Pharmacoepidemiological studies of breast and colorectal cancer:

The association between statins and cancer outcomes

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Declaration

Declaration

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Summary

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Breast and colorectal cancer are two of the most commonly diagnosed cancers in Ireland and worldwide, and a significant cause of cancer deaths. Statins, which are drugs that are commonly used in the prevention of cardiovascular disease, have been identified as having a potential role in the treatment of these cancers. Pre-clinical, clinical, and epidemiological studies have highlighted these potential pleiotropic effects of statins; however, results are conflicting and research is on-going.

Pharmacoepidemiological studies provide the opportunity to investigate the effects of drug exposures on breast and colorectal cancer outcomes using existing datasets. Records from the National Cancer Registry Ireland, which have been linked to prescription claims data from the Health Service Executive Primary Care Reimbursement Service, for patients diagnosed with breast or colorectal cancer between 2001 and 2011, were used in this thesis.

Analyses of the patterns of statin use in the time prior to death from breast or colorectal cancer showed that the probability of continuing statin use was significantly lower in the three to six months prior to death from these cancers. These results suggest that it is important to account for peri-mortality changes in statin exposure in pharmacoepidemiological studies, to minimise potential reverse causation bias.

In analyses of de-novo statin use and breast cancer-specific mortality, no association was found between de-novo statin initiation and breast cancer-specific mortality, after adjusting for important covariates (HR 0.88, 95% CI 0.66, 1.17). Subgroup analyses also yielded null associations. Analyses of de-novo statin use on colorectal cancer-specific mortality also found no significant association in multivariate adjusted analyses (HR 0.96, 95% CI 0.78, 1.19). These studies suggest there may be limited benefit for statins in an adjuvant setting for an unselected population.

While no significant association was found between pre-diagnostic statin use and lymph node status at breast cancer diagnosis, pre-diagnostic statin use was associated with a significant, 19% reduction in breast cancer-specific mortality (HR 0.81, 95% CI 0.68, 0.96). Pre-diagnostic statin use was associated with a more marked, statistically significant, 31% reduction in breast cancer-specific mortality in patients with ER+ tumours (HR 0.69, 95% CI 0.55, 0.85).

Finally, in analyses of pre-diagnostic statin use and lymph node status in colorectal cancer, no association was found in multivariate adjusted analyses. However, pre-diagnostic statin use was associated with a non-significant, 14% reduction in colorectal cancer-specific mortality (HR 0.86, 95% CI 0.73, 1.00). In analyses stratified by type of statin received, colorectal cancer survival benefit was significant in those who received a lipophilic statin (HR 0.75, 95% CI 0.61, 0.93) but not a hydrophilic statin.

Strengths of these studies include the use of high-quality national-level cancer data, which is linked to detailed statin exposure data, enabling the robust study of the exposure-outcome relationship. However, the data used in these studies is a subset of the general population, defined by eligibility for General Medical Services scheme who are generally older and of lower socioeconomic status.

The results from these studies are broadly consistent with previous research on associations between statins and breast and colorectal cancer. These studies contribute novel data on the importance of considering peri-mortality changes in statin exposure, and on the associations between pre and post-diagnostic statin exposure and breast and colorectal cancer outcomes. This is important due to the high prevalence of statin use in Ireland and worldwide, and the growing investigation of the effects of commonly used medications and cancer outcomes.

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Smith, A. *et al*. De novo post-diagnosis statin use, breast cancer-specific and overall mortality in women with stage I-III breast cancer. Br. J. Cancer (2016). doi:10.1038/bjc.2016.232

Smith, A., Murphy, L., Bennett, K. & Barron, T. I. Patterns of statin initiation and continuation in patients with breast or colorectal cancer, towards end-of-life. Support. Care Cancer Off. J. Multinatl. Assoc. Support. Care Cancer (2017). doi:10.1007/s00520-017-3576-0

Smith, A., Murphy, L., Zgaga, L., Barron, T. I. & Bennett, K. Pre-diagnostic statin use, lymph node status and mortality in women with stages I–III breast cancer. Br. J. Cancer (2017). doi:10.1038/bjc.2017.227

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Smith, A., Zgaga, L., Bennett, K., Pre-diagnostic statin use, lymph node status and mortality in people with stage I–III colorectal cancer.

Abbreviations

List of abbreviations

AI	Aromatase inhibitor
AJCC	American Joint Committee on Cancer
Akt	(Akt/PKB) Protein kinase B
ASCO	American Society of Clinical Oncology
ATC	Anatomical Therapeutic Chemical
Bcl-XL	B-cell lymphoma-extra large
BMI	Body mass index
BRAF	Proto-oncogene B-Raf
BRCA	Breast cancer susceptibility gene
CD8	Cluster of differentiation 8
CHD	Coronary heart disease
CI	Confidence interval
CMF	Cyclophosphamide, methotrexate, fluorouracil
c-myc	Avian myelocytomatosis virus oncogene cellular homolog
COX	Cyclooxygenase
CPR	Complete pathological response
CRC	Colorectal Cancer
CSO	Central Statistics Office
CVD	Cardiovascular disease
DCIS	Ductal carcinoma in-situ
DNA	Deoxyribonucleic acid
DPS	Drugs payment scheme
EBCTCG	Early Breast Cancer Trialists' Collaborative Group
EGFR	Epidermal growth factor receptor
ER	Estrogen receptor
ERBB2	Erythroblastic oncogene B
Erk	Extracellular receptor kinase
ET	Endocrine therapy
FAP	Familial adenomatous polyposis
FDA	Food and Drug Administration
FGF-2	Fibroblast growth factor 2
FIT	Faecal immunochemical test
FPP	Farnesyl pyrophosphate
GDPR	General data protection regulation
GPP	Geranyl pyrophosphate
GMS	General Medical Services (Scheme)
Gy	Gray
HER2	Human epidermal growth factor receptor-2
HIF	Hypoxia inducible factor
HMGCR	3-hydroxy-3-methylglutaryl-coenzyme-A reductase
HNPCC	Hereditary non-polyposis colorectal cancer
HR	Hazard ratio
HSE	Health Services Executive
HTD	High-tech drug

IARC	International Agency for Research on Cancer
ICD	International classification of diseases
IDC	Invasive ductal carcinoma
ILC	Invasive lobular carcinoma
IQR	Interquartile range
ki67	Antigen KI-67
KRAS	Kirsten rat sarcoma viral oncogene homolog
LCIS	Lobular carcinoma in-situ
LDL	Low-density lipoprotein
LDL-C	Low-density lipoprotein cholesterol
LN	Lymph node
LTI	Long term illness
M1	Macrophage type 1
MAPK	Mitogen-activated protein kinase
MMR	Mismatch repair
NCCP	National Cancer Control Programme
NCRI	National Cancer Registry of Ireland
NFkB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NSAID	Non-steroidal anti-inflammatory drug
OATP1B1	Organic-anion-transporting polypeptide 1B1
OR	Odds ratio
OS	Overall survival
OTC	Over-the-counter
Pcr	Pathologic complete response
PCRS	Primary Care Reimbursement Services
PI3K	Phosphoinositide 3-Kinase
pMEK	Phospho-Mitogen-activated protein kinase
PR	Progesterone receptor
PTEN	Phosphatase and tensin homolog
Ras	Renin-angiotensin system
RD	Risk difference
RR	Relative risk
RT	Radiation therapy
SAHRU	Small Area Health Research Unit
SCORE	Systematic COronary Risk Evaluation
SEER	Surveillance Epidemiology and End Results (database)
SERM	Selective estrogen receptor modulator
TEM	Transanal endoscopic microsurgery
TK	Tyrosine kinase
TNBC	Triple negative breast cancer
TNM	Tumour, node, metastasis
VEGF-A	Vascular endothelial growth factor-A
WBRT	Whole breast radiation therapy
WHO	World Health Organisation
27HC	27-hydroxycholesterol

Chapter One

1. Introduction

This chapter begins by giving an overview of breast and colorectal cancer epidemiology, classification and treatment, as these cancers are the focus of this PhD thesis. Statins, the drugs examined in this thesis, are introduced, and finally the overall research aim and objectives of the individual studies are outlined.

1.1. Breast Cancer

1.1.1. Burden of disease

According to the International Agency for Research on Cancer (IARC) Globocan data, breast cancer is the most frequent cancer among women worldwide, with an estimated 1.67 million new breast cancer cases diagnosed in 2012 [1]. In Ireland, breast cancer is the most commonly diagnosed invasive cancer in women (excluding non-melanoma skin cancer) and it accounts for just over 30% of all invasive cancers diagnosed [2]. Breast cancer trends in Ireland, from 1994 to 2013, have been described by the National Cancer Registry of Ireland (NCRI) [3]. Approximately 2,880 new cases of invasive breast cancer and 360 non-invasive (in-situ) breast cancers were diagnosed per year during from 2011 to 2013. The incidence rate has increased by ~1.5% annually from 1994 onwards, with a peak in incidence in 2002 [4]. This is partly explained by the influence of the national screening programme, BreastCheck, which was introduced in 2000 [5]. Breast cancer occurs almost predominantly in women, but up to 20 men are diagnosed with breast cancer in Ireland each year [3].

Cancer is the second most common cause of death registered in Ireland, after diseases of the circulatory system [6]. Breast cancer accounts for approximately 20% of cancer deaths in women [3]. On average, there are 690 deaths attributable to breast cancer each year in Ireland - an age-standardised rate of 27 deaths per 100,000 per year [2].

Breast cancer Screening

In February 2000, the Republic of Ireland commenced its national breast cancer screening programme, BreastCheck. It was initially rolled out in the East of the country, to women aged 50-64 years, covering approximately 50% of the eligible population. In December 2007, the programme was extended to the rest of the Republic of Ireland. The programme is delivered through postal invitation to all eligible women every two years, and is free of charge. BreastCheck is currently being extended and by the end of 2021, all eligible women aged 50 to 69 will be invited for routine screening. This is being done on a phased basis and will be achieved by inviting women who were aged between 50 and 66, on the 1st January 2018, for mammograms until they reach the age of 69. The uptake of BreastCheck has remained quite stable over time, ranging from 68%-76%. Women with abnormal screen results are referred to a breast assessment clinic for further investigations [5].

1.1.2. Risk factors associated with breast cancer

Approximately 5–10% of breast cancers have a strong genetic component. These inherited genetic mutations confer a 40-85% lifetime risk of developing breast cancer, and most commonly include mutations in Breast Cancer susceptibility gene 1 (BRCA1) and Breast Cancer susceptibility gene 2 (BRCA2) mutations [7]. Breast cancers arising due to BRCA mutations typically occur at a younger age (typically between 30-40 years of age) [8].

More commonly diagnosed, and accounting for at least 90% of breast cancer cases, is sporadic breast cancer. There are a number of risk factors associated with developing sporadic breast cancer; older age, nulliparity and low parity [9,10]; late age at first pregnancy [9,10]; late natural menopause [9,10]; oral contraceptives (oestrogen/progestogen combined pill) [11]; hormone replacement therapy [11]; exposure to diethylstilbestrol, a synthetic estrogen, in-utero [11]; body fatness, abdominal fatness and weight gain in adulthood [12,13]; alcohol [14,15] and smoking [15].

