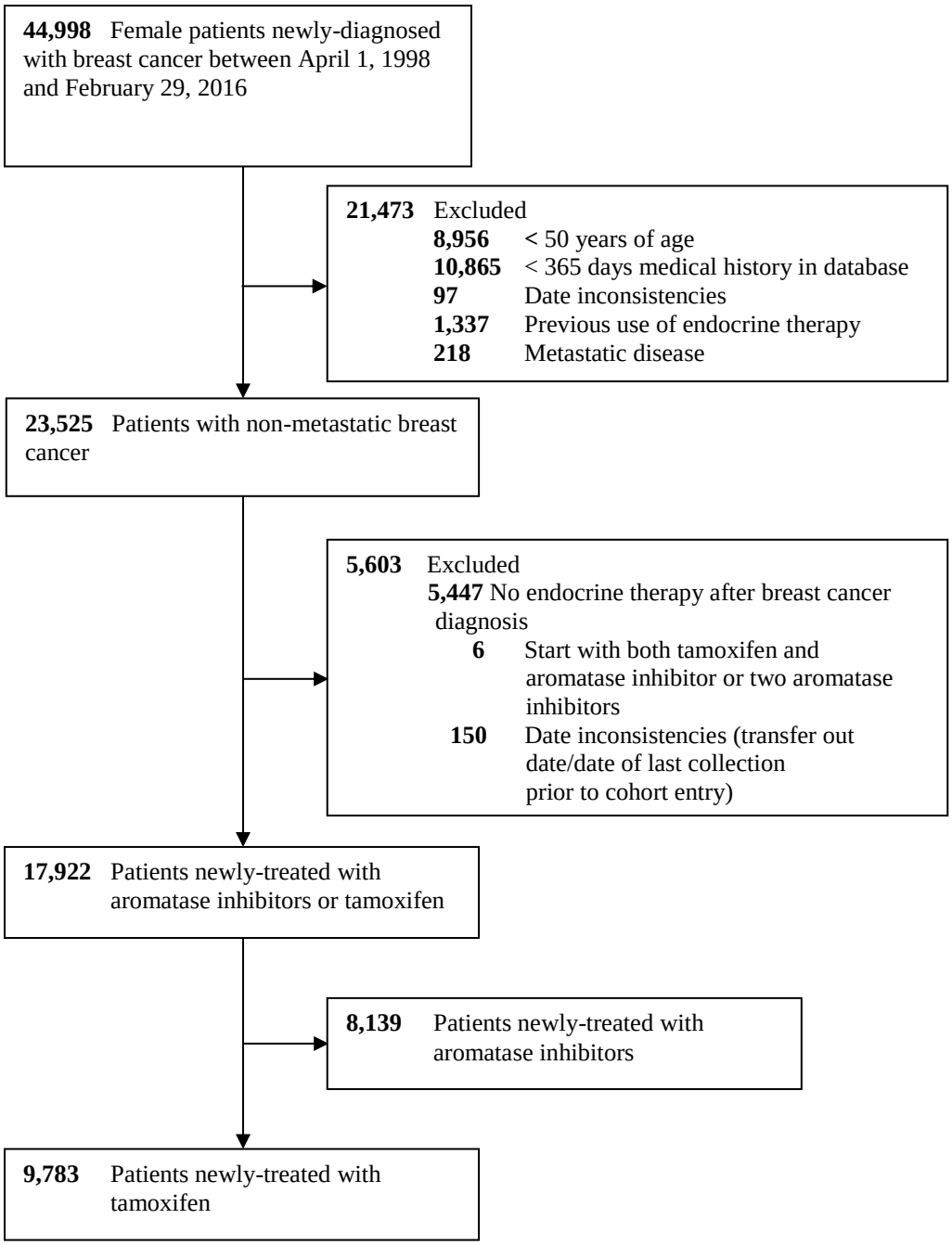
b. Hazard ratio obtained from matched population

c. Patients who were lost to follow-up, discontinued or switched treatment, or died before end of lag period were censored (AI=1,653, tam=3,527)

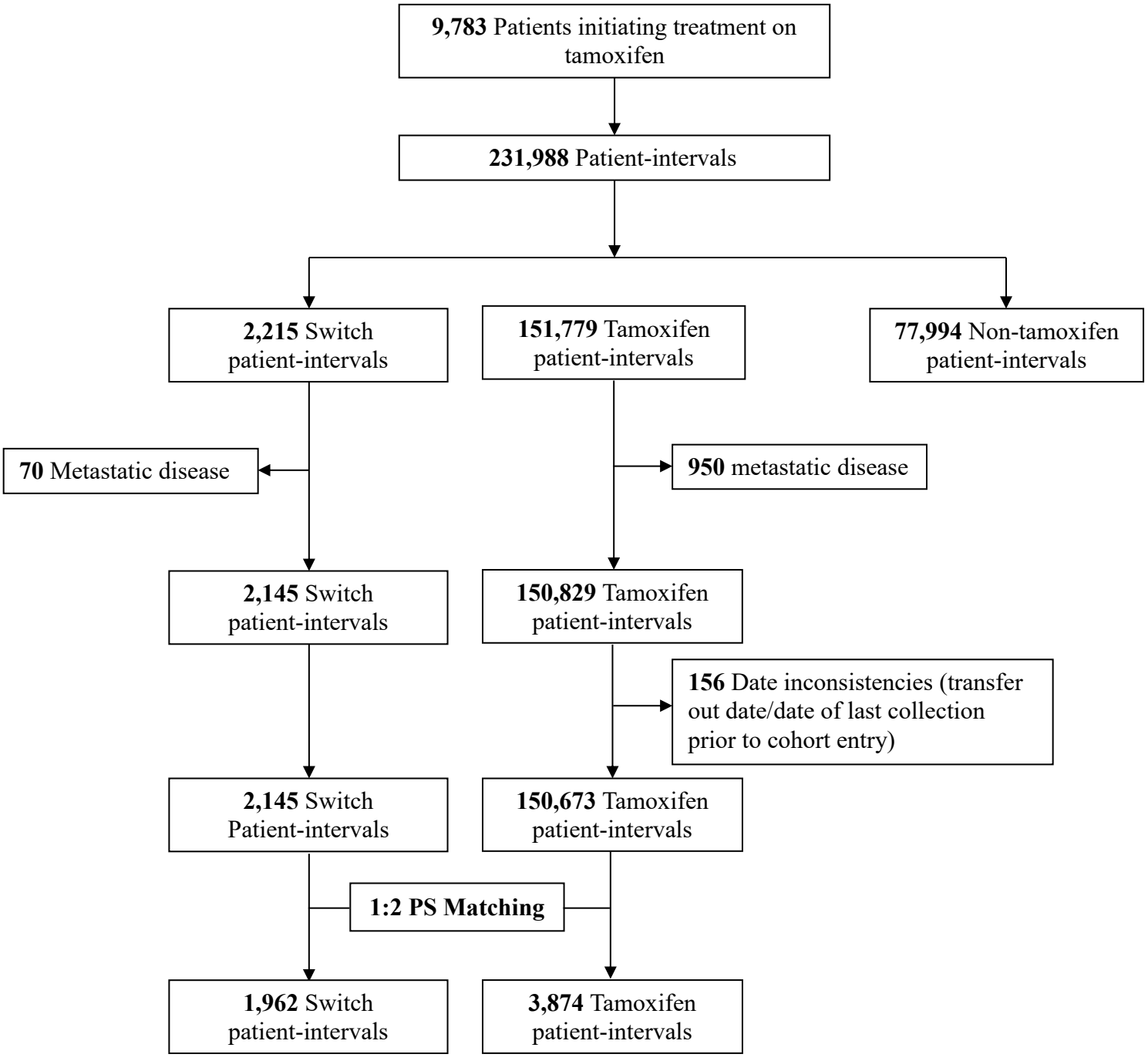
Web Table 6.9 The Risk of Myocardial Infarction, Ischemic Stroke, Heart Failure, and Cardiovascular Mortality When Comparing Aromatase Inhibitors Switchers versus Continuing Tamoxifen in Women with Breast Cancer When Adjusting for Calendar Time in Outcome Model

Outcome	Exposure	Events	Person-years	Incidence rate [a]		HR [b]	
				IR	95% CI	HR	95% CI
Myocardial Infarction	Tamoxifen	14	7,126	2.0	1.1, 3.3	1.00	Referent
	Aromatase inhibitors	18	3,820	4.7	2.8, 7.5	2.03	0.99, 4.17
Ischemic Stroke	Tamoxifen	22	7,120	3.1	1.9, 4.7	1.00	Referent
	Aromatase inhibitors	19	3,831	5.0	3.0, 7.7	1.67	0.89, 3.11
Heart Failure	Tamoxifen	14	7,128	2.0	1.1, 3.3	1.00	Referent
	Aromatase inhibitors	13	3,835	3.4	1.8, 5.8	1.80	0.83, 3.89
Cardiovascular Mortality	Tamoxifen	36	7,134	5.0	3.5, 7.0	1.00	Referent
	Aromatase Inhibitors	19	3,843	4.9	3.0, 7.7	0.86	0.49, 1.54