1.1.3. Breast Cancer Anatomy

Ductal carcinoma in situ (DCIS) and lobular carcinoma in situ (LCIS) are the earliest stages of breast cancer. DCIS may also be called intraductal, non-invasive cancer. The cancer cells are confined to the ducts and have not invaded surrounding tissues. Similarly, LCIS is non-invasive cancer confined to the lobules of the breast [16]

Invasive ductal carcinoma (IDC) is the most common type of breast cancer, accounting for almost 80% of breast cancers. It is also known as infiltrating ductal carcinoma. IDC develops in the ducts but has spread to the surrounding tissue. This type of breast cancer is most common in women over the age of 55 years. Invasive lobular carcinoma (ILC) accounts for less than 10% of breast cancer cases, and occurs in the lobules of the breast [16].

Inflammatory breast cancer and Paget's disease of the nipple are rare types of breast cancer, accounting for 1% of breast cancer cases.

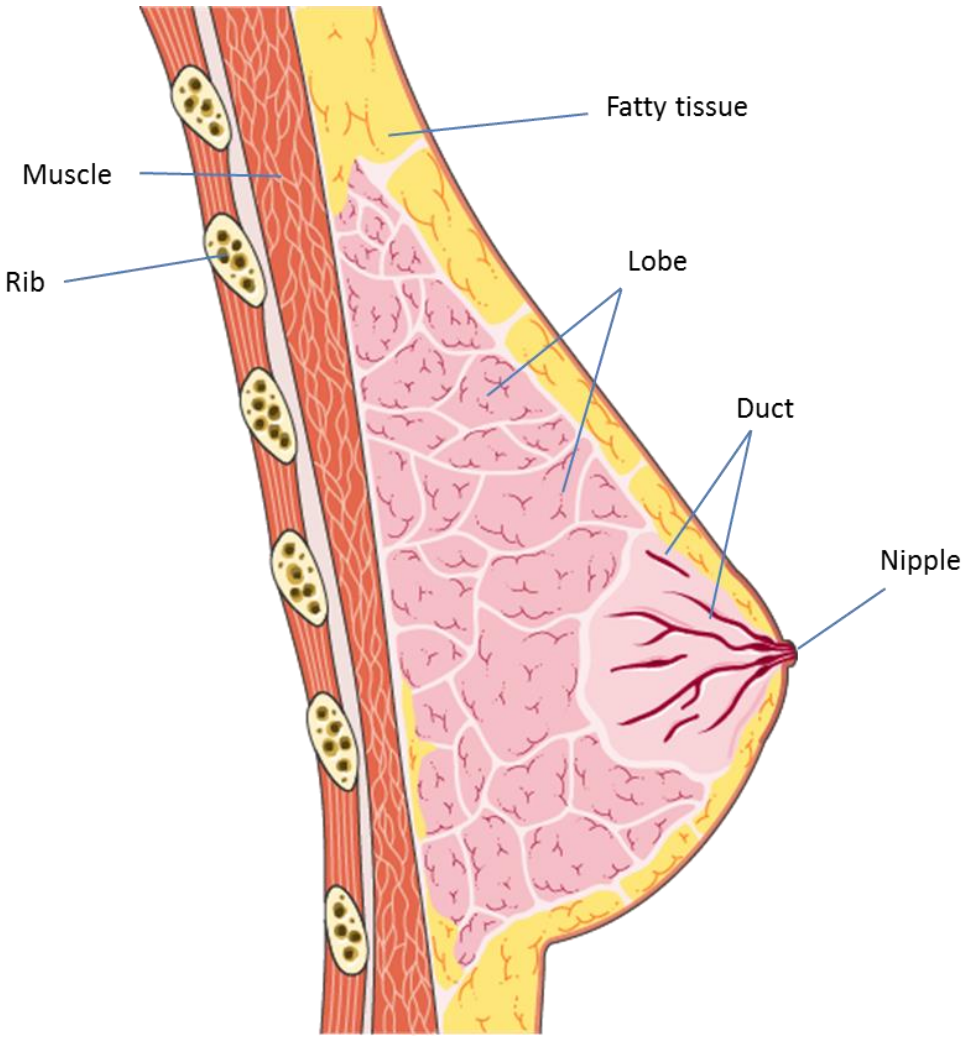

Figure 1.1: Anatomy of the female breast.

Produced in part using Servier Medical Art (www.servier.com).

1.1.4. Molecular Classification of Breast Cancers

In the early 2000's, with the advance of molecular techniques, breast cancers began to be classified based on the expression of specific receptors. Breast cancers are commonly classified based on their expression of: estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Around 70% of all breast cancers are positive for the hormone receptors ER and PR. These ER and/or PR positive tumours are dependent on estrogen/progesterone hormones for growth and, therefore, respond to endocrine therapy and generally have better prognosis [17].

HER2 amplified tumours are present in approximately 20% of cases and are characterised by ERBB2 amplification and overexpression, with the consequent dependency on HER2 signalling. HER2 amplified breast cancer typically has a worse prognosis, however, HER2 targeted therapies are now available, which provide significant improvements in prognosis for these women [18].

Triple negative breast cancer (TNBC) accounts for 10-15% of breast cancer cases and is categorised by tumours that do not express ER, PR, or HER2. Patients with TNBC have the worst clinical outcomes, and a far shorter disease-free survival and overall survival (OS) [19]. There are no molecular-targeted therapies for TNBC and approximately only 20% of these tumours benefit from standard chemotherapeutic agents [20].

1.1.5. Breast Cancer Staging

Breast cancer staging involves determining the extent of disease in the affected breast, evaluating the regional lymph nodes, and identifying sites of distant metastatic disease [21]. Breast cancers are staged according to the tumour, node, metastasis (TNM) system, published by the American Joint Committee on Cancer (AJCC) [22]. In the AJCC staging system, the tumour (T) size is measured, and the size of the invasive component is recorded. Lymph node (N) staging is extremely important for treatment planning and for providing prognostic information. An ipsilateral axillary lymph node with a metastasis is classified as N1. Sentinel lymph node dissection is routinely used in breast cancer staging, as there is one (or more) sentinel lymph node which receives primary drainage from the tumour, and metastatic involvement of the sentinel lymph node is reflective of the entire nodal basin [21,22]. The TNM system assesses metastatic disease in a binary fashion. M0 means no evidence of distant metastatic disease, and M1 signifies the presence of distant metastases [22]. Accurate staging of breast cancers is extremely important for appropriate treatment planning and determining prognostic information.

1.1.6. Breast Cancer Treatment

The choice of breast cancer treatment is based on the following; size and location of primary tumour, number of lesions, extent of lymph node involvement, biomarker and gene expression, as

well as on the age and general health status of the patient and personal preferences [23]. The main treatment options are; surgery, radiation therapy, hormone therapy, and chemotherapy, each described below.

Surgery

Currently, between 60% and 80% of newly diagnosed breast cancers are treated with breast conservation surgery, also known as a lumpectomy. A careful histological assessment of resection margins is essential, with no tumour at the inked margin required and a minimum 1 mm margin preferred for the invasive component and >2 mm of normal tissue required for *in situ* disease [24]. In some patients, a mastectomy, or full breast removal, is carried out due to tumour size (relative to breast size), tumour multi-centricity, inability to achieve negative surgical margins after resection, or patient choice [24].

Radiation Therapy

Postoperative radiation therapy (RT) is strongly recommended after breast conservation surgery. Whole breast radiation therapy (WBRT) in low-risk patients after breast conservation surgery reduces the risk of local recurrence by two-thirds [24]. Radiation therapy has also been shown to be effective in node-positive patients, post-mastectomy. A large meta-analysis by Clarke *et al*. showed that post-mastectomy RT reduces 15-year breast cancer mortality by approximately 5% [25].

Doses used for local and/or regional adjuvant irradiation have traditionally been 45–50 gray (Gy) in 25–28 fractions of 1.8–2.0 Gy with a typical boost dose of 10–16Gy in 2 Gy single doses. Shorter fractionation schemes (e.g. 15–16 fractions with 2.5–2.67Gy single dose) have shown similar effectiveness and comparable side-effects [26].

Endocrine Therapy

Endocrine therapy (ET) is indicated in patients with detectable ER expression [27]. There are two main categories of endocrine therapy agents: selective estrogen receptor modulators (SERMs) and aromatase inhibitors (AIs). SERMs competitively bind to estrogen receptors to interfere with DNA synthesis, and inhibit G0 to G1 cell cycle progression [28]. The three main SERMs are tamoxifen, raloxifen, and toremifene. AIs inhibit the aromatase enzyme that converts circulating testosterone to estradiol (E2), and androstenedione to estrone, by aromatization. Such peripheral conversion of other hormones to estradiol is the main source of estrogen in post-menopausal women. Exemestane, anastrazole and letrozole are three main AIs used clinically [29].

The choice of ET medication is generally determined by the patient's menopausal status. In premenopausal patients, tamoxifen at 20 mg/day for 5–10 years has become the standard treatment option [30]. In patients who become postmenopausal during the first 5 years of tamoxifen, it has been shown that treatment-switching to letrozole, an AI, is beneficial for improved disease-free survival [31]. In postmenopausal patients, both AIs and tamoxifen are valid treatment options. AIs have largely replaced tamoxifen as the preferred treatment for hormone receptor-positive breast cancer in postmenopausal women [32]. Although there is no proven benefit for the routine use of AIs for >5 years, extended adjuvant treatment should be discussed with patients [23].

HER2-directed therapy

About 20–25% of breast cancers are characterized by the over-expression of HER2 protein [33]. HER2 is a transmembrane glycoprotein that has both an intracellular receptor tyrosine kinase (TK) domain and an extracellular ligand binding domain [33]. Trastuzumab is the first monoclonal antibody developed as an anti-HER2 therapeutic [34]. Since its first US Food and Drug Administration (FDA) approval in 1997, trastuzumab has become routine in the treatment of HER2 overexpressing breast cancer. A large multi-centre study has shown that trastuzumab, administered after adjuvant chemotherapy, significantly improves disease-free survival and recurrence-free survival among women with HER2-positive breast cancer [35].

Chemotherapy

Surgery followed by adjuvant treatment has been the gold standard for breast cancer treatment for a long time. More recently, neoadjuvant treatment (or, chemotherapy prior to surgery) has been recognized as an important treatment strategy, particularly for patients with large tumour size, high nodal involvement, and those with an inflammatory component [29]. In 2001, The Early Breast Cancer Trialists' Collaborative Group (EBCTCG) reported the collective data of the randomized trials in early breast cancer adjuvant systemic chemotherapy from 1985 to 2000. This report showed long term benefits of adjuvant endocrine therapy, and also a 50% reduction of the overall mortality in 15 years, when hormone receptor positive breast cancer patients received adjuvant chemotherapy and tamoxifen for 5 years following surgery [27].

Most frequently used chemotherapy regimens contain anthracyclines; such as doxorubicin (Adriamycin) and epirubicin (Ellence), and/or taxanes; such as paclitaxel (Taxol) and docetaxel (Taxotere). Although in some patients, combinations of cyclophosphamide, methotrexate, and fluorouracil (CMF) may still be used. Chemotherapy is usually administered for 12–24 weeks (four to eight cycles), depending on the individual recurrence risk and the selected regimen [36].

'Oncotype DX' is a multi-gene assay that can categorize patients into low, intermediate, and high risk groups corresponding to 6.8%, 14.3% and 30.5% risk of distant recurrence at 10 years after 5 years of tamoxifen therapy, respectively [37]. Use of the 'Oncotype DX' for gene expression profiling to support chemotherapy decision making was recommended by the National Cancer Control Programme (NCCP) Technology Review Committee in August 2011, in line with eligibility guidelines drafted by the Irish Society of Medical Oncology. However, this assay came into clinical practice after the collection of the data used in this thesis, and will not be discussed in much detail. In the next section, the other cancer considered in the thesis, colorectal cancer, is described.

1.2. Colorectal Cancer

1.2.1. Burden of Disease

According to the IARC globocan data, colorectal cancer (CRC) is the third most common cancer in men (746,000 cases diagnosed in 2012) and the second most common in women (614,000 cases diagnosed in 2012) worldwide [1]. In Ireland, CRC is the third most commonly diagnosed invasive cancer (excluding non-melanoma skin cancer), across men and women, and it accounts for 12% of all newly diagnosed invasive cancers [37]. The NCRI has previously described CRC trends in Ireland, from 1994 to 2010 [38]. There are approximately 2,775 new cases of CRC diagnosed in Ireland each year, with the incidence being higher in the male population (65% of cases) [38].

Colorectal Cancer Screening

A nationwide faecal immunochemical test (FIT)-based screening programme, called BowelScreen, was commenced in Ireland in late 2013. As the CRC data used in this PhD thesis was collected prior to the initiation of BowelScreen, it will not be discussed in much detail. In brief, the national screening programme is delivered through a postal invitation to all eligible residents of Ireland (aged 60-69) every two years, and is free of charge. Participants with abnormal results will be referred for colonoscopy and further investigations.

1.2.2. Risk factors associated with colorectal cancer

Similar to breast cancer, a proportion of CRC are familial cases in which affected individuals tend to be younger (<50 years of age) and have a significant familial history and/or genetic predisposition. It is estimated that up to 30% of CRC cases are hereditary, however, the exact mechanisms are to be elucidated [39]. A significant proportion of familial cases are attributed to the presence of Lynch syndrome (also called hereditary nonpolyposis colorectal cancer [HNPCC]), or familial adenomatous polyposis (FAP). Lynch syndrome is an autosomal dominant condition, predisposing to CRC and other malignancies at a young age due to a germline mutation in one of the mismatch repair (MMR) genes [40]. Individuals with FAP develop many hundreds of colonic adenomas, often beginning in

adolescence, leading to inevitable CRC if left untreated - 95% of people with untreated FAP will develop CRC by age 50. Attenuated FAP is a less-severe form, with much less colonic polyps, and a later age of polyp and CRC development [39].

The majority of CRC cases are sporadic. A recent meta-analysis examined the potential risk factors for CRC incidence, and inflammatory bowel disease (such as Crohn's disease or Ulcerative Colitis) was found to considerably increase an individual's risk of developing CRC. The following were associated with moderate increases in risk of CRC: increased body mass index (BMI), low physical activity, cigarette smoking, consumption of red meat, low consumption of fruit and/or vegetables [41].

1.2.3. Colorectal Cancer Anatomy

CRC is defined as a tumour of the digestive tract located in the colon, rectum or rectosigmoid junction (Figure 1.2). Undiagnosed CRC is often presented with the following symptoms: abdominal pain, change in bowel habit, and rectal bleeding or anaemia [42]. Investigation of symptoms can include faecal occult blood testing and referral for colonoscopy, which is the primary method of diagnosis [42]. The majority of CRCs arise from pre-cancerous adenomatous polyps which gradually transform into malignant adenocarcinomas, typically over a period of 10-15 years [43].

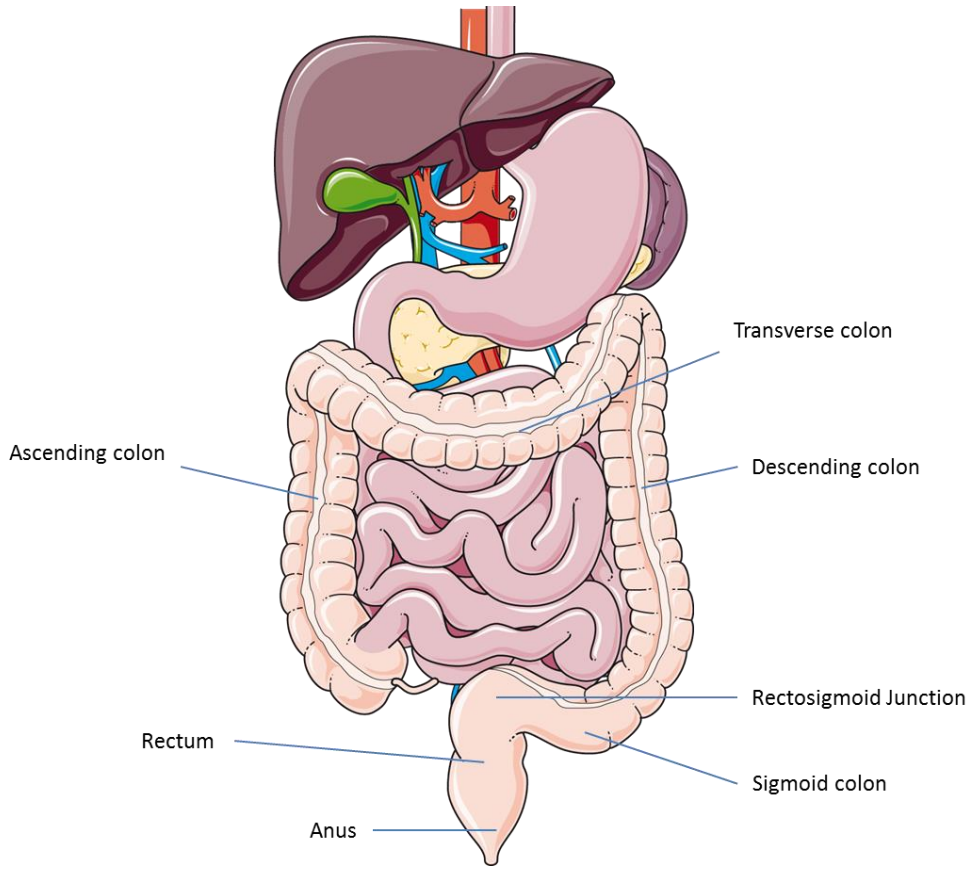

Figure 1.2: Anatomy of the human digestive tract.

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1.2.4. Colorectal Cancer Staging

Staging at the time of diagnosis is essential for determining course of adjuvant/neoadjuvant treatment. The TNM staging system of the AJCC is the standard for colorectal cancer staging [22]. In the TNM staging system for CRC, (T) describes: the size of the tumour and extent of spread of the tumour through the colorectal wall; (N) describes the presence, if any, of lymph node metastasis. The number of lymph nodes recovered from resection samples varies widely, but it has been shown that a minimum of 12 lymph nodes must be examined to accurately determine regional node status in colorectal cancer and (M) describes if the tumour has metastasised to distant organs: M1 disease encompasses pathologically documented spread to any non-regional lymph node, the parenchyma of any distant organ or tissue, and/or the peritoneum [44].

1.2.5. Colorectal Cancer Treatment

Colorectal cancer treatment is based on various factors such as: the TNM stage of the tumour, the age and overall health of the patient, and personal preference. The main treatment options are; surgery, radiation therapy, and chemotherapy, each described below.

Surgery

Currently, up to 80% of CRC cases are treated with surgery, and this is the first line treatment option for this cancer. Local excisional procedures such as trans-anal endoscopic microsurgery (TEM) are appropriate for early cancers, without any lymph node or metastatic spread. More advanced tumours are usually treated with more radical surgery, whereby part of the healthy colon/rectum and lymph nodes are also removed, due to higher risks of recurrence and the higher risk of lymph node involvement [45].

Radiation Therapy

Pre-operative neoadjuvant RT is recommended for patients with large rectal tumours (those that invade >5mm into the surrounding fat tissue), in order to reduce the size of the tumour prior to surgery [46]. This is often given alongside pre-operative chemotherapy and may result in a complete pathological response (cPR), whereby no viable tumour cells remain in the resected specimen. A recent study found that patients with a cPR had much lower rates of local recurrence (odds ratio (OR) 0.45, 95% CI 0.22, 0.90), distant recurrence (OR 0.15, 95% CI 0.07, 0.31), and increased disease-free survival (OR 3.53, 95% CI 1.62, 7.72) [47].

Chemotherapy

As mentioned above in section 1.1.6, chemotherapy can be administered pre-operatively in order to reduce tumour burden prior to surgery [47]. The use of adjuvant (post-surgery) fluorouracil-based chemotherapy is recommended in patients with stage II/III colon cancer at high risk of recurrence. For example; those with a suboptimal number of removed lymph nodes, or poor tumour differentiation [42]. The addition of new chemotherapeutic agents has been investigated

in the adjuvant setting. The FOLFOX combination (oxaliplatin, fluorouracil, and leucovorin) is associated with improved 3-year disease-free survival in patients with stage II/III colon cancer, however, no difference is observed for overall survival [48].

Alongside these chemotherapy regimens, targeted agents are used for metastatic colorectal cancer treatment. These include: monoclonal antibodies against EGFR (cetuximab), and monoclonal antibodies against VEGF-A (bevacizumab). Cetuximab in addition to chemotherapy is associated with progression-free survival in CRC patients with wild-type KRAS and BRAF (Hazard Ratio (HR) 0.84, 95% CI 0.72, 0.99) [49]. Bevacizumab, in combination with chemotherapy (5-fluorouracil and irinotecan), was shown to significantly improve median progression-free survival (HR 0.54; P<0.001) in chemotherapy-naïve metastatic colorectal cancer patients [50]. These targeted agents are costly and provide modest survival benefits to patients, warranting the need for additional, cost-beneficial therapies.

In the next section, I will discuss how drugs may be repurposed in the treatment of cancer, by giving some examples of non-cancer drugs which are now used in this setting.

1.3. Drug repurposing in cancer treatment

Typically, cancer drug discovery and development involves identification and optimization of lead compounds, followed by pre-clinical and clinical studies to extensively test and determine their pharmacological properties, anti-neoplastic effects and toxicity. This process is costly and time consuming, with the average time span from initial experiments to completed regulatory review varying between 11–13 years [51]. A study investigating the cost of CRC to the healthcare payer, the Health Services Executive (HSE) in Ireland, revealed that the use of chemotherapy and biological agents such as bevacizumab and cetuximab had a major impact on costs, particularly for stage II and III disease [52].

Drug repurposing refers to the application of a drug for another indication other than the original indication [53]. A major advantage is that extensive pre-clinical, clinical, and utilization data are

often available, reducing the need for additional studies to investigate pharmacokinetic properties and toxicity. The safety profile is likely to resemble that of the original indication, thus increasing the likelihood of the drug making it through the trial [53]. In recent years, a number of drugs have been investigated, at the biological and epidemiological level, for their potential chemopreventative and chemotherapeutic effects. I will briefly discuss two such examples; bisphosphonates and aspirin. The focus of this thesis is statin drugs, and these will be discussed in detail in the next section (section 1.4).

The bisphosphonates alendronate sodium, zoledronic acid and clodronic acid are frequently used to treat osteoporosis. Twenty years ago, Diel *et al*. showed that the addition of clodronate to standard adjuvant treatment decreased the incidence and number of new bone and visceral metastases in breast cancer patients with known bone marrow micro metastases [54]. More recently, a meta-analysis of bisphosphonate use in post-menopausal women with breast cancer showed significant reductions in recurrence (Relative Risk (RR) 0.86, 95% CI 0.78, 0.94), distant recurrence (RR 0.82, 95% CI 0.74, 0.92), bone recurrence (RR 0.72, 95% CI 0.60, 0.86), and breast cancer mortality (RR 0.82, 95% CI 0.73, 0.93) [55]. Based on this wealth of evidence from observational and clinical studies, the use of bisphosphonates as adjuvant therapy should now be considered for all postmenopausal women with early breast cancer who are deemed to be candidates for adjuvant therapy, according to the American Society of Clinical Oncology (ASCO) and a European Stakeholder Panel [56,57].