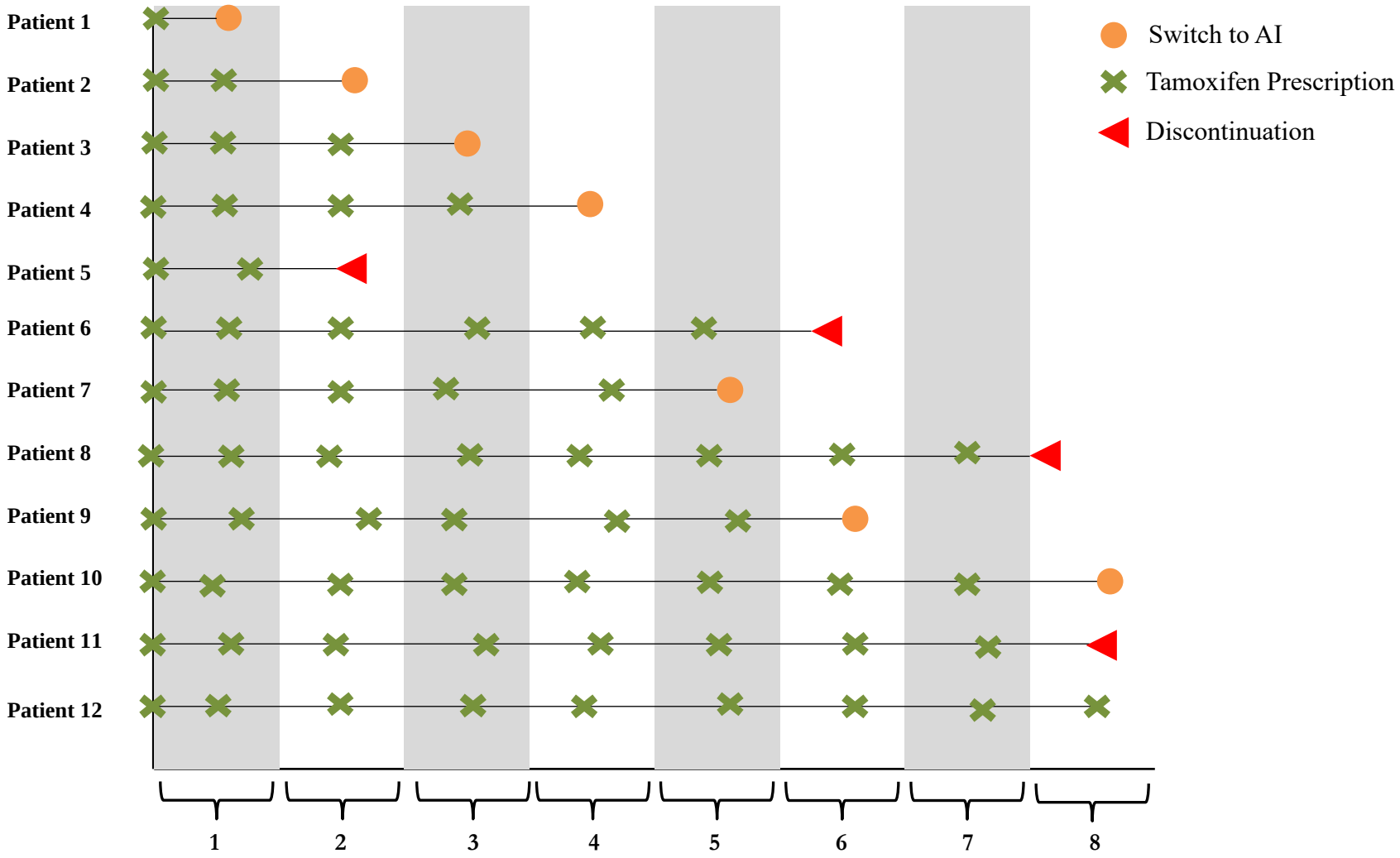
Web Figure 6.1 Flow Diagram of Study Population Depicting Selection of Women with Diagnosis of Breast Cancer Initiating Treatment on Tamoxifen



Web Figure 6.2 Flow Diagram Depicting Selection of Patients Switching to Aromatase Inhibitors Who Were Matched with Patients Who Continued Tamoxifen Treatment

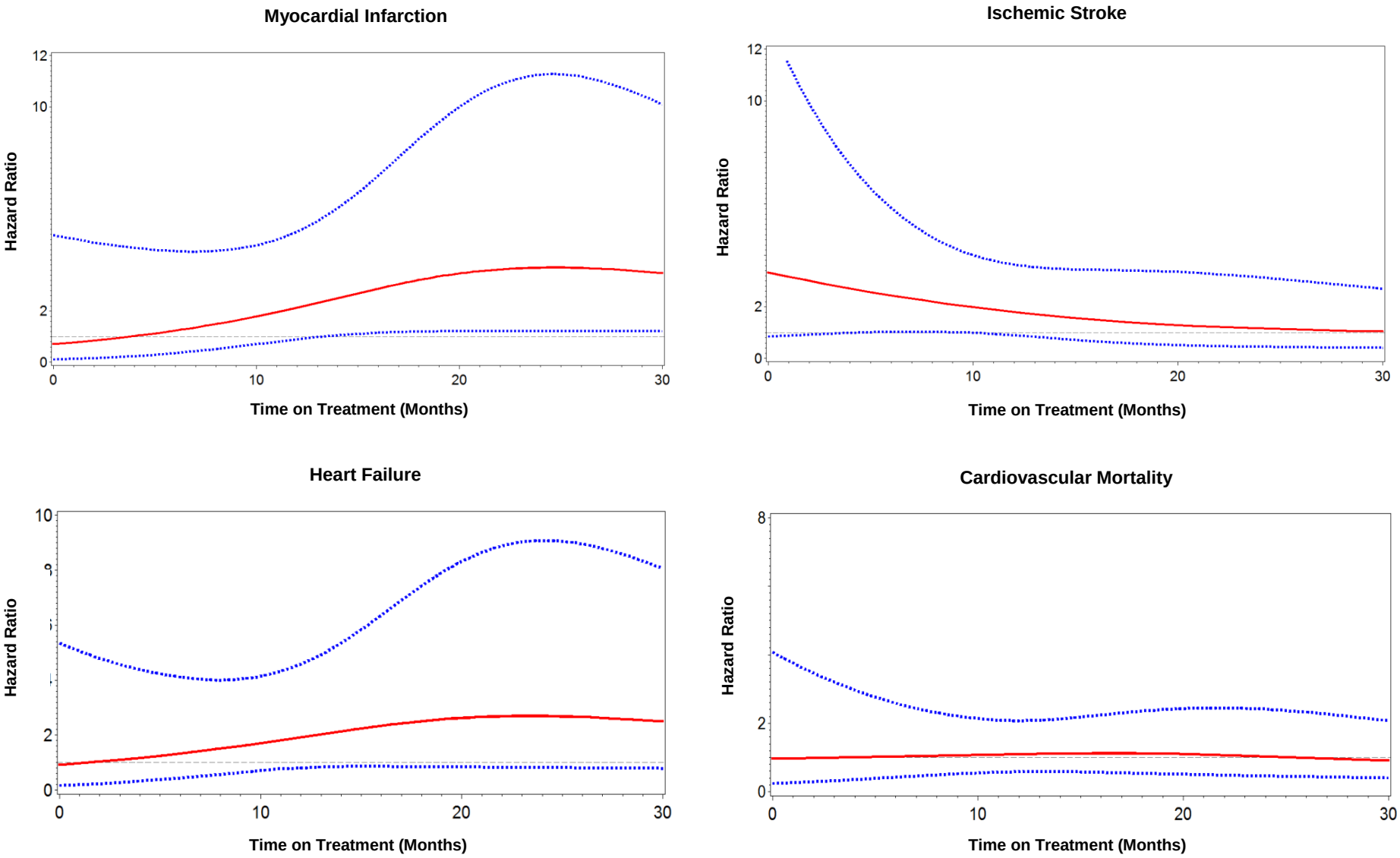


Web Figure 6.3 Schematic of Prevalent New-User Design Depicting Matching of Patients Switching to Aromatase Inhibitors with Patients Continuing Tamoxifen Treatment