Aspirin is a cyclooxygenase (COX) 1 and 2 inhibitor, used in low doses (75mg) for the prevention and treatment of cardiovascular disease, and in higher doses as an analgesic (300-600mg). Aspirin exerts effects on platelet aggregation and platelet adherence, which play a role in tumour cell immune evasion. In addition, COX2 is responsible for the production of prostaglandin E2, which can promote proliferation [83]. A recent meta-analysis investigating the effect of aspirin on cancer-specific mortality from colon cancer, breast cancer, and prostate cancer suggests that aspirin may have clinical utility in the adjuvant setting; (HR 0.76, 95% CI 0.66, 0.88), (HR 0.87, 95% CI 0.69, 1.09),

and (HR 0.89, 95% CI 0.79, 0.99), respectively. Currently, there are a number of ongoing clinical trials of aspirin in both the chemoprevention and adjuvant setting (clinicaltrials.gov).

Another class of medicines that has been considered as a potential chemopreventative therapy are statins. These are a commonly prescribed medication, and will be discussed further in the next section.

1.4. Statins

Statins are commonly used medications for the primary and secondary prevention of cardiovascular disease (CVD). The FDA in the United States approved the first commercial statin, lovastatin, to the pharmaceutical company Merck in September 1987 [58]. Numerous long-term, placebo-controlled clinical trials and subsequent meta-analyses have conclusively demonstrated that statins reduce the risk of morbidity and mortality from cardiovascular disease across a wide range of cholesterol levels [59]. A recent overview of systematic reviews suggested that statins were associated with a 25% reduction in CVD (RR 0.75, 95% CI 0.70, 0.81), when compared to placebo [60]. However, there is ongoing debate over the use of statins in the primary prevention setting, as studies have shown conflicting results [61].

1.4.1. Statin Pharmacology and Mode of Action

Statins are competitive inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), the key enzyme in the cholesterol synthesis pathway. HMGCR catalyses the conversion of 3-hydroxy-3-methylglutaryl-coenzyme A to mevalonate, which is the precursor of cholesterol. Inhibition of HMGCR leads to a decrease in mevalonate levels and thereby to an increase in the number of low-density lipoprotein (LDL) receptors on the surface of cells and, finally, to an increase in LDL catabolism [62].

Statins can be classified as either natural or synthetic, according to their origin. Natural statins (lovastatin and pravastatin) are secondary metabolites of fungi and are structurally similar. They can be obtained from different types and species of filamentous fungi. Lovastatin is a product of fermentation carried out by *Aspergillus terreus* or *Monascus ruber*. Pravastatin is obtained as a

result of the biotransformation of mevastatin which is most efficiently carried out by *Streptomyces carbophilus*. [63]. Simvastatin is a semisynthetic derivative of lovastatin, generated as part of a process which requires chemical modification of lovastatin [64]. Atorvastatin, cerivastatin, fluvastatin, and rosuvastatin are fully synthetic statins. Atorvastatin and fluvastatin are obtained synthetically from mevalonate and pyridine, respectively. Cerivastatin, because of its many side effects, was withdrawn from the market in 2001 [65].

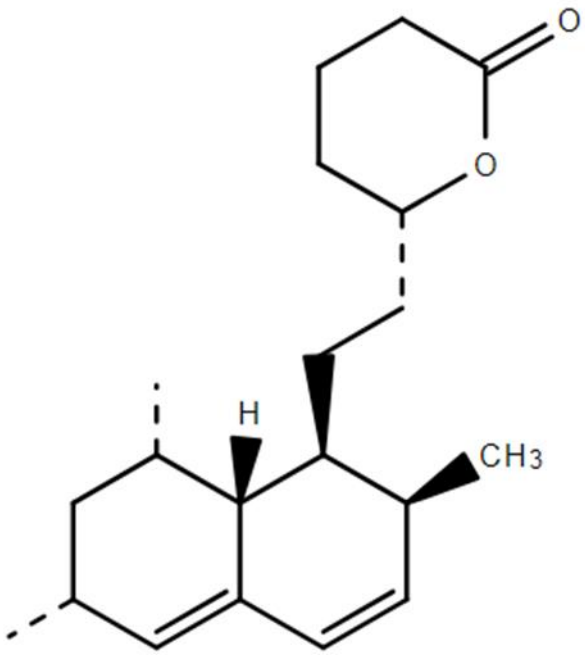

Figure 1.3: The base structure of natural statins.

Produced using **www.chemspider.com**.

The liver is the target organ for statins, as 60% of the total cholesterol in the body is synthesised here [66]. The effect that statins exert on cells may depend on structure of the statin and its ability

to penetrate cell membrane. Lipophilic statins, such as simvastatin, passively penetrate through the plasma membrane, including extra-hepatic cell membranes. Hydrophilic statins cannot penetrate passively, as extra-hepatic cells do not express the OATP1B1 transporter, which is required for uptake of hydrophilic statins [67]. Therefore hydrophilic statins are more hepatoselective.

Statins are administered orally, in active or prodrug form. The time to reach peak plasma concentration is typically 4 hours, and the percentage of drug absorbed varies from 30-90% [68].

Table 1.1: Hydrophilic and Lipophilic statins (adapted from [64]**).**

	Hydrophilic statins	**Lipophilic statins**
Type of statin	Pravastatin, rosuvastatin	Simvastatin, lovastatin, fluvastatin, atorvastatin
Origin	Pravastatin—natural and rosuvastatin—synthetic	Lovastatin—natural; simvastatin—semisynthetic; and cerivastatin, fluvastatin, atorvastatin – synthetic
Distribution in the body	Accumulate mainly in the liver (uptake by OATP1B1)	Distributed to various tissues
Plasma membrane penetration	Poor; the OATP1B1 transporter is needed	Passively penetrate through the plasma membrane

1.4.2. Clinical Use of Statins

Dyslipidaemias cover a broad spectrum of lipid abnormalities, most of which are due to the interaction between genetic predisposition and lifestyle factors, such as obesity and comorbid conditions [69]. Increased total cholesterol and low density lipoprotein cholesterol (LDL-C) are major risk factors for CVD, and therefore management of lipid levels (through lifestyle changes and lipid lowering therapies) is the aim of CVD prevention. Familial hyperlipidaemia is an inherited

disorder in which there are elevated plasma LDL-C levels from birth, and early diagnosis and prompt initiation of diet and lipid-lowering therapy are critical to the prevention of CVD [70].

In addition to clinical evaluation (e.g. age, presence of comorbidities) a risk assessment tool may be used to estimate CVD risk. The Systematic Coronary Risk Evaluation (SCORE) risk tool is based on large European data and is recommended for use in Ireland [71]. It uses validated clinical endpoints and enables the risk of CVD and stroke to be estimated. Risk assessment tools are useful in helping to identify those without CVD who are at risk, and may aid patient-doctor communication in order to optimise treatment compliance. Statins are the lipid lowering agents for which there is the largest body of clinical evidence. The aim of statin treatment is typically to reduce LDL-C to <1.8mmol/L or to achieve a >50% reduction from the pre-treatment level [72].

In the last 30 years, there has been a large increase in the use of statins for the primary and secondary prevention of cardiovascular disease. In Ireland, the use of statins increased significantly between 2000 and 2003, according to a study of statin utilization in nine European countries [73].

Before offering statin treatment for primary prevention of CVD, it is recommended that clinicians and patients discuss the benefits of lifestyle modification and, if possible, optimising the management of all other modifiable CVD risk factors, such as smoking and obesity. Should statins be initiated, the dose range is 10 mg to 80 mg given as a single oral dose. An 80 mg dose is recommended only in patients with severe hyper-cholesterolaemia at high risk of cardiovascular events, or post-cardiac event in the secondary prevention setting [74].

1.4.3. Statin Safety

While statins are generally well tolerated, they are associated with muscle, metabolic, neurological, and other possible side effects, known as statin-associated symptoms (SAS). Statin associated muscle symptoms are the most common statin side effect, and have been reported by 10% to 25% of patients receiving statin therapy. Additionally, in a survey of former statin users, approximately 60% reported stopping statin therapy because of side effects [75]

In particular, clinicians should prescribe statins with caution in patients with pre-disposing factors for myopathy or rhabdomyolysis. A creatine kinase (CK) level should be measured before starting treatment in the case of the following: renal impairment, hypothyroidism, history of hereditary muscular disorders, alcohol abuse, and in elderly patients. In these situations, the risk of treatment should be considered in relation to potential benefit. Clinical monitoring is recommended in these patients. If CK levels are significantly elevated (> 5 x upper limit of normal) at baseline, treatment should not be started. In addition, if muscle pain, weakness or cramps occur whilst a patient is receiving treatment, their CK levels should be measured. If these levels are found to be significantly elevated (> 5 x upper limit of normal), then statin treatment should be stopped [76]. Muscle symptoms often appear soon after starting stain therapy or after an increase in dose, and will generally resolve within weeks after cessation of therapy. Different statins usually produce similar symptoms, however, it has been noted that some patients will tolerate one statin better than another [75].

Increased serum statin concentrations or reduced body muscle mass increases the risk of muscle symptoms, as well as; older age, female sex, physical disability, and lower body mass index. Similarly, higher statin doses increase the risk of muscle symptoms, resulting in the clinical observation that symptoms appeared after an increase in statin dose. Alcohol use is also associated with increased risk of muscle symptoms, due to alterations in statin catabolism by the liver's cytochrome P450 system [75].

A large review was conducted to summarise the available evidence on the potential non-cardiovascular harms associated with the use of statins [77]. It was found that statins cause a modest increase in the incidence of severe myopathy (muscle pain, with a CK level >10 times the upper limit of normal), and statins were not significantly associated with an increased risk of myalgias (muscle pain, but with normal creatine kinase levels). There is some evidence to suggest statins may increase the risk of diabetes, however, this was largely attributable to patients who were already at high-risk of diabetes [78]. The authors suggest that further work is needed to

determine the associations between statin use and; cognition, erectile dysfunction, and cataracts [77]. Therefore, based on currently available evidence, it is generally accepted that the overall cardiovascular benefits of statins outweigh the non-cardiovascular effects.

1.4.4. Anti-cancer properties of statins

The potential role for statins in the inhibition of cancer cell growth was suggested by Buchwald over 25 years ago [79]. Since then, there have been many pre-clinical studies into the potential pleiotropic effects of statins, mainly due to the downstream effects of mevalonate pathway inhibition. As well producing cholesterol, the mevalonate pathway also produces geranyl pyrophosphate (GPP) and farnesyl pyrophosphate (FPP), which are involved in many cancer cell processes [80]. Inhibition of GPP and FPP production causes downstream effects on Ras and Rho, two intracellular proteins involved in: proliferation, apoptosis, and angiogenesis [80].

A recent study has shown that simvastatin can inhibit the growth of four breast cancer cell lines, in a dose dependant manner. Simvastatin caused depletion of FPP and GPP, deactivating the PI3K/Akt and MAPK/ERK pathway, and subsequently inducing apoptosis and inhibiting proliferation. Independent of the mevalonate pathway, treatment of the cell lines with simvastatin significantly decreased the expression of c-myc and cyclin D1, and increased p21 and p27. This study suggests that simvastatin inhibits cell cycle progression from G1 to S phase, therefore suppressing breast cancer cell proliferation [81]. In a colon cancer cell line, lovastatin was shown to induce apoptosis through the blocking of Ras isoprenylation, causing a subsequent decrease in survivin expression [82]. In breast cancer cell lines, simvastatin has also been shown to cause a significant, dose-responsive, reduction in HIF-1α, a factor which induces angiogenesis. Further, in in-vivo models, simvastatin-treated tumours had significantly lower protein levels of both vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF-2) than controls [83]. Simvastatin was also shown to inhibit VEGF protein expression in a CRC cell lines [84].

The chemopreventative effects of statins have also been investigated, with many observational studies and meta-analyses published on statin use and cancer risk. In a meta-analyses including

6,662 incident cancers, statins did not reduce the incidence of cancer (OR 1.02; 95% CI 0.97, 1.07). No reductions were observed for any specific cancer types, or by type of statin received [85]. Similar results were found in a CRC-specific meta-analysis by Lytras *et al*. They carried out a meta-analysis of randomised controlled trials (RR 0.89, 95% CI 0.74, 1.07), and meta-analysis of cohort studies (RR 0.91, 95% CI 0.83, 1.00) [86]. For breast cancer risk, a meta-analysis of 24 studies found that statin use did not affect breast cancer risk (RR 0.99, 95% CI 0.94, 1.04) [87]. A number of studies investigating the association between statin use and cancer outcomes, such as cancer-specific mortality have also been published, and these will be discussed in further detail in chapters 4-7. Each study chapter (Chapters 4-7) will have a study-specific introduction and discussion, with an overall thesis discussion and conclusion in Chapter 8.

1.5. Research aims and objectives

The aim of my research is to use pharmacoepidemiological methods to examine the association between statins and breast and colorectal cancer outcomes. Research efforts will seek to add to the knowledge base by investigating the patterns of statin use in these patient groups, and by determining the association between exposure to statins before and after a diagnosis of breast/colorectal cancer and outcomes, in particular lymph node status and survival, using data from linked national resources.

Specific research objectives include the following:

1. To describe the patterns of statin initiation and continuation in patients with breast or colorectal cancer, towards end-of-life
2. To examine associations between de-novo statin use and breast cancer survival, in women with stage I-III breast cancer
3. To examine associations between de-novo statin use and colorectal cancer survival, in those with stage I-III colorectal cancer
4. To examine association between pre-diagnostic statin use and LN status and breast cancer survival, in women with stage I-III breast cancer

5. To examine associations between pre-diagnostic statin use and LN status and colorectal cancer survival, in those with stage I-III colorectal cancer

In the next chapter, I will provide a description of the data used in these studies, and where the data was sourced from. I will also discuss the strengths and limitations of these resources, and also some methodological considerations.

Chapter Two

2. Data used in this thesis

This chapter will first describe the data used in the studies presented in this thesis and then I will discuss the strengths and limitations of these data.

2.1. Data sources

All of the data analysed in this thesis have been collected and provided by the National Cancer Registry of Ireland (NCRI). Individual patient records have been linked to prescription dispensing data from the Health Services Executive (HSE) Primary Care Reimbursement Services (PCRS) pharmacy claims database. These datasets will be described further in this chapter.

2.1.1. Patient and tumour data

The NCRI was established in in 1991, and began registering all incident cancers in the population normally resident in Ireland from 1994. Tumour registration officers are in place in hospitals throughout the country to register newly diagnosed cancer cases. Cases are primarily ascertained through pathology reports, but may also be picked up through the 'Hospital Inpatient Enquiry' system which collects administrative data on hospital discharges, and through death certificates [88]. Further information on: patient characteristics, tumour details, and treatment(s) received are obtained from a variety of sources, mainly: pathology/radiology/laboratory reports and individual patient records. Death certificates are also supplied to the NCRI from the Central Statistics Office (CSO) for linkage. Patients are followed up passively, whereby cancer cases are linked to death certificate information provided regularly by the CSO and the General Register Office [88].

Data quality at the NCRI has been examined for completeness and validity. For all cancers, excluding non-melanoma skin cancer, the NCRI data completeness is estimated to be 97%. The indicators used to assess validity were the percentage of cases which were morphologically verified or were listed as primary site unknown. The total percentage of cases which are listed as primary site

unknown in the period 2003-2007 is 2.1%. The percentage of cases with stage unknown was also examined; this was 10% for colorectal cancer and 5% for breast cancer [89].

A separate dataset of all breast cancers [International Statistical Classification of Diseases-10 (ICD-10) C50] and all colorectal cancers (ICD-10, C18, 19, 20) diagnosed between January 1st 2001 and December 31st 2011, was provided by the NCRI for this research.

2.1.2. Exposure data

The Health Services Executive (HSE) in Ireland funds a scheme known as the General Medical Services (GMS) scheme. The GMS scheme is designed to provide a number a healthcare services at no or minimal cost to those "for whom acquiring such services would present undue hardship". These services include: GP visits, dental procedures, hospital visits, and prescription medicines. Eligibility for the scheme is determined through a combination of means testing and age, with all persons aged 70 and over being eligible from July 2001 to December 2008. From January 2009, means-testing was introduced for those over the age of 70. At the end of 2011, the GMS scheme was provided to approximately 37% of the population (1.6 million people), known as medical card holders [90]. Additionally, the HSE has discretion when deciding to grant a medical card to a person whose income is in excess of the usual cut-off, but due to certain circumstances, such as a chronic or serious illness like cancer, may be under financial pressure [91]. In order to obtain a discretionary medical card, the person affected must provide evidence in support of these circumstances, for example, a medical report [92].

There are additional HSE schemes through which certain people may receive their medications at no or reduced cost. Such schemes are: the Long Term Illness Scheme (LTI) - persons who suffer from one or more specified chronic illnesses (such as diabetes or cystic fibrosis) are entitled to obtain, without charge, irrespective of income, necessary medicines and/or appliances [93] and less than 5% of the Irish population is eligible for this scheme [94], the High Tech Drugs (HTD) – some medicines are generally only prescribed or initiated in hospital, and include items such as anti-rejection drugs for transplant patients or medicines used in conjunction with chemotherapy or

growth hormones [93], and the Drugs Payment Scheme (DPS) - an individual or family in Ireland is required to pay up to €134 per month, and no more, for approved prescribed drugs. The scheme is recommended to those without LTI or GMS cover, and just under 30% of the Irish population avail of this scheme [93].

The HSE-PCRS pharmacy claims data was used in this thesis. The PCRS claims database contains detailed information on medicines dispensed under the GMS scheme. Drugs are coded according to the World Health Organization (WHO) Anatomical Therapeutic Chemical (ATC) classification system [94].

2.2. Linkage of NCRI and HSE-PCRS

The NCRI and PCRS databases have been linked by the NCRI. There are two software applications used in the data linkage: DataPipe was used to standardise the data, and Automatch was used to perform probabilistic record-linkage. Probabilistic record-linkage is used to link two sources of information together based on multiple, possibly non-unique, keys. Probabilistic linkage is a method that creates "comparisons" between individuals across at least two relatively large datasets, using linking variables. By way of an example, in a cancer registry-based study, patient information may be linked to prescribing data using non-unique first and last name combinations, and date of birth. These linked data have been used in many pharmacoepidemiological studies in the last number of years [94–97].

2.3. Ethical approval

A formal ethical approval for use of this dataset is not required as all traceable patient identifiers were removed from the data, and it is completely anonymised. The use for research of anonymised data held by the NCRI is covered by the Health (Provision of Information) Act 1997. In May 2018, the GDPR (General Data Protection Regulation) law was introduced in Ireland, European Union (EU) and the European Economic Area (EEA) [98]. This means that all patients whose data is recorded by the NCRI have enhanced rights around the collection and protection of their personal information. As such, the NCRI are currently reviewing their policy and procedures on the provision of

anonymised datasets to external researchers [99]. However, all datasets provided for this thesis were supplied and analysis completed prior to the introduction of the GDPR law.

2.4. Covariates

Covariates available in the NCRI-PCRS linked database and used in this thesis are described here. They will be considered further in each individual study, also.

2.4.1. Patient characteristics

The following socio-demographic information was collected by the NCRI, and is included in the data: gender (male/female), age at diagnosis (years), smoking status at diagnosis (current/former/never/unspecified), and deprivation level. The deprivation level is derived from a census-based measure and is a categorical, five-level indicator from 1 (least deprived) to 5 (most deprived). The deprivation measure was developed by the Small Area Health Research Unit (SAHRU) to give an estimate of material disadvantage, and is based on the following indicators: unemployment, low social class, car ownership, rented accommodation, and overcrowding [100].

Based on prescribing information in the PCRS dataset, a medication-based comorbidity score was calculated, as there are no other diagnostic codes recorded in the data. The comorbidity score was calculated based on the number of distinct drug classes which were dispensed in the year prior to diagnosis. Distinct drug classes were identified using the 4^{th} level of the ATC classification system (for example, antithrombotic agents have the 4^{th} level ATC code B01A). Medication-based comorbidity scores have been used in many pharmacoepidemiology and provide a good proxy of comorbidity [101].

2.4.2. Tumour characteristics

There are a variety of tumour-related variables collected by the NCRI and included in the linked database: AJCC TNM staging as discussed in sections 1.1.5 and 1.2.4 (I, IIa, IIb, IIIa, IIIb-c), histologic tumour grade (low, intermediate, high, unspecified), and tumour size (T1, T2, T3, T4).

For breast cancer only, the following variables were recorded: tumour morphology (lobular, ductal, other), tumour presentation (organised screening, opportunistic screening, incidental, symptomatic, unknown), oestrogen (ER), progesterone (PR), human epidermal growth factor-2 (HER-2) receptor status (positive, negative, unspecified), and anti-oestrogen therapy started in the year after breast cancer diagnosis (yes, no). For colorectal cancer only: tumour site (colon, rectum).

As the NCRI actively follow-up cases for one year, the database also records receipt of chemotherapy/radiotherapy/surgery (yes, no) in the year after diagnosis. After this one year period, follow-up is passive until notification of death via the CSO. The HSE-PCRS database was also used to identify other medication use in the year prior to diagnosis as possible confounders (exposed, unexposed): vitamin D, aspirin, anti-diabetics, non-steroidal anti-inflammatory drugs and bisphosphonates. Where a variable had a missing value, the value was coded as 'unspecified' for that individual and retained in the analyses.

2.5. Strengths and limitations of the individual and linked datasets

Strengths of the PCRS data are that it is considered to be reliable and accurate, as it is based on administrative claims data. Patients receive all their prescribed medications at no or minimal cost, so it unlikely that they will obtain medications through another means, at an increased personal cost; and claims made through the GMS scheme are mostly submitted electronically and in order for reimbursement, the pharmacist must submit the correct PCRS product code and quantity (for example, 10mg Atorvastatin (Actavis) has product code 15451) [102].

However, limitations include that there are a small proportion of patients eligible for the GMS scheme who may also receive medications through other HSE schemes as discussed in section 2.1.2, but due to lack of information to link across these schemes, this was not considered further. The PCRS data contains only information on the medication dispensed, and not patient diagnoses, nor over-the-counter (OTC) medications. Also, medication dispensing does not ensure compliance, so some misclassification may occur. The limitations of the studies included in this thesis will be discussed further in each corresponding chapter, and in the overall conclusion (Chapter 8).

Strengths of the NCRI dataset are that it is nationally representative and as discussed earlier, is both complete and valid [89]. The dataset contains comprehensive information on the patient, treatment and the tumour. However, there is no information on tumour recurrence provided in this dataset, thus limiting analyses to those investigating mortality, or a particular characteristic at diagnosis. Together, the linked HSE-PCRS and NCRI datasets provide comprehensive information on patient characteristics, tumour characteristics, and drug usage, allowing for reliable pharmacoepidemiologic research.