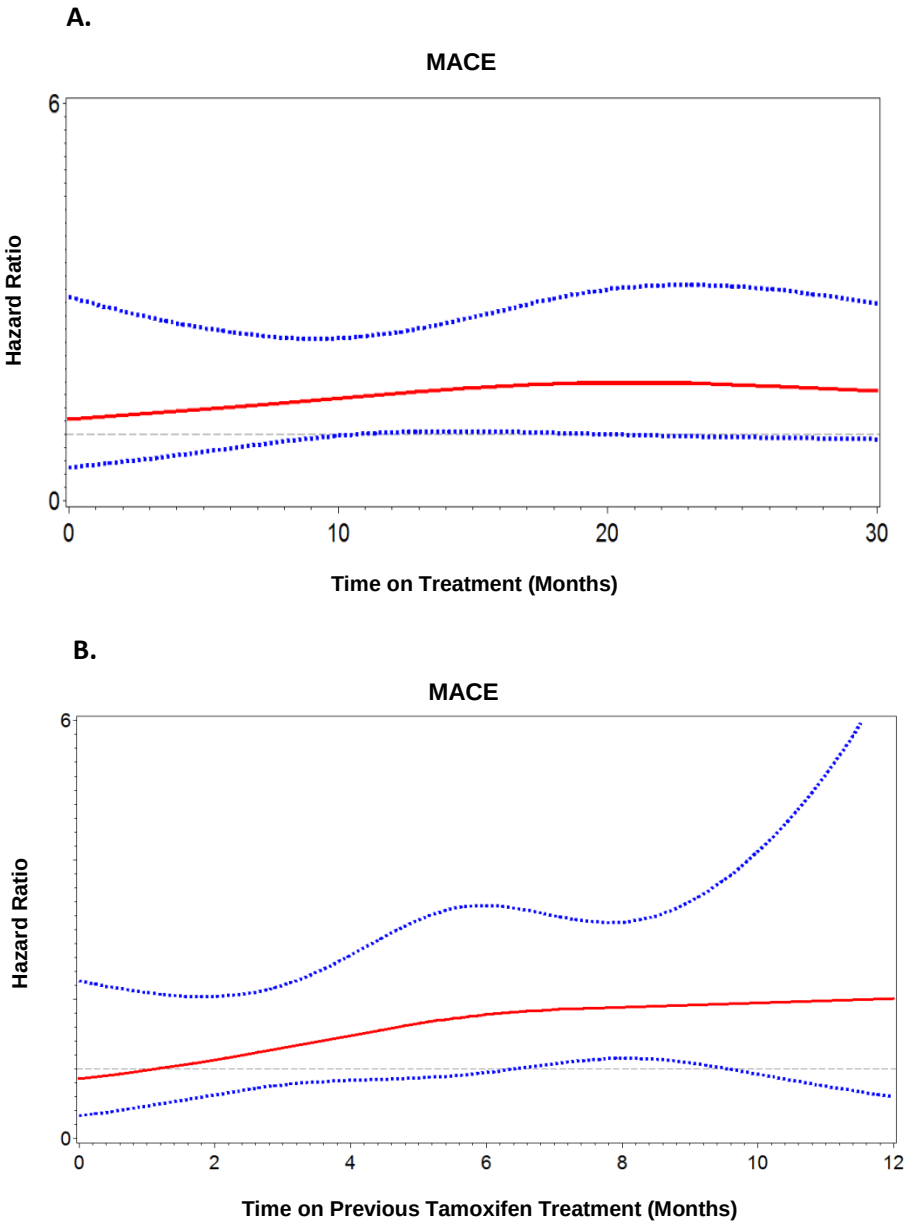


At each 30-day interval, patients who switched to aromatase inhibitors (AIs) were identified (exposed group). In each interval, a patient who switched to AIs was matched to two distinct patients who continued on tamoxifen therapy and received a tamoxifen prescription in the interval and based on propensity score matching using nearest neighbor matching without replacement with a caliper of 0.2 standard deviation of the logit of the propensity score.

Web Figure 6.4 Restricted Cubic Spline of the Hazard Ratio as a Function of Time on Treatment When Comparing Patients Switching to Aromatase Inhibitors with Patients Continuing Tamoxifen Treatment



Web Figure 6.5 Restricted Cubic Spline of the Hazard Ratio for Major Adverse Cardiovascular Events (MACE) as a Function of Time on Treatment (Panel A) and Duration of Previous Tamoxifen Treatment (Panel B) When Comparing Patients Switching to Aromatase Inhibitors with Patients Continuing Tamoxifen Treatment



Chapter 7. Discussion and Conclusions

7.1 Summary of Findings

The overarching aim of this thesis was to determine whether AIs are associated with an increased risk of cardiovascular outcomes in post-menopausal women with breast cancer. Findings from previous meta-analyses of RCTs have been discordant, with some studies indicating that AIs, in comparison with tamoxifen, increase the risk of cardiovascular outcomes in post-menopausal women with breast cancer.[23,26] As a result, organizations such as ASCO indicate ischemic heart disease as a safety concern for anastrozole while the US FDA product label for anastrozole also indicates ischemic heart disease as a safety concern in women with pre-existing heart disease.[10,120] Results from four observational studies have been discordant with one study reporting an increased risk of MI associated with AIs[94] while others studies did not find an increased risk of MI or stroke when comparing AIs with tamoxifen or no treatment.[95-97] However, the interpretation of the results from these studies are limited due to potential sources of biases. The potential cardiovascular safety of AIs is a concern given breast cancer shares common risk factors with cardiovascular disease and post-menopausal women represent a population already at increased risk of cardiovascular disease.[32,143]

The aim of the first objective was to determine the risk of cardiovascular outcomes in RCTs comparing AIs with tamoxifen, AIs with placebo or no treatment in patients previously treated with five years of tamoxifen, and tamoxifen with placebo in post-menopausal women with breast cancer. We conducted a comprehensive systematic review to capture RCTs reporting on cardiovascular outcomes in these setting.[144] Overall, the systematic search identified a total of 19 RCTs assessing efficacy of AIs or tamoxifen in post-menopausal women with early stage breast cancer which reported on cardiovascular outcomes. Consistent with some previous meta-

analyses, we found that AIs were associated with a 19% increased risk of cardiovascular events (RR: 1.19, 95% CI: 1.07-1.34) in RCTs directly comparing AIs with tamoxifen. However, this increased risk was not observed in the extended adjuvant setting when comparing AIs with placebo or no treatment (RR: 1.01, 95% CI: 0.85-1.20). In addition, tamoxifen was associated with a 33% reduction in the risk of cardiovascular outcomes compared with placebo or no treatment (RR: 0.67, 95% CI: 0.45-0.98) in the adjuvant setting and a modest 9% decreased risk in the extended adjuvant setting (RR: 0.91, 95% CI: 0.77-1.07). Consistent results were observed when restricting the outcome definition to ischemic heart disease. Based on results from this study, a scientific statement from the American Heart Association has indicated that the increased risk of cardiovascular outcomes in RCTs comparing AIs with tamoxifen may be secondary to cardioprotective effects of tamoxifen.[32] The results from this study are also consistent with a recent systematic review and meta-analysis of RCTs and observational studies[145] and a previous meta-analysis which have indicated that tamoxifen is associated with decreased risk of cardiovascular events.[146]

The reduction in ischemic heart disease may be related to the effect of tamoxifen on cholesterol levels. Tamoxifen has been associated with an approximately 20% reduction in levels of LDL and total cholesterol within one year of initiation of tamoxifen treatment, with effects persisting to five years when on tamoxifen treatment.[93,106,107,147] Other proposed cardioprotective mechanisms for tamoxifen include anti-inflammatory effects and decrease in C-reactive protein and fibrinogen levels which are strongly associated with cardiovascular disease.[31,111-114] Tamoxifen also has antioxidant properties which may inhibit LDL cholesterol from harmful oxidation.[116,117] Finally, tamoxifen has been shown to have favorable effects on endothelial function by increasing flow-mediated dilation leading to subsequent decrease in carotid intima-

media thickness.[109,118] We did not find an association between AIs and cardiovascular outcomes in RCTs comparing AIs with placebo or no-treatment in extended adjuvant setting after five years of previous treatment with tamoxifen. These results are consistent with a potential rebound effect of tamoxifen after discontinuation where lipid levels have been shown to return to baseline levels after five-years of treatment discontinuation.[110]

The objective of the second manuscript was to compare the risk of cardiovascular outcomes between AIs and tamoxifen in post-menopausal women with breast cancer in setting of routine clinical practice. To address this question, a population-based cohort study was conducted using the UK CPRD linked to the Hospital Episode Statistics and Office for National Statistics. The study population consisted of 8,139 patients newly diagnosed with breast cancer and initiating treatment with AIs and 9,783 post-menopausal with breast cancer initiating treatment with tamoxifen. Patients were followed until cardiovascular outcomes (MI, ischemic stroke, heart failure, or cardiovascular mortality), non-cardiovascular death, treatment discontinuation or switch, end of registration with general practice, or end of the study period (February 29, 2016). We found that AIs, when compared with tamoxifen, were associated with an 86% increased risk of heart failure (HR: 1.86, 95% CI: 1.14-3.03) and 50% increase in cardiovascular-mortality (HR: 1.50, 95% CI: 1.11-2.04). There was also a non-significant increased risk of MI (HR: 1.37, 95% CI: 0.88-2.13) and ischemic stroke (HR: 1.19, 95% CI: 0.82-1.72). Consistent results were found by time since initiation of treatment and across various sensitivity analyses addressing potential limitations such as missing data, exposure and outcome misclassification, and residual confounding. The findings from this observational study were consistent with results from meta-analysis of RCTs in Objective 1 and indicate that AIs, in comparison with tamoxifen, are associated with an increased risk of cardiovascular outcomes in

the real-world setting. The increased risk of heart failure in this study is consistent with higher risk of heart failure associated with letrozole when compared with tamoxifen in the BIG 1-98 trial (letrozole: 26/3975 vs tamoxifen: 13/3988).[101] In this observational study, the risk of heart failure increased within three months after treatment initiation with AIs when compared with tamoxifen.This increased risk may also be related to differential effect of hormonal therapy on serum cholesterol levels. There is evidence that that heart failure may be related to ischemic events and myocardial ischemia has been shown to lead to cardiac remodelling.[148,149] The increased risk of heart failure may also be related to the differential effects of AIs and tamoxifen on hypertension[16,86,150] and endothelial function.[109,118,119,151] However, further studies are required to elucidate these mechanisms.

The aim of the third manuscript was to determine whether AIs in sequential treatment with tamoxifen, when compared with continuous tamoxifen treatment, are associated with increased risk of cardiovascular outcomes. The motivation for this study was based on current clinical guidelines which recommend treatment of post-menopausal women with hormone-receptor positive breast cancer with AIs either as upfront treatment or in sequential setting with tamoxifen.[9-11] These recommendations are based on results from RCTs that have shown similar efficacy when comparing sequential treatment of AIs with tamoxifen compared with upfront treatment with AIs in the adjuvant setting in regards to breast cancer recurrence, breast cancer related mortality and all-cause mortality.[17] The cardiotoxicity of sequential treatment with AIs could be an important consideration in deciding on the optimal treatment strategy for patients with hormone-receptor positive breast cancer. However, there was limited data regarding the risk of cardiovascular outcomes with administration of AIs in the sequential setting with tamoxifen in RCTs. In addition, no observational studies had previously assessed the cardiovascular safety of

this treatment regimen. To address this question, we conducted a retrospective cohort study with a prevalent new-user design where 1,962 patients who switched from tamoxifen to AIs were matched to 3,874 patients continuing on tamoxifen treatment based on previous duration of tamoxifen use and time-conditional propensity scores. Overall, we found an approximately doubling in risk of MI (HR: 2.08, 95% CI: 1.02-4.27) when comparing AIs with tamoxifen and a non-significant increased risk of ischemic stroke (HR: 1.58, 95% CI: 0.85-2.93) and heart failure (HR: 1.69, 95% CI: 0.79-3.62) but not cardiovascular mortality (HR: 0.87, 95% CI: 0.49-1.54). The results from this study are consistent with meta-analysis RCTs in Objective 1, where we found an increased risk of composite cardiovascular outcomes (RR: 1.20, 95% CI: 1.02-1.41) and ischemic heart disease (RR: 1.21, 95% CI: 0.93-1.57) in RCTs comparing sequential treatment with AIs compared with upfront treatment with tamoxifen.[144]

7.2 Strengths and Limitations

This thesis has strengths and limitations. The major overall strength of this thesis is that we synthesized new evidence from RCTs and generated new evidence from observational studies to comprehensively assess the risk of cardiovascular outcomes associated with AIs and tamoxifen in the upfront and sequential setting in post-menopausal women with breast cancer. The studies in this thesis addressed methodological limitations in previous studies systematic review and meta-analyses of RCTs and observational studies to generate high quality evidence regarding the cardiovascular safety of AIs and tamoxifen. Previous systematic reviews and meta-analyses only considered RCTs directly comparing the AIs with tamoxifen and found discordant results.[26,27,93,152-154] Thus, the interpretation of these studies was limited as the underlying assumption was no effect of tamoxifen on cardiovascular outcomes. We conducted the first systematic review and meta-analysis which included results from all RCTs of AIs and tamoxifen

in the adjuvant and extended adjuvant setting in post-menopausal women with breast cancer and which reported on cardiovascular outcomes.When considering the totality of evidence from RCTs, our study demonstrated that tamoxifen is associated with decreased risk of cardiovascular outcomes and there was no increased risk of cardiovascular outcomes observed in RCTs comparing AIs with placebo or no treatment in the extended adjuvant setting. Based on this study, a scientific statement from the American Heart Association has recently indicated that the increased risk of cardiovascular outcomes in RCTs comparing AIs with tamoxifen may be secondary to cardioprotective effects of tamoxifen.[32]

Nevertheless, there were limitations to data from RCTs included in our systematic review. First, there was heterogeneity in definition of cardiovascular and cerebrovascular endpoints between RCTs, although we found similar results with ischemic heart disease as definition of cardiovascular event. There was also heterogeneity between RCTs with respect to duration of follow-up, calendar time of patient recruitment, and patient characteristics. Second, RCTs were designed to assess efficacy as the primary endpoint and not cardiovascular safety and included a patient population with less comorbidities than observed in clinical practice. We were not able to assess the risk of specific cardiovascular outcomes including cardiovascular mortality, which was reported sparsely across trials. Last, there were sparse data regarding cardiovascular safety of sequential treatment strategy with tamoxifen and AIs which has been shown to have similar efficacy to upfront treatment with AIs.[17]

To address the aforementioned gaps in knowledge from RCTs, we conducted the largest observational study to date to directly compare the risk of clinically relevant cardiovascular outcomes between use of AIs and tamoxifen in post-menopausal with breast cancer in Objective 2. In addition, we conducted the first observational study to specifically examine the association

between AIs and cardiovascular outcomes in the sequential setting in Objective 3. The observational studies examined the association between AIs and specific cardiovascular endpoints, including MI, ischemic stroke, heart failure, and cardiovascular mortality. We used the UK HES and ONS databases which have shown to have high specificity for cardiovascular outcomes and thus minimized outcome misclassification.[136,137] In Objective 2, we implemented a new user, active comparator design which eliminated prevalent-user bias and reduced confounding by indication[140,155] while in Objective 3, we applied a rigorous study design where patients who switched to AIs were matched to patients on tamoxifen on duration of previous tamoxifen use and time-conditional propensity scores. Finally, the observational studies were conducted in setting of clinical practice which represents patients treated in real-world setting. Overall, we found consistent results by time since initiation and across many sensitivity analyses addressing potential limitations such as missing data and residual confounding.

The observational studies conducted in Objectives 2 and 3 had limitations. First, we used prescriptions in the CPRD to ascertain exposure. CPRD captures prescriptions issued by GPs which may result in exposure misclassification if AIs or tamoxifen are prescribed by specialists or the patients do not adhere to the prescriptions. However, in the UK, GP are involved in the management and treatment of breast cancer, including the administration of endocrine therapy to post-menopausal women with hormone-receptor positive breast cancer.[133,134] Accordingly, we found that approximately 76% of the study population initiated treatment with either an AI or tamoxifen which is consistent with the prevalence of hormone-receptor positive breast cancer reported in other studies.[156,157] Second, residual confounding is possible given the observational nature of studies in Objective 2 and Objective 3. We included a range of potential confounders in the propensity score model including demographic, lifestyle variables such as smoking,

anthropometric measures including body mass index, comorbidities, cardiovascular history, prescription drugs, and breast-cancer related variables. In addition, in Objective 2, we conducted analyses using high dimensional propensity scores to minimize residual confounding at baseline while marginal structural models were implemented to account for time-varying confounding and informative censoring.

In Objective 3, we found that the patients switching to AIs were similar to patients who remained on tamoxifen therapy in the study population. Thus, switching between tamoxifen and AIs is likely due to physician preference as a treatment strategy rather than due to toxicity of tamoxifen or disease progression. Thus, it is also unlikely that treatment with sequential AIs in comparison with upfront tamoxifen was influenced by baseline cardiovascular risk.[133,134] Nevertheless, in the sequential setting, we also observed an increased risk of cardiovascular outcomes with AIs in comparison with tamoxifen. Finally, some of our secondary analyses had limited power, such as assessing the association with specific AIs and stratification by history of cardiovascular disease. Further large observational studies are required to examine the risk of cardiovascular outcomes by type of AIs and history of cardiovascular disease.

7.3 Implication of findings

Overall, the results from this study suggest that that AIs, in comparison with tamoxifen, are associated with increased risk of cardiovascular outcomes in post-menopausal women with breast cancer. The differential risk of cardiovascular outcomes between AIs and tamoxifen could be weighed with the differences in efficacy and other adverse events of these drugs. An individual patient data meta-analysis demonstrated that five years of treatment with AIs in comparison with five years of tamoxifen is associated with a 20% decreased risk of recurrence (RR: 0.80, 95% CI: 0.73-0.88), 15% decreased risk of breast cancer mortality (RR: 0.85, 95%

CI: 0.75-0.96), and modest decrease in all-cause mortality (RR: 0.89, 95% CI: 0.80-0.97). This corresponded to a risk difference of 3.6% (95% CI: 1.7-5.4%) for breast cancer recurrence, 2.1% (95% CI: 0.5-3.7%) for breast-cancer mortality, and 2.7% (95% CI: 0.1-4.7%) decrease in death from any cause. Some experts have proposed that despite improvements in breast-cancer related outcomes, the differential risk of cardiovascular outcomes may act as a competing risk and account for modest overall survival benefit with AIs.[4,28]

The differential cardiovascular effects of AIs and tamoxifen should also be considered in the context of other adverse events of these drugs. The Early Breast Cancer Trialists' patient-level meta-analysis demonstrated a lower risk of endometrial cancer (10-year incidence: 0.4% vs 1.2%, RR: 0.33: 0.21-0.51) but higher risk of bone fractures (5-year risk: 8.2% vs 5.5%, RR: 1.42, 95% CI: 1.28-1.57) associated with AIs in comparison with tamoxifen.[17] In contrast, tamoxifen has been consistently associated with higher risk of venous thromboembolism in RCTs. In the BIG 1-98 RCT, the rate for thromboembolic events was lower when comparing letrozole with tamoxifen (2.0% vs 3.8%)[85] and similar finding were reported in ATAC trial comparing anastrozole with tamoxifen at approximately 4 years of follow-up (2.2% vs 3.8%).[98] In regards to long-term adverse events, we did not find an association between use of AIs and risk of colorectal cancer in this patient population.[158] There have been mixed findings in regards to use of AIs and risk of Parkinson's disease and a recent study did not find an association between use of AIs and risk of dementia.[158-161]

Overall, the findings of this thesis provide an important addition to the toxicity profile of AIs and tamoxifen. Cardiovascular disease is the leading cause of mortality in women with breast cancer[143] and thus the differential effects of AIs and tamoxifen on cardiovascular outcomes is an important safety consideration. Although the choice of AIs or tamoxifen will

primarily be based on the efficacy for recurrence of breast cancer, the individual patient's risk of cardiovascular disease is an important secondary consideration when deciding on treatment choice between AIs and tamoxifen.[32] The addition of new evidence regarding the differential cardiovascular effects of AIs and tamoxifen could be an important consideration when deciding on the optimal treatment choice for post-menopausal women with hormone-receptor positive breast cancer. Given common risk factors between breast cancer and cardiovascular disease, active monitoring of cardiometabolic biomarkers including lipid levels and interventions on modifiable cardiovascular risk factors may also be part of the management of patients with breast cancer, especially those at high risk of cardiovascular disease.