2.6. Background to pharmacoepidemiology

Pharmacoepidemiology is a field of observational research which studies the utilisation and effects of drugs in large numbers of people [103]. Epidemiology means 'the study of what is upon the people', comes from the Greek; "*epi"* upon, *"demos"* people, *"logos"* the study. Epidemiology includes the study of disease occurrence, prevalence, and outcomes as well as the study of the causal associations between environmental or lifestyle exposures and disease. Pharmacology is the study of the effects of drugs, and pharmacological studies can range from pre-clinical lab-based studies, to clinical trials in human participants. Pharmacoepidemiological studies bring these two disciplines together and are essential to learning more about the effects of drugs in large patient populations.

2.6.1. Pharmacoepidemiological study designs

The most commonly used study designs in pharmacoepidemiology, and the designs used in this thesis, include cohort and case-control studies. A cohort is defined as a "group of people with defined characteristics who are followed up to determine incidence of, or mortality from, some specific disease, cause of death, or some other outcome." [104]. In a cohort study, the population is identified by the exposure (eg. statin use) and followed in time until the outcome of interest occurs (eg. death), with rates of the outcome of interest then compared between the exposed and unexposed. As exposure is identified before the outcome, cohort studies have a temporal framework to assess causality and have the potential to provide the strong scientific evidence [104].

Additionally, the investigator can examine multiple outcomes simultaneously. However, this will generally require a large sample size and, depending on the outcomes, a long follow-up time [104].

Case-control studies differ from cohort studies in that they compare cases (with the outcome of interest) to controls (without the outcome of interest), and compare antecedent exposures. Case-control studies are useful in investigation of rare outcomes, or outcomes with a long latency period, as subjects are selected from the outset by their outcome status [104]. An important consideration of case-control studies is that the selected control group must be at similar risk of developing the outcome. Matching is a method that can be used to ensure comparability between cases and controls, and reduces systematic differences in the compared groups. Each case is typically paired with a control, or multiple controls, with respect to the certain variables (eg. age, sex, and race). The exposure of interest is then compared between the matched cases and the controls. [105].

2.7. Methodological challenges to pharmacoepidemiological studies of statin exposure and cancer outcomes

Observational studies of statin use and cancer outcomes pose both study design and analytical challenges due to risks of bias. The most common of which are discussed in this section.

2.7.1. Healthy user bias

This form of bias is highly prevalent in pharmacoepidemiology. The results from observational studies of statin use and cancer outcomes must be interpreted with care as there is evidence that statins are preferentially prescribed for, and taken by, patients who make better healthcare choices, engage in healthier behaviours and have superior health outcomes [106–109]. This has been shown to cause appreciable residual confounding if unaccounted for in analyses, and a tendency to overestimate any beneficial effect of statins [110,111]. Observational studies have frequently attributed a variety of non-cardiovascular health benefits to statin use [108,112,113] including protection from cancer incidence and mortality [114,115]. However, secondary analyses of randomized trial data have not confirmed these associations [113,116], and many of the findings from observational studies have subsequently been attributed to the preferential prescribing of

statins to healthier patients [106,113]. Dealing with this type of bias can be done through design approaches, for example; an active comparator group of subjects who initiated a different preventive therapy, such as glaucoma medication, rather than non-users serve as a control group. However, in this thesis I took a methods approach, whereby I adjusted for use of preventive services (mammography screening, where possible) and lifestyle factors, such as smoking [117]

2.7.2. Selective prescribing

There is some evidence to indicate that statins are also selectively prescribed, or channelled, for women with better prognosis (eg. lower stage) breast cancer. In studies by Snyder *et al*, women with later stage breast cancer were considerably less likely to be screened for hypercholesterolemia after their diagnosis [118,119]. Fully accounting for the selective prescribing of statins in analyses of cancer outcomes is challenging. For example, in an often-cited study reporting a significant association between statin use and breast cancer recurrence [120], the observed benefit was solely attributable to reductions in locoregional (ipsilateral, lymph node) and contralateral recurrences, with no reduction in distant recurrence. While standard baseline prognostic information (e.g. stage, grade, receptor status) was adjusted for, there are additional strong clinical predictors of locoregional recurrence (such as the presence of residual disease after neo-adjuvant therapy, sub-optimal lymph node evaluation at surgery, and the presence of positive tumour margins after surgery [121–124]), which may influence the prescribing and use of statins. Additionally, locoregional recurrences are strongly influenced by patients' healthcare choices, in particular decisions to forego additional surgery to re-excise positive tumour margins [124] and non-compliance with adjuvant radiation [125,126], chemotherapy or hormonal therapy [127]. The presence of residual confounding must therefore be carefully considered in studies reporting beneficial effects of statins on cancer outcomes.

2.7.3. Reverse causation

Reverse causation occurs when occurrence of the outcome of interest (eg. cancer recurrence or death) leads to changes in the exposure or measurement of the exposure. A common form of this

is called protopathic bias, whereby patients start or stop a particular medication because of symptoms of the disease under study [128]. For example, if an undetected colorectal cancer recurrence causes symptoms which leads to the use of a certain gastric drug, that drug may appear to be associated with an increased risk of recurrence when, in fact, medication initiation was a result of recurrence. A method called 'lagging' exposure may help to minimise this bias. Lagging exposure means individuals are not considered exposed to the drug of interest until a certain window of time following initiation of the drug has passed [129]. However, the use of an exposure lag may result in reduced statistical power as patients who die within the lag time window do not contribute survival time to the exposure group in the analysis.

2.7.4. Immortal time bias

Immortal time refers to a period of follow-up during which, by design, the study outcome cannot occur. In pharmacoepidemiology studies, immortal time bias is introduced when the period of immortal time is either incorrectly attributed to the treated group through a time fixed analysis [130]. For example, the time between cohort entry and the first statin prescription is ''immortal'' for the exposed participants because to have received the treatment implies that the subject ''survived'' until the first prescription. One way to reduce this bias is to use time-varying analyses whereby this immortal person-time is classified as unexposed, prior to the first statin prescription, and the subsequent person-time as exposed [131]. In all studies of association between statin use and breast or colorectal cancer outcomes in this thesis, a time-varying analysis is used to account for immortal time bias.

In the next five chapters, I will describe the individual studies included in this thesis. I will give a brief introduction to each study, followed by the methods used; results found, and brief study-specific discussion.

Chapter Three

3. Describing the patterns of statin initiation and continuation in patients with breast or colorectal cancer, towards end-of-life.[1]

3.1. Introduction

Several studies have investigated statin use in those with reduced life expectancy [132–136]. However, these are largely cross-sectional studies reporting statin exposure at the time of death. Observational studies investigating the association between statin exposure and cancer outcomes, not accounting for changes in statin utilisation towards the end-of-life, are likely to be associated with reverse causation bias, as discussed in section 2.5.3. This occurs when changes in prognosis or disease status (ie. approaching end-of-life), lead to a change in the exposure of interest (ie. statins, or other preventative, medications). There is little empirical evidence informing the choice of an appropriate exposure lag time for adjusting for reverse causation bias, as investigators do not always have an *a priori* assumption based on biological evidence [129]. It is well established that many cancer patients will have ceased statin treatment by the time of death [132,133,136,137]; in patients with reduced life expectancy, such as after a diagnosis of metastatic cancer, there may be a substantial increase in pharmacotherapeutic burden [138]. Approaching end-of-life, there can often be a treatment paradigm shift to that of palliative care. Accordingly, medications prescribed to patients with advanced cancer may be reviewed regularly and those unlikely to provide benefit, or those associated with increased risk of side-effects, can be discontinued [138]. The potential benefit of statin use in those with reduced life expectancy may be limited to high-risk patients [139], and may be considered for discontinuation in those who are unlikely to benefit. However, there is little data to describe the changes in statin exposure longitudinally prior to death.

[1] A version of this chapter has been published in Supportive Care in Cancer, PMID: 28101676

The aim of this study was to describe the changes in statin exposure longitudinally prior to death in patients in Ireland with breast or colorectal cancer, two of the most prevalent cancers [140], to establish an appropriate statin-exposure lag time for observational studies investigating cancer outcomes. In particular, to:

i) estimate the probability of initiating statin therapy in the five years prior to a death from cancer,

ii) estimate the probability of continuing statin-use towards end-of-life

iii) compare these longitudinal statin exposures with statin exposures measured in matched cancer-survivors over the same time period.

3.2. Methods

3.2.1. Setting and data sources

This study was carried out using the individual-level patient records from the National Cancer Registry Ireland (NCRI), which are linked to prescription dispensing records from Ireland's Health Services Executive (HSE) Primary Care Reimbursement Services (PCRS) pharmacy claims database. These linked datasets have been described previously in Chapter 2 (section 2.2), and also in the literature [95].

3.2.2. Cohort and exposure definitions

The study population was defined as all patients diagnosed with stage I-III, invasive breast (ICD-10 C50) or colorectal cancer (ICD-10 C18-C20), between 1st January 2001 and 31st December 2009, with continuous eligibility for the GMS scheme starting at least one year prior to diagnosis. Patients with prior invasive cancers (other than non-melanoma skin cancer) were excluded, as were men with a diagnosis of breast cancer.

From this defined study population, I identified patients who died of their cancer (cases) between 1st January 2001 and 31st December 2009, using Surveillance, Epidemiology, and End Results (SEER)

Program definitions for breast and colorectal cancer-specific death (Appendix One, Table A1.2)[141]. Patients who were alive on 31st December 2011 were identified as cancer survivors, and were used as controls. Using a greedy matching algorithm, (also known as nearest neighbour matching) [142] controls were matched to cases, separately for breast (2:1) and colorectal cancer (1:1), by tumour stage (I, II, III), age at diagnosis (5 year caliper), gender (colorectal cancer only), and pre-diagnostic statin use (yes/no). The rationale for this type of matching was to maintain similarity between cases and controls, but maximise difference in disease state – controls were alive for at least two years longer than the cases. Where cases have pre-diagnostic statin exposure, I also matched controls on the intensity of statin exposure in the year prior to diagnosis (10% caliper). The date of death for each case was used as an index date for matched controls and a reference point to calculate statin exposure. It should be noted that people were stage IV cancer were not included, due to inadequate stage IV survivors (controls) for matching.

All prescriptions for statins were identified using the PCRS database; drugs are coded using WHO-ATC drug classifications [143] (Appendix One, Table A1.1). I used the dose and number of days' supply on each statin prescription to establish longitudinal exposure histories for each patient by assigning the days' supply from each prescription (normally of 1 month duration) to sequential days from the date of dispensing [144].

For cases and matched controls, I used these statin exposure histories to calculate measures of statin use in sequential pre-defined exposure windows starting at date of death for cases, or index date for controls, and continuing up to diagnosis, or for a maximum of 5 years (whichever comes first). I chose a duration of maximum 5 years prior to death as the median survival post breast or colorectal cancer recurrence is 2-2.5 years [145,146]. First, I identified patients without statin exposure prior to diagnosis who initiated statin treatment in the time post-diagnosis within each exposure window. Second, for those patients with statin exposure prior to diagnosis, I identified patients with a supply of statins during each exposure window. From these pre-mortality measures of statin exposure, I estimated, for each exposure window;

i) the probability of starting statin use as death approached

ii) the probability of continuing statin use towards end-of-life

3.2.3. Statistical analyses

The frequency of cases and controls were tabulated by clinical and socio-demographic characteristics. Descriptive statistics were weighted by the inverse number of controls matched to each case, and standardised differences were used to assess balance ($d<0.1$) in the matched covariates [142]. The probability of i) statin initiation, and ii) maintaining statin use in each exposure window were plotted for cases and matched controls with respect to the length of time prior to death/index date.