7.4 Future directions

Overall, we found an increased risk of cardiovascular outcomes in the meta-analysis of published data from RCTs comparing AIs with tamoxifen, albeit this increased risk may be at least partially due to cardioprotective effect of tamoxifen as observed in placebo-controlled RCTs of tamoxifen. In addition, the observational studies conducted suggest that AIs are also associated with increased risk of cardiovascular outcomes in the upfront and sequential setting when compared with tamoxifen in setting of clinical practice. The differential cardiovascular risk of AIs and tamoxifen is an important consideration when deciding on the optimal treatment strategy for post-menopausal women with hormone-receptor positive breast cancer. However, there remain gaps in knowledge. To date, studies have not established the effect of AIs or tamoxifen on cholesterol levels or biomarkers of cardiovascular disease. Evidence from RCTs suggest that tamoxifen decreases levels of LDL and total cholesterol.[93,106,107,147] Nevertheless, there was heterogeneity in reports across trials on the effect of tamoxifen on cholesterol levels and the magnitude of their effects on LDL and total cholesterol.[93] Thus, further well-conducted studies are needed to comprehensively evaluate the long-term effect of AIs and tamoxifen on serum cholesterol levels. One study has suggested that the effect of tamoxifen on cholesterol level may occur through inhibition of enzymes involve in cholesterol metabolism pathway including sterol-Δ8,7 isomerase and Acetyl-Coenzyme A acetyltransferase.[29] Additional research is required to elucidate the mechanism by which tamoxifen may exert its effect on serum lipid levels. In addition, studies are needed to establish the effect of AIs and tamoxifen on markers of cardiovascular disease and endothelial function. Further mechanistic studies are required to evaluate the effect of these drugs on glucose metabolism and body composition which have been associated with cardiovascular disease.[162]

Our observational studies were underpowered to assess the risk of cardiovascular outcomes by history of cardiovascular disease. Thus, larger observational studies are needed to examine the cardiovascular effects of AIs and tamoxifen by baseline risk of cardiovascular disease. Further studies are also needed to compare cardiovascular mortality and all-cause mortality between use of AIs and tamoxifen in post-menopausal women with different underlying risk of breast cancer recurrence and baseline risk of cardiovascular disease. These studies could provide important additional evidence that may guide a tailored treatment strategy for individual patients with hormone receptor-positive breast cancer. Current clinical guidelines by ASCO recommend the extension of treatment with AIs to ten years based on decreased risk of breast cancer recurrence associated with AIs in MA.17 trial.[9,22] However, long-term cardiovascular risk of AIs should also be assessed, specifically given the modest improvement in efficacy outcomes with treatment extension.[17,22] While this thesis fills an important gap with respect to cardiovascular safety of endocrine therapy, more studies are also needed to understand the effects of AIs and tamoxifen on other biological systems including observational studies with long duration of follow-up to examine the long-term safety of AIs and tamoxifen.

7.5 Conclusions

Overall, this thesis provides an important addition regarding the cardiovascular safety of AIs in treatment of hormone-receptor positive breast cancer. Specifically, findings from this thesis suggest that AIs in the upfront setting or in sequential setting with tamoxifen, when compared with upfront tamoxifen treatment, are associated with increased risks of cardiovascular outcomes. However, the results from the systematic review and meta-analysis of RCTs suggest that cardioprotective effects of tamoxifen may at least partially account for the observed increased risk associated with AIs when directly compared with tamoxifen. Although choice of AIs or tamoxifen will primarily be based on the efficacy of these drugs against recurrence of breast cancer and breast-cancer mortality, the individual patient's risk of cardiovascular outcomes could be taken into account when considering the net clinical benefit of these drugs and deciding on the optimal treatment choice for patients diagnosed with hormone receptor-positive breast cancer.[143] Further large observational studies are required to establish the risk of cardiovascular outcomes associated with AIs by baseline risk of cardiovascular disease. Given that cardiovascular disease is the leading cause of mortality in women with breast cancer,[32] further studies are also required to examine the association between AIs and risk of all-cause mortality in patients with different underlying risks of breast cancer recurrence and cardiovascular disease. These studies can provide evidence that will inform a tailored treatment strategy for post-menopausal women with hormone receptor-positive breast cancer.

8. List of References

1. International Agency for Research On Cancer WHO. World Population Fact Sheets 2018; https://gco.iarc.fr/today/data/factsheets/populations/900-world-fact-sheets.pdf.

2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2018;68(6):394-424.

3. Clemons M, Goss P. Estrogen and the risk of breast cancer. *N EnglJMed.* 2001;344(4):276-285.

4. Lonning PE, Eikesdal HP. Aromatase inhibition 2013: clinical state of the art and questions that remain to be solved. *EndocrRelat Cancer.* 2013;20(4):R183-R201.

5. Kelly E, Lu CY, Albertini S, Vitry A. Longitudinal trends in utilization of endocrine therapies for breast cancer: an international comparison. *Journal of clinical pharmacy and therapeutics.* 2015;40(1):76-82.

6. Alberta Health Services. *Aromatase Inhibitors as Adjuvant Therapy in Post-Menopausal Women with Early-Stage Hormone Receptor Positive Breast Cancer.* 2011.

7. Cancer Care Ontario. *The Role of Aromatase Inhibitors in Adjuvant Therapy for Postmenopausal Women with Hormone Receptor-positive Breast Cancer.* 2012.

8. Kennecke HF, Ellard S, O'Reilly S, Glemon KA. New guidelines for treatment of early hormone-positive breast cancer with tamoxifen and aromatase inhibitors. *BCMJ.* 2006;48(3):121-126.

9. Burstein HJ, Lacchetti C, Anderson H, et al. Adjuvant Endocrine Therapy for Women With Hormone Receptor-Positive Breast Cancer: ASCO Clinical Practice Guideline Focused Update. *J Clin Oncol.* 2018:JCO1801160.

10. Burstein HJ, Temin S, Anderson H, et al. Adjuvant endocrine therapy for women with hormone receptor-positive breast cancer: american society of clinical oncology clinical practice guideline focused update. *J Clin Oncol.* 2014;32(21):2255-2269.

11. Cardoso F, Kyriakides S, Ohno S, et al. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol.* 2019.

12. Forbes JF, Cuzick J, Buzdar A, Howell A, Tobias JS, Baum M. Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 100-month analysis of the ATAC trial. *Lancet Oncol.* 2008;9(1):45-53.

13. Colleoni M, Giobbie-Hurder A, Regan MM, et al. Analyses adjusting for selective crossover show improved overall survival with adjuvant letrozole compared with tamoxifen in the BIG 1-98 study. *J Clin Oncol.* 2011;29(9):1117-1124.

14. Thurlimann B, Keshaviah A, Coates AS, et al. A comparison of letrozole and tamoxifen in postmenopausal women with early breast cancer. *New England Journal of Medicine.* 2005;353(26):2747-2757.

15. Jakesz R, Jonat W, Gnant M, et al. Switching of postmenopausal women with endocrine-responsive early breast cancer to anastrozole after 2 years' adjuvant tamoxifen: combined results of ABCSG trial 8 and ARNO 95 trial. *Lancet.* 2005;366(9484):455-462.

16. Coombes RC, Kilburn LS, Snowdon CF, et al. Survival and safety of exemestane versus tamoxifen after 2-3 years' tamoxifen treatment (Intergroup Exemestane Study): a randomised controlled trial. *Lancet.* 2007;369(9561):559-570.

17. Early Breast Cancer Trialists' Collaborative G, Dowsett M, Forbes JF, et al. Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. *Lancet.* 2015;386(10001):1341-1352.

18. Davies C, Pan H, Godwin J, et al. Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial. *The Lancet.* 2013;381(9869):805-816.

19. McDonald CC, Alexander FE, Whyte BW, Forrest AP, Stewart HJ. Cardiac and vascular morbidity in women receiving adjuvant tamoxifen for breast cancer in a randomised trial. The Scottish Cancer Trials Breast Group. *BMJ.* 1995;311(7011):977-980.

20. Tamoxifen for early breast cancer: an overview of the randomised trials. Early Breast Cancer Trialists' Collaborative Group. *Lancet.* 1998;351(9114):1451-1467.

21. van Leeuwen FE, Benraadt J, Coebergh JW, et al. Risk of endometrial cancer after tamoxifen treatment of breast cancer. *Lancet.* 1994;343(8895):448-452.

22. Goss PE, Ingle JN, Pritchard KI, et al. Extending Aromatase-Inhibitor Adjuvant Therapy to 10 Years. *N Engl J Med.* 2016.

23. Amir E, Seruga B, Niraula S, Carlsson L, Ocana A. Toxicity of adjuvant endocrine therapy in postmenopausal breast cancer patients: a systematic review and meta-analysis. *J Natl Cancer Inst.* 2011;103(17):1299-1309.

24. Goss PE, Ingle JN, Martino S, et al. Randomized trial of letrozole following tamoxifen as extended adjuvant therapy in receptor-positive breast cancer: updated findings from NCIC CTG MA.17. *J Natl Cancer Inst.* 2005;97(17):1262-1271.

25. Bliss JM, Kilburn LS, Coleman RE, et al. Disease-related outcomes with long-term follow-up: an updated analysis of the intergroup exemestane study. *J Clin Oncol.* 2012;30(7):709-717.

26. Aydiner A. Meta-analysis of breast cancer outcome and toxicity in adjuvant trials of aromatase inhibitors in postmenopausal women. *Breast.* 2013;22(2):121-129.

27. Cuppone F, Bria E, Verma S, et al. Do adjuvant aromatase inhibitors increase the cardiovascular risk in postmenopausal women with early breast cancer? Meta-analysis of randomized trials. *Cancer.* 2008;112(2):260-267.

28. Foglietta J, Inno A, de Iuliis F, et al. Cardiotoxicity of Aromatase Inhibitors in Breast Cancer Patients. *Clinical breast cancer.* 2016.

29. Grainger DJ, Schofield PM. Tamoxifen for the prevention of myocardial infarction in humans: preclinical and early clinical evidence. *Circulation.* 2005;112(19):3018-3024.

30. Guetta V, Lush RM, Figg WD, Waclawiw MA, Cannon RO, 3rd. Effects of the antiestrogen tamoxifen on low-density lipoprotein concentrations and oxidation in postmenopausal women. *Am J Cardiol.* 1995;76(14):1072-1073.

31. Love RR, Wiebe DA, Feyzi JM, Newcomb PA, Chappell RJ. Effects of tamoxifen on cardiovascular risk factors in postmenopausal women after 5 years of treatment. *J Natl Cancer Inst.* 1994;86(20):1534-1539.

32. Mehta LS, Watson KE, Barac A, et al. Cardiovascular Disease and Breast Cancer: Where These Entities Intersect: A Scientific Statement From the American Heart Association. *Circulation.* 2018;137(8):e30-e66.

33. Canadian Breast Cancer Foundation. Breast Cancer Statistics. 2015; http://www.cbcf.org/central/AboutBreastCancerMain/FactsStats/Pages/Breast-Cancer-Canada.aspx.

34. Canadian Cancer Society. Survival statistics for breast cancer https://www.cancer.ca/en/cancer-information/cancer-type/breast/prognosis-and-survival/survival-statistics/?region=on.

35. Winters S, Martin C, Murphy D, Shokar NK. Breast Cancer Epidemiology, Prevention, and Screening. *Prog Mol Biol Transl Sci.* 2017;151:1-32.

36. DeSantis CE, Ma J, Goding Sauer A, Newman LA, Jemal A. Breast cancer statistics, 2017, racial disparity in mortality by state. *CA Cancer J Clin.* 2017;67(6):439-448.

37. Collaborative Group on Hormonal Factors in Breast C. Menarche, menopause, and breast cancer risk: individual participant meta-analysis, including 118 964 women with breast cancer from 117 epidemiological studies. *Lancet Oncol.* 2012;13(11):1141-1151.

38. Ahern TP, Sprague BL, Bissell MCS, et al. Family History of Breast Cancer, Breast Density, and Breast Cancer Risk in a U.S. Breast Cancer Screening Population. *Cancer Epidemiol Biomarkers Prev.* 2017;26(6):938-944.

39. Visser LL, Elshof LE, Schaapveld M, et al. Clinicopathological Risk Factors for an Invasive Breast Cancer Recurrence after Ductal Carcinoma In Situ-A Nested Case-Control Study. *Clin Cancer Res.* 2018;24(15):3593-3601.

40. Yue W, Wang JP, Li Y, et al. Effects of estrogen on breast cancer development: Role of estrogen receptor independent mechanisms. *Int J Cancer.* 2010;127(8):1748-1757.

41. Thomas HV, Reeves GK, Key TJ. Endogenous estrogen and postmenopausal breast cancer: a quantitative review. *Cancer Causes Control.* 1997;8(6):922-928.

42. Christopoulos PF, Msaouel P, Koutsilieris M. The role of the insulin-like growth factor-1 system in breast cancer. *Mol Cancer.* 2015;14:43.

43. Wang M, Wu X, Chai F, Zhang Y, Jiang J. Plasma prolactin and breast cancer risk: a meta-analysis. *Sci Rep.* 2016;6:25998.

44. Lambe M, Hsieh CC, Chan HW, Ekbom A, Trichopoulos D, Adami HO. Parity, age at first and last birth, and risk of breast cancer: a population-based study in Sweden. *Breast Cancer Res Treat.* 1996;38(3):305-311.

45. Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. Collaborative Group on Hormonal Factors in Breast Cancer. *Lancet.* 1997;350(9084):1047-1059.

46. Hunter DJ, Spiegelman D, Adami HO, et al. Non-dietary factors as risk factors for breast cancer, and as effect modifiers of the association of fat intake and risk of breast cancer. *Cancer Causes Control.* 1997;8(1):49-56.

47. Victora CG, Bahl R, Barros AJ, et al. Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. *Lancet.* 2016;387(10017):475-490.

48. Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50302 women with breast cancer and 96973 women without the disease. *Lancet.* 2002;360(9328):187-195.

49. Dieterich M, Stubert J, Reimer T, Erickson N, Berling A. Influence of lifestyle factors on breast cancer risk. *Breast Care (Basel).* 2014;9(6):407-414.

50. Manson JE, Chlebowski RT, Stefanick ML, et al. Menopausal hormone therapy and health outcomes during the intervention and extended poststopping phases of the Women's Health Initiative randomized trials. *JAMA.* 2013;310(13):1353-1368.

51. Pijpe A, Andrieu N, Easton DF, et al. Exposure to diagnostic radiation and risk of breast cancer among carriers of BRCA1/2 mutations: retrospective cohort study (GENE-RAD-RISK). *BMJ.* 2012;345:e5660.

52. Klarenbach S, Sims-Jones N, Lewin G, et al. Recommendations on screening for breast cancer in women aged 40-74 years who are not at increased risk for breast cancer. *CMAJ.* 2018;190(49):E1441-E1451.

53. Myers ER, Moorman P, Gierisch JM, et al. Benefits and Harms of Breast Cancer Screening: A Systematic Review. *JAMA.* 2015;314(15):1615-1634.

54. McDonald ES, Clark AS, Tchou J, Zhang P, Freedman GM. Clinical Diagnosis and Management of Breast Cancer. *J Nucl Med.* 2016;57 Suppl 1:9S-16S.

55. Amin MB, Greene FL, Edge SB, et al. The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. *CA Cancer J Clin.* 2017;67(2):93-99.

56. American Cancer Society. Cancer Facts and Figures 2019. https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2019.html.

57. Rakha EA, Reis-Filho JS, Baehner F, et al. Breast cancer prognostic classification in the molecular era: the role of histological grade. *Breast Cancer Res.* 2010;12(4):207.

58. American Cancer Society. Invasive Breast Cancer (IDC/ILC). https://www.cancer.org/cancer/breast-cancer/understanding-a-breast-cancer-diagnosis/types-of-breast-cancer/invasive-breast-cancer.html.

59. Li CI. Risk of mortality by histologic type of breast cancer in the United States. *Horm Cancer.* 2010;1(3):156-165.

60. Li CI, Moe RE, Daling JR. Risk of mortality by histologic type of breast cancer among women aged 50 to 79 years. *Arch Intern Med.* 2003;163(18):2149-2153.

61. Makki J. Diversity of Breast Carcinoma: Histological Subtypes and Clinical Relevance. *Clin Med Insights Pathol.* 2015;8:23-31.

62. Yager JD, Davidson NE. Estrogen carcinogenesis in breast cancer. *NEnglJMed.* 2006;354(3):270-282.

63. Edwards DP. Regulation of signal transduction pathways by estrogen and progesterone. *Annu Rev Physiol.* 2005;67:335-376.

64. Yue W, Yager JD, Wang JP, Jupe ER, Santen RJ. Estrogen receptor-dependent and independent mechanisms of breast cancer carcinogenesis. *Steroids.* 2013;78(2):161-170.

65. Chumsri S, Howes T, Bao T, Sabnis G, Brodie A. Aromatase, aromatase inhibitors, and breast cancer. *JSteroid BiochemMolBiol.* 2011;125(1-2):13-22.

66. Iqbal N, Iqbal N. Human Epidermal Growth Factor Receptor 2 (HER2) in Cancers: Overexpression and Therapeutic Implications. *Mol Biol Int.* 2014;2014:852748.

67. Howlader N, Altekruse SF, Li CI, et al. US incidence of breast cancer subtypes defined by joint hormone receptor and HER2 status. *J Natl Cancer Inst.* 2014;106(5).

68. Dai X, Xiang L, Li T, Bai Z. Cancer Hallmarks, Biomarkers and Breast Cancer Molecular Subtypes. *J Cancer.* 2016;7(10):1281-1294.

69. Ruddy KJ, Ganz PA. Treatment of Nonmetastatic Breast Cancer. *JAMA.* 2019;321(17):1716-1717

70. Waks AG, Winer EP. Breast Cancer Treatment: A Review. *JAMA.* 2019;321(3):288-300.

71. Early Breast Cancer Trialists' Collaborative G, Darby S, McGale P, et al. Effect of radiotherapy after breast-conserving surgery on 10-year recurrence and 15-year breast cancer death: meta-analysis of individual patient data for 10,801 women in 17 randomised trials. *Lancet.* 2011;378(9804):1707-1716.

72. Fisher B, Anderson S, Bryant J, et al. Twenty-year follow-up of a randomized trial comparing total mastectomy, lumpectomy, and lumpectomy plus irradiation for the treatment of invasive breast cancer. *N Engl J Med.* 2002;347(16):1233-1241.

73. Denduluri N, Chavez-MacGregor M, Telli ML, et al. Selection of Optimal Adjuvant Chemotherapy and Targeted Therapy for Early Breast Cancer: ASCO Clinical Practice Guideline Focused Update. *J Clin Oncol.* 2018;36(23):2433-2443.

74. Denduluri N, Somerfield MR, Giordano SH. Selection of Optimal Adjuvant Chemotherapy and Targeted Therapy for Early Breast Cancer: ASCO Clinical Practice Guideline Focused Update Summary. *J Oncol Pract.* 2018;14(8):508-510.

75. Early Breast Cancer Trialists' Collaborative G, Peto R, Davies C, et al. Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. *Lancet.* 2012;379(9814):432-444.

76. Gianni L, Dafni U, Gelber RD, et al. Treatment with trastuzumab for 1 year after adjuvant chemotherapy in patients with HER2-positive early breast cancer: a 4-year follow-up of a randomised controlled trial. *Lancet Oncol.* 2011;12(3):236-244.

77. von Minckwitz G, Procter M, de Azambuja E, et al. Adjuvant Pertuzumab and Trastuzumab in Early HER2-Positive Breast Cancer. *N Engl J Med.* 2017;377(2):122-131.

78. Burstein HJ, Prestrud AA, Seidenfeld J, et al. American Society of Clinical Oncology clinical practice guideline: update on adjuvant endocrine therapy for women with hormone receptor-positive breast cancer. *J Clin Oncol.* 2010;28(23):3784-3796.