Conditional binomial models were used to estimate relative risks (RR) and risk differences (RD) with 95% confidence intervals (CI) for: i) initiating statin treatment, and ii) continuing statin use in cases versus controls. These analyses were carried out for statin exposure immediately prior to death/index date, and repeated for consecutive preceding windows. All analyses were performed with SAS®, version 9.3 (SAS® Institute Inc, Cary, NC). Results were considered statistically significant at a two-sided α-level of 0.05.

3.3. Results

3.3.1. Study population

The selection of breast (N=8,711) or colorectal cancer (N=8,520) patients from the NCRI database is shown in a flow diagram (Figure 3.1), and the patient characteristics are shown in Table 3.1. The median age at diagnosis of patients with breast or colorectal cancer was 69 and 75 years respectively, and 28.8% of breast and 11.5% of colorectal cancer patients were taking statins in the year prior to cancer diagnosis. From these breast and colorectal cancer cohorts, I matched 1,055 breast or 1,688 colorectal cancer cases (deaths) to 1,557 and 1,668 cancer controls (survivors) respectively. The patient characteristics of these matched groups are also shown in Table 3.1.

3.3.2. Statin initiation approaching end-of-life

The results from analyses investigating statin initiation in the five years prior to death/index date are shown in Tables 3.2 and 3.3. Rates of statin initiation did not seem to differ as breast or colorectal cancer patients approached end of life. In the 6 months prior to death/index date, 1.4% of breast and 1.2% of colorectal cases initiated statin use, as compared to 2.1% and 1.4% of controls, respectively.

3.3.3. Continued statin use at end-of-life

In the five years prior to death or matched index date, I calculated the proportion of statin users who maintained statin use for both breast and colorectal cancer patients in consecutive time windows and the results are shown in Tables 3.4 and 3.5. This data is presented graphically for breast (Figure 3.2) and colorectal cancer (Figure 3.3). For both breast and colorectal cancer patients, the probability of continuing statin use was comparable in cases and controls up to approximately one year prior to death/index date. Subsequently, statin use declined for cancer cases when compared to matched cancer survivors. When compared to matched controls, the probability of continued statin use in breast cancer cases was significantly lower from 3 months prior to death (RR 0.86, 95% CI 0.79, 0.94)(Table 3.4). In colorectal cancer cases, when compared to matched controls, the probability of continued statin use was lower from 12 months prior to death (RR 0.90, 95% CI 0.81, 1.00), and significantly lower at 3 months prior to death from colorectal cancer (RR 0.77, 95% CI 0.68, 0.88)(Table 3.5).

In the week prior to death, the probability of continued statin use was 45.7% for breast cancer cases, compared to 76.5% of breast cancer controls (RR 0.60, 95% CI 0.52, 0.69); and 30.8% for colorectal cancer cases, versus 77.4% for cancer controls (HR 0.40, 95% CI 0.32, 0.49).

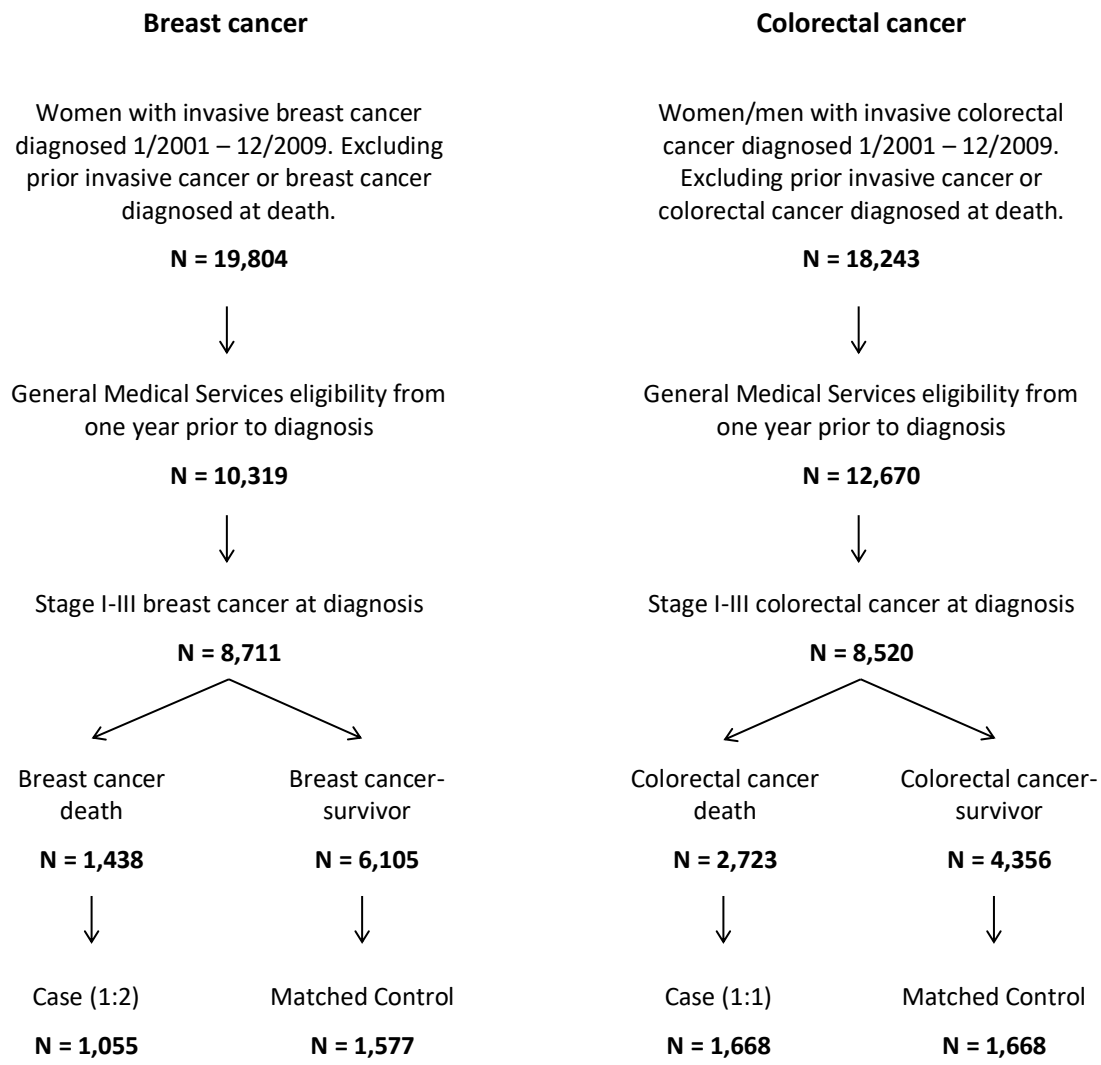

Figure 3.1: Flow chart of patient selection into the study

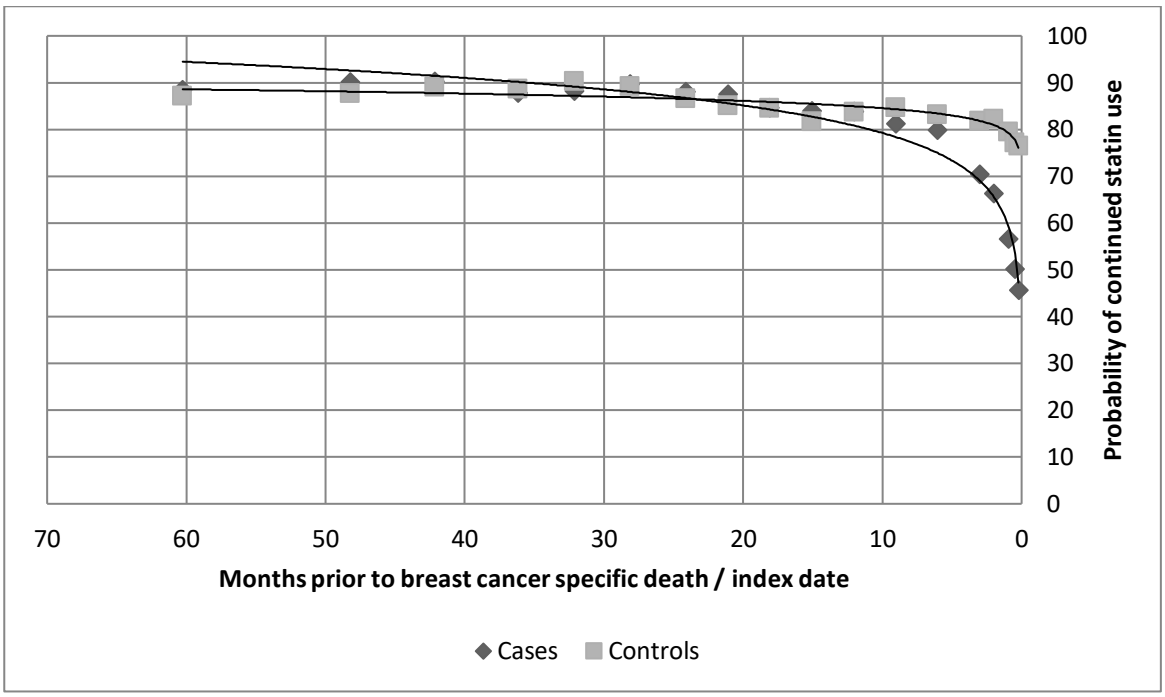

Figure 3.2: Probability of continued statin use prior to breast cancer-specific death

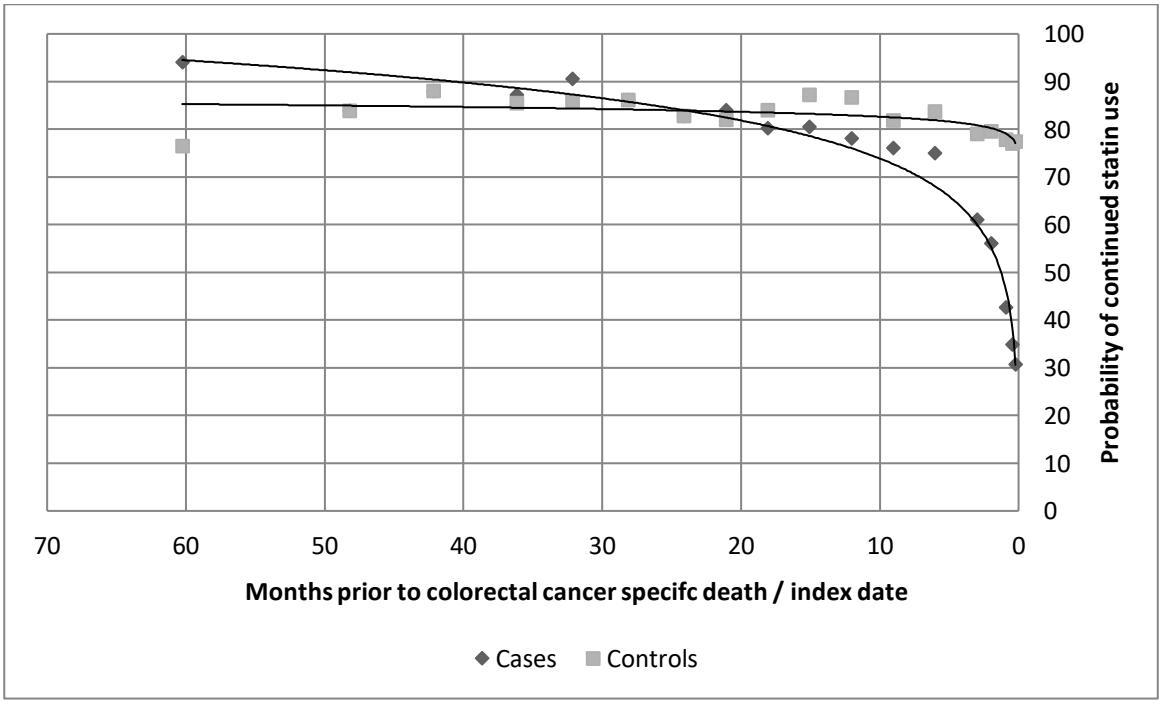

Figure 3.3: Probability of continued statin use prior to colorectal cancer-specific death