79. Buzdar AU, Hortobagyi G. Update on endocrine therapy for breast cancer. *Clin Cancer Res.* 1998;4(3):527-534.

80. Buzdar AU. Pharmacology and pharmacokinetics of the newer generation aromatase inhibitors. *Clin Cancer Res.* 2003;9(1 Pt 2):468S-472S.

81. U.S. Food and Drug Administration. Novaldex Tamoxifen Citrate. https://www.accessdata.fda.gov/drugsatfda_docs/label/1998/17970.pdf.

82. Bosco-Levy P, Jove J, Robinson P, Moore N, Fourrier-Reglat A, Bezin J. Persistence to 5-year hormonal breast cancer therapy: a French national population-based study. *British journal of cancer*. 2016.

83. Hadji P, Ziller V, Kyvernitakis J, et al. Persistence in patients with breast cancer treated with tamoxifen or aromatase inhibitors: a retrospective database analysis. *Breast Cancer Res Treat.* 2013;138(1):185-191.

84. Huiart L, Dell'Aniello S, Suissa S. Use of tamoxifen and aromatase inhibitors in a large population-based cohort of women with breast cancer. *British journal of cancer.* 2011;104(10):1558-1563.

85. Coates AS, Keshaviah A, Thurlimann B, et al. Five years of letrozole compared with tamoxifen as initial adjuvant therapy for postmenopausal women with endocrine-responsive early breast cancer: update of study BIG 1-98. *J Clin Oncol.* 2007;25(5):486-492.

86. Cuzick J, Sestak I, Baum M, et al. Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 10-year analysis of the ATAC trial. *Lancet Oncol.* 2010;11(12):1135-1141.

87. Smith I, Yardley D, Burris H, et al. Comparative Efficacy and Safety of Adjuvant Letrozole Versus Anastrozole in Postmenopausal Patients With Hormone Receptor-Positive, Node-Positive Early Breast Cancer: Final Results of the Randomized Phase III Femara Versus Anastrozole Clinical Evaluation (FACE) Trial. *J Clin Oncol.* 2017;35(10):1041-1048.

88. Goss PE, Ingle JN, Pritchard KI, et al. Exemestane versus anastrozole in postmenopausal women with early breast cancer: NCIC CTG MA.27--a randomized controlled phase III trial. *J Clin Oncol.* 2013;31(11):1398-1404.

89. Goldvaser H, Barnes TA, Seruga B, et al. Toxicity of Extended Adjuvant Therapy With Aromatase Inhibitors in Early Breast Cancer: A Systematic Review and Meta-analysis. *J Natl Cancer Inst.* 2018;110(1).

90. Baum M, Budzar AU, Cuzick J, et al. Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early breast cancer: first results of the ATAC randomised trial. *Lancet.* 2002;359(9324):2131-2139.

91. Buzdar A, Howell A, Cuzick J, et al. Comprehensive side-effect profile of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: long-term safety analysis of the ATAC trial. *Lancet Oncol.* 2006;7(8):633-643.

92. Goss PE, Ingle JN, Martino S, et al. A randomized trial of letrozole in postmenopausal women after five years of tamoxifen therapy for early-stage breast cancer. *NEnglJMed.* 2003;349(19):1793-1802.

93. Younus M, Kissner M, Reich L, Wallis N. Putting the cardiovascular safety of aromatase inhibitors in patients with early breast cancer into perspective: a systematic review of the literature. *Drug Saf.* 2011;34(12):1125-1149.

94. Abdel-Qadir H, Amir E, Fischer HD, et al. The risk of myocardial infarction with aromatase inhibitors relative to tamoxifen in post-menopausal women with early stage breast cancer. *European journal of cancer.* 2016;68:11-21.

95. Haque R, Shi J, Schottinger JE, et al. Cardiovascular Disease After Aromatase Inhibitor Use. *JAMA Oncol.* 2016.

96. Kamaraju S, Shi Y, Smith E, Nattinger AB, Laud P, Neuner J. Are aromatase inhibitors associated with higher myocardial infarction risk in breast cancer patients? A Medicare population-based study. *Clinical cardiology.* 2018.

97. Ligibel JA, James OMA, Fisher M, Daniel GW, Winer EP, Keating NL. Risk of myocardial infarction, stroke, and fracture in a cohort of community-based breast cancer patients. *Breast Cancer ResTreat.* 2012;131(2):589-597.

98. Baum M, Buzdar A, Cuzick J, et al. Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early-stage breast cancer: results of the ATAC (Arimidex, Tamoxifen Alone or in Combination) trial efficacy and safety update analyses. *Cancer.* 2003;98(9):1802-1810.

99. Boccardo F, Rubagotti A, Guglielmini P, et al. Switching to anastrozole versus continued tamoxifen treatment of early breast cancer. Updated results of the Italian tamoxifen anastrozole (ITA) trial. *Ann Oncol.* 2006;17 Suppl 7:vii10-14.

100. Boccardo F, Rubagotti A, Guglielmini P, et al. Switching to anastrozole versus continued tamoxifen treatment of early breast cancer. Updated results of the Italian tamoxifen anastrozole (ITA) trial. *Ann Oncol.* 2006;17 Suppl 7:vii10-vii14.

101. Mouridsen H, Keshaviah A, Coates AS, et al. Cardiovascular adverse events during adjuvant endocrine therapy for early breast cancer using letrozole or tamoxifen: safety analysis of BIG 1-98 trial. *J Clin Oncol.* 2007;25(36):5715-5722.

102. Markopoulos C, Chrissochou M, Michailidou A, et al. Effect of exemestane on the lipidemic profile of post-menopausal operable breast cancer patients following 5-7 years of adjuvant tamoxifen: preliminary results of the ATENA substudy. *Anticancer Drugs.* 2005;16(8):879-883.

103. Markopoulos C, Polychronis A, Zobolas V, et al. The effect of exemestane on the lipidemic profile of postmenopausal early breast cancer patients: preliminary results of the TEAM Greek sub-study. *Breast Cancer Res Treat.* 2005;93(1):61-66.

104. Hozumi Y, Suemasu K, Takei H, et al. The effect of exemestane, anastrozole, and tamoxifen on lipid profiles in Japanese postmenopausal early breast cancer patients: final

results of National Surgical Adjuvant Study BC 04, the TEAM Japan sub-study. *Ann Oncol.* 2011;22(8):1777-1782.

105. Wasan KM, Goss PE, Pritchard PH, et al. The influence of letrozole on serum lipid concentrations in postmenopausal women with primary breast cancer who have completed 5 years of adjuvant tamoxifen (NCIC CTG MA.17L). *Ann Oncol.* 2005;16(5):707-715.

106. Love RR, Newcomb PA, Wiebe DA, et al. Effects of tamoxifen therapy on lipid and lipoprotein levels in postmenopausal patients with node-negative breast cancer. *J Natl Cancer Inst.* 1990;82(16):1327-1332.

107. Dewar JA, Horobin JM, Preece PE, Tavendale R, Tunstall-Pedoe H, Wood RA. Long term effects of tamoxifen on blood lipid values in breast cancer. *BMJ.* 1992;305(6847):225-226.

108. Sahebkar A, Serban MC, Penson P, et al. The Effects of Tamoxifen on Plasma Lipoprotein(a) Concentrations: Systematic Review and Meta-Analysis. *Drugs.* 2017;77(11):1187-1197.

109. Stamatelopoulos KS, Lekakis JP, Poulakaki NA, et al. Tamoxifen improves endothelial function and reduces carotid intima-media thickness in postmenopausal women. *American heart journal.* 2004;147(6):1093-1099.

110. Markopoulos C, Chrissochou M, Antonopoulou Z, et al. Duration of tamoxifen effect on lipidemic profile of postmenopausal breast cancer patients following deprivation of treatment. *Oncology.* 2006;70(4):301-305.

111. Bonanni B, Johansson H, Gandini S, et al. Effect of tamoxifen at low doses on ultrasensitive C-reactive protein in healthy women. *J Thromb Haemost.* 2003;1(10):2149-2152.

112. Cushman M, Costantino JP, Tracy RP, et al. Tamoxifen and cardiac risk factors in healthy women: Suggestion of an anti-inflammatory effect. *Arterioscler Thromb Vasc Biol.* 2001;21(2):255-261.

113. Stec JJ, Silbershatz H, Tofler GH, et al. Association of fibrinogen with cardiovascular risk factors and cardiovascular disease in the Framingham Offspring Population. *Circulation.* 2000;102(14):1634-1638.

114. Emerging Risk Factors Collaboration, Kaptoge S, Di Angelantonio E, et al. C-reactive protein, fibrinogen, and cardiovascular disease prediction. *N Engl J Med.* 2012;367(14):1310-1320.

115. Grainger DJ, Witchell CM, Metcalfe JC. Tamoxifen elevates transforming growth factor-beta and suppresses diet-induced formation of lipid lesions in mouse aorta. *Nat Med.* 1995;1(10):1067-1073.

116. Wiseman H. Tamoxifen as an antioxidant and cardioprotectant. *Biochem Soc Symp.* 1995;61:209-219.

117. Wiseman H, Laughton MJ, Arnstein HR, Cannon M, Halliwell B. The antioxidant action of tamoxifen and its metabolites. Inhibition of lipid peroxidation. *FEBS Lett.* 1990;263(2):192-194.

118. Clarke SC, Schofield PM, Grace AA, Metcalfe JC, Kirschenlohr HL. Tamoxifen effects on endothelial function and cardiovascular risk factors in men with advanced atherosclerosis. *Circulation.* 2001;103(11):1497-1502.

119. Maor R, Sara JDS, Wanous AA, et al. Attenuated peripheral endothelial function among women treated with aromatase inhibitors for breast cancer. *Coronary artery disease.* 2018;29(8):687-693.

120. U.S. Food and Drug Administration. Arimidex (anastrozole) Table Label. 2011.

121. Eisen A, Fletcher GG, Gandhi S, et al. Optimal systemic therapy for early breast cancer in women: a clinical practice guideline. *Current oncology.* 2015;22(Suppl 1):S67-81.

122. European Medicines Agency. Arimidex-Article 30 referral-Annex III. 2011; https://www.ema.europa.eu/en/medicines/human/referrals/arimidex.

123. European Medicines Agency. Femara-Article 30 referral-Assessment Report. 2012; https://www.ema.europa.eu/en/documents/referral/femara-article-30-referral-assessment-report_en.pdf

124. Herrett E, Gallagher AM, Bhaskaran K, et al. Data Resource Profile: Clinical Practice Research Datalink (CPRD). *International journal of epidemiology.* 2015;44(3):827-836.

125. Chisholm J. The Read clinical classification. *BMJ.* 1990;300(6732):1092.

126. Gillam SJ, Siriwardena AN, Steel N. Pay-for-performance in the United Kingdom: impact of the quality and outcomes framework: a systematic review. *Ann Fam Med.* 2012;10(5):461-468.

127. Herrett E, Thomas SL, Schoonen WM, Smeeth L, Hall AJ. Validation and validity of diagnoses in the General Practice Research Database: a systematic review. *British journal of clinical pharmacology.* 2010;69(1):4-14.

128. Khan NF, Harrison SE, Rose PW. Validity of diagnostic coding within the General Practice Research Database: a systematic review. *The British journal of general practice : the journal of the Royal College of General Practitioners.* 2010;60(572):e128-136.

129. Dregan A, Moller H, Murray-Thomas T, Gulliford MC. Validity of cancer diagnosis in a primary care database compared with linked cancer registrations in England. Population-based cohort study. *Cancer epidemiology.* 2012;36(5):425-429.

130. Arhi CS, Bottle A, Burns EM, et al. Comparison of cancer diagnosis recording between the Clinical Practice Research Datalink, Cancer Registry and Hospital Episodes Statistics. *Cancer epidemiology.* 2018;57:148-157.

131. Boggon R, van Staa TP, Chapman M, Gallagher AM, Hammad TA, Richards MA. Cancer recording and mortality in the General Practice Research Database and linked cancer registries. *Pharmacoepidemiol Drug Saf.* 2013;22(2):168-175.

132. Margulis AV, Fortuny J, Kaye JA, et al. Validation of Cancer Cases Using Primary Care, Cancer Registry, and Hospitalization Data in the United Kingdom. *Epidemiology.* 2018;29(2):308-313.

133. National Institute for Clinical Excellence. Improving Outcomes in Breast Cancer. 2002; www.nice.org.uk/guidance/csg1/resources/improving-outcomes-in-breast-cancer-update-pdf-773371117.

134. National Institute for Health and Care Excellence. Early and locally advanced breast cancer: diagnosis and management. 2018; https://www.nice.org.uk/guidance/ng101/chapter/Recommendations#endocrine-therapy.

135. Hospital Episode Statistics. https://digital.nhs.uk/data-and-information/data-tools-and-services/data-services/hospital-episode-statistics.

136. Herrett E, Shah AD, Boggon R, et al. Completeness and diagnostic validity of recording acute myocardial infarction events in primary care, hospital care, disease registry, and national mortality records: cohort study. *BMJ.* 2013;346:f2350.

137. Kivimaki M, Batty GD, Singh-Manoux A, Britton A, Brunner EJ, Shipley MJ. Validity of Cardiovascular Disease Event Ascertainment Using Linkage to UK Hospital Records. *Epidemiology.* 2017;28(5):735-739.

138. Office for National Statistics. https://www.ons.gov.uk/aboutus/whatwedo.

139. UK Data Service. 2011 UK Townsend Deprivation Scores. https://www.statistics.digitalresources.jisc.ac.uk/dataset/2011-uk-townsend-deprivation-scores.

140. Ray WA. Evaluating medication effects outside of clinical trials: new-user designs. *Am J Epidemiol.* 2003;158(9):915-920.

141. Nakasian SS, Rassen JA, Franklin JM. Effects of expanding the look-back period to all available data in the assessment of covariates. *Pharmacoepidemiol Drug Saf.* 2017;26(8):890-899.

142. Durrleman S, Simon R. Flexible regression models with cubic splines. *Stat Med.* 1989;8(5):551-561.

143. Mehta PA, Cowie MR. Gender and heart failure: a population perspective. *Heart.* 2006;92 Suppl 3:iii14-18.

144. Khosrow-Khavar F, Filion KB, Al-Qurashi S, et al. Cardiotoxicity of Aromatase Inhibitors and Tamoxifen in Post-Menopausal Women with Breast Cancer: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Ann Oncol.* 2016.

145. Matthews A, Stanway S, Farmer RE, et al. Long term adjuvant endocrine therapy and risk of cardiovascular disease in female breast cancer survivors: systematic review. *BMJ.* 2018;363:k3845.

146. Braithwaite RS, Chlebowski RT, Lau J, George S, Hess R, Col NF. Meta-analysis of vascular and neoplastic events associated with tamoxifen. *Journal of general internal medicine.* 2003;18(11):937-947.

147. Sawada S, Sato K, Kusuhara M, et al. Effect of anastrozole and tamoxifen on lipid metabolism in Japanese postmenopausal women with early breast cancer. *Acta oncologica.* 2005;44(2):134-141.

148. Remme WJ. Overview of the relationship between ischemia and congestive heart failure. *Clinical cardiology.* 2000;23(7 Suppl 4):IV4-8.

149. Cleland JG, McGowan J. Heart failure due to ischaemic heart disease: epidemiology, pathophysiology and progression. *J Cardiovasc Pharmacol.* 1999;33 Suppl 3:S17-29.

150. Messerli FH, Rimoldi SF, Bangalore S. The Transition From Hypertension to Heart Failure: Contemporary Update. *JACC Heart Fail.* 2017;5(8):543-551.

151. Blaes A, Beckwith H, Florea N, et al. Vascular function in breast cancer survivors on aromatase inhibitors: a pilot study. *Breast Cancer Res Treat.* 2017;166(2):541-547.

152. Amir E, Seruga B, Niraula S, Carlsson L, Ocana A. Toxicity of adjuvant endocrine therapy in postmenopausal breast cancer patients: a systematic review and meta-analysis. *J Natl Cancer Inst.* 2011;103(17):1299-1309.

153. Josefsson ML, Leinster SJ. Aromatase inhibitors versus tamoxifen as adjuvant hormonal therapy for oestrogen sensitive early breast cancer in post-menopausal women: meta-analyses of monotherapy, sequenced therapy and extended therapy. *Breast.* 2010;19(2):76-83.

154. Xu HB, Liu YJ, Li L. Aromatase inhibitor versus tamoxifen in postmenopausal woman with advanced breast cancer: a literature-based meta-analysis. *Clinical breast cancer.* 2011;11(4):246-251.

155. Lund JL, Richardson DB, Sturmer T. The active comparator, new user study design in pharmacoepidemiology: historical foundations and contemporary application. *Current epidemiology reports.* 2015;2(4):221-228.

156. Kohler BA, Sherman RL, Howlader N, et al. Annual Report to the Nation on the Status of Cancer, 1975-2011, Featuring Incidence of Breast Cancer Subtypes by Race/Ethnicity, Poverty, and State. *J Natl Cancer Inst.* 2015;107(6):djv048.

157. Lumachi F, Santeufemia DA, Basso SM. Current medical treatment of estrogen receptor-positive breast cancer. *World J Biol Chem.* 2015;6(3):231-239.

158. Khosrow-Khavar F, Yin H, Barkun A, Bouganim N, Azoulay L. Aromatase inhibitors and the risk of colorectal cancer in postmenopausal women with breast cancer. *Ann Oncol.* 2018;29(3):744-748.

159. Bromley SE, Matthews A, Smeeth L, Stanway S, Bhaskaran K. Risk of dementia among postmenopausal breast cancer survivors treated with aromatase inhibitors versus tamoxifen: a cohort study using primary care data from the UK. *J Cancer Surviv.* 2019;13(4):632-640.

160. Latourelle JC, Dybdahl M, Destefano AL, Myers RH, Lash TL. Risk of Parkinson's disease after tamoxifen treatment. *BMC neurology.* 2010;10:23.

161. Lin HF, Liao KF, Chang CM, Lin CL, Lai SW. Tamoxifen usage correlates with increased risk of Parkinson's disease in older women with breast cancer: a case-control study in Taiwan. *Eur J Clin Pharmacol.* 2018;74(1):99-107.

162. Cheung YM, Ramchand SK, Yeo B, Grossmann M. Cardiometabolic Effects of Endocrine Treatment of Estrogen Receptor-Positive Early Breast Cancer. *J Endocr Soc.* 2019;3(7):1283-1301.

Appendix

Certificates of Ethical Approval

Manuscript 2: Aromatase Inhibitors and Risk of Cardiovascular Outcomes in Post-Menopausal Women with Brest Cancer: Population-Based Cohort Study

- Jewish General Hospital Research Ethics Board approved
- CPRD International Scientific Advisory Committee (Protocol No. 17_072RA)

Manuscript 3: Cardiotoxicity of Aromatase Inhibitors and Tamoxifen Sequential Therapy in Post-Menopausal Women with Breast Cancer

- Montreal Jewish General Hospital Research Ethics Board approved
- CPRD International Scientific Advisory Committee (Protocol No. 17_072RA)