Table 3.1: Characteristics of matched cases and controls for breast cancer and colorectal cancer

Characteristic		Breast cancer			Colorectal cancer		
		All *N* = 8711	**Breast cancer death** *N* = 1,055	**Matched control** [A B C] *N* = 1,577	**All** *N* =8,520	**Colorectal cancer death** *N* = 1,668	**Matched control** [A B] *N* = 1,668
Matched covariates							
Age in years - Median (IQR) [D]		69 (57,77)	71 (58,78)	70 (59,77)	75 (70,80)	75 (69,79)	74 (68,78)
Tumour stage - N (%) [E]	I	2,651 (30.4)	141 (13.3)	244 (13.3)	1,546 (18.2)	184 (11.0)	184 (11.0)
	II	4,603 (52.9)	623 (59.1)	995 (59.1)	3,608 (42.3)	756 (45.3)	756 (45.3)
	III	1,457 (16.7)	291 (27.6)	338 (27.6)	3,366 (39.5)	728 (43.7)	728 (43.7)
Pre-diagnostic statin use - N (%) [F]		2,508 (28.8)	248 (23.5)	377 (23.5)	977 (11.5)	198 (11.9)	198 (11.9)
Pre-diagnosis statin intensity - Mean (SD) [F]		0.77 (0.3)	0.77 (0.32)	0.77 (0.26)	0.73 (0.32)	0.74 (0.32)	0.75 (0.32)
Gender Male – N (%)		- -	- -	- -	4682 (54.9)	915 (54.9)	874 (52.4)
Unmatched covariates							
Treatments - N (%) [G]	Chemo	3,260 (37.4)	449 (42.6)	772 (49.0)	2,697 (31.7)	570 (34.1)	745 (44.6)
	Radiation	5,355 (61.5)	566 (53.7)	1,102 (69.9)	1,370 (16.1)	370 (22.2)	260 (15.6)
	Surgery	7487 (86.0)	803 (76.1)	1,480 (94.9)	7971 (91.4)	1362 (81.7)	1628 (97.6)
	All three modalities	2519 (29.0)	324 (30.7)	624 (39.6)	885 (10.4)	204 (12.2)	211 (12.7)

		Breast cancer			Colorectal cancer		
Characteristic		**All** ***N* = 8711**	**Breast cancer death** ***N* = 1,055**	**Matched control [A B C]** ***N* = 1,577**	**All** ***N* =8,520**	**Colorectal cancer death** ***N* = 1,668**	**Matched control [A B]** ***N* = 1,668**
Tumour grade - N (%) [D]	1	862 (9.9)	30 (2.8)	141 (8.9)	530 (6.2)	80 (4.8)	104 (6.2)
	2	4,275 (49.1)	369 (35.0)	821 (52.1)	6,017 (70.6)	1,071 (64.2)	1,252 (45.1)
	3	2,762 (31.7)	509 (48.3)	504 (32.0)	1,045 (12.3)	270 (16.2)	184 (11.0)
	Unspecified	812 (9.3)	147 (13.9)	111 (7.0)	900 (10.6)	247 (14.8)	128 (7.7)
Smoking - N (%) [D]	Current	1,747 (20.1)	235 (22.3)	298 (18.9)	1,165 (13.7)	282 (16.9)	200 (12.0)
	Past	3,993 (45.8)	118 (11.2)	199 (12.6)	3,415 (40.1)	302 (18.1)	347 (20.8)
	Never	1,004 (11.5)	437 (41.4)	783 (49.7)	1,731 (20.3)	640 (38.4)	749 (44.9)
	Unspecified	1,967 (22.6)	265 (25.1)	297 (18.8)	2,209 (25.9)	444 (26.6)	372 (22.3)
Deprivation Score - N (%) [D]	1 - Low	1,098 (12.6)	133 (12.6)	207 (13.1)	1,225 (14.4)	211 (12.7)	269 (16.1)
	2	954 (11.0)	113 (10.7)	184 (11.6)	926 (10.9)	168 (10.1)	192 (11.5)
	3	1,091 (12.5)	129 (12.2)	196 (12.4)	1,157 (13.6)	255 (15.3)	202 (12.1)
	4	1,563 (17.9)	181 (17.1)	297 (18.8)	1,550 (18.2)	300 (18.0)	288 (16.8)
	5 - High	3,428 (39.4)	424 (40.2)	602 (38.2)	3,163 (37.1)	633 (38.0)	617 (17.3)
	Unspecified	577 (6.6)	75 (7.1)	91 (5.8)	499 (5.9)	101 (6.1)	100 (6.0)

IQR: Inter-Quartile Range; **SD**: Standard Deviation.
A) Breast cancer cases & controls matched in ratio of 1:2
B) Matched on tumor stage (I, II, III), age (5 year caliper) and pre-diagnostic statin use (yes/no). Pre-diagnostic statin users were also matched on the intensity of pre-diagnostic statin exposure (10% caliper)
C) Means and percentages for controls were weighted by the inverse number of controls matched to each case
D) At the time of cancer diagnosis
E) AJCC Cancer Staging Manual 6th Edition. Springer, 2002
F) In the year pre cancer diagnosis
G) Any chemotherapy, radiation, or surgery in the year post cancer diagnosis

Table 3.2: Relative risks (RR) & risk differences (RD) for statin initiation in five years prior to breast cancer-specific death

Statin initiation within exposure windows -	Breast cancer [A B] Cases-N (Statin Initiation %)	Controls-N (Statin Initiation %) [C]	RR (95%CI)	RD (95%CI)
49-60 months prior to death/index	243 (3.7)	361 (3.9)	1.03 (0.44, 2.41)	0.00 (-0.03, 0.03)
37-48 months prior to death/index	368 (4.1)	544 (4.1)	1.11 (0.57, 2.17)	0.00 (-0.02, 0.03)
25-36 months prior to death/index	509 (3.3)	757 (4.1)	0.63 (0.36, 1.1)	-0.02 (-0.04, 0.00)
19-24 months prior to death/index	594 (1.7)	889 (3.0)	0.6 (0.29, 1.26)	-0.01 (-0.03, 0.00)
13-18 months prior to death/index	662 (3.0)	990 (2.8)	1.03 (0.58, 1.83)	0.00 (-0.02, 0.02)
7-12 months prior to death/index	733 (1.8)	1,092 (2.2)	0.75 (0.38, 1.46)	-0.01 (-0.02, 0.01)
0-6 months prior to death/index	807 (1.4)	1,200 (2.1)	0.56 (0.29, 1.11)	-0.01 (-0.02, 0.00)

A) Breast cancer cases & cancer controls matched in ratio of 1:2.
B) Matched on tumor stage (I, II, III), age (5 year caliper) and pre-diagnostic statin use (yes/no).
C) Percentages for controls were weighted by the inverse number of controls matched to each case

Table 3.3: Relative risks (RR) & risk differences (RD) for statin initiation in five years prior to colorectal cancer-specific death

Statin initiation within exposure windows -	Colorectal cancer [A B] Cases-N (Statin Initiation %)	Controls-N (Statin Initiation %)	RR (95%CI)	RD (95%CI)
49-60 months prior to death/index	147 (5.4)	147 (5.4)	1.0 (0.37, 2.66)	0.00 (-0.05, 0.05)
37-48 months prior to death/index	279 (3.3)	279 (1.8)	1.8 (0.6, 5.37)	0.02 (-0.01, 0.04)
25-36 months prior to death/index	507 (3.6)	507 (4.1)	0.86 (0.46, 1.58)	-0.01 (-0.02, 0.01)
19-24 months prior to death/index	660 (1.1)	660 (1.2)	0.88 (0.31, 2.41)	-0.00 (-0.01, 0.01)
13-18 months prior to death/index	838 (1.2)	838 (1.1)	1.11 (0.45, 2.73)	0.00 (-0.01, 0.01)
7-12 months prior to death/index	1,042 (1.5)	1,042 (2.7)	0.57 (0.31, 1.04)	-0.01 (-0.02, 0.00)
0-6 months prior to death/index	1,470 (1.2)	1,470 (1.4)	0.85 (0.45, 1.62)	-0.00 (-0.01, 0.01)

A) Colorectal cancer cases & cancer-controls matched in ratio of 1:1.
B) Matched on tumor stage (I, II, III), age (5 year caliper) and pre-diagnostic statin use (yes/no). Colorectal cancer patients are also matched on gender (male, female)

Table 3.4: Relative risks & risk differences for continued statin use in the five years prior to breast cancer-specific death

	Breast cancer [A B]					
Statin use within exposure windows -	**Cases-N (Statin Use %)**	**Controls-N (Statin Use %) [C]**	**RR (95%CI)**		**RD (95%CI)**	
49-60 months prior to death/index	26 (88.5)	31 (87.1)	1.02	(0.85, 1.21)	0.01	(-0.14, 0.17)
43-48 months prior to death/index	51 (90.2)	65 (87.7)	1.03	(0.91, 1.16)	0.03	(-0.08, 0.13)
37-42 months prior to death/index	62 (90.3)	82 (89.0)	1.01	(0.91, 1.13)	0.01	(-0.08, 0.11)
33-36 months prior to death/index	90 (87.8)	123 (88.6)	0.99	(0.90, 1.09)	-0.01	(-0.09, 0.07)
29-32 months prior to death/index	110 (88.2)	154 (90.3)	0.98	(0.91, 1.05)	-0.02	(-0.09, 0.05)
25-28 months prior to death/index	126 (89.7)	175 (89.1)	1.01	(0.94, 1.08)	0.01	(-0.06, 0.07)
22-24 months prior to death/index	143 (88.1)	202 (86.6)	1.02	(0.95, 1.09)	0.01	(-0.05, 0.08)
19-21 months prior to death/index	160 (87.5)	229 (85.2)	1.03	(0.96, 1.1)	0.02	(-0.04, 0.08)
16-18 months prior to death/index	176 (84.7)	258 (84.5)	1.00	(0.93, 1.08)	0.00	(-0.06, 0.06)
13-15 months prior to death/index	188 (84.0)	278 (81.7)	1.03	(0.96, 1.1)	0.02	(-0.03, 0.08)

Statin use within exposure windows -	Breast cancer [A B] Cases-N (Statin Use %)	Controls-N (Statin Use %) [C]	RR (95%CI)		RD (95%CI)	
10-12 months prior to death/index	198 (83.8)	294 (83.7)	1.00	(0.93, 1.08)	0.00	(-0.06, 0.06)
7-9 months prior to death/index	214 (81.3)	321 (84.7)	0.96	(0.89, 1.03	-0.03	(-0.10, 0.03)
4-6 months prior to death/index	224 (79.9)	338 (83.1)	0.96	(0.89, 1.04)	-0.03	(-0.09, 0.03)
3 months prior to death/index	234 (70.5)	352 (81.8)	**0.86**	**(0.79, 0.94)***	**-0.11**	**(-0.18, -0.05)***
2 months prior to death/index	238 (66.4)	359 (82.2)	**0.81**	**(0.73, 0.89)***	**-0.16**	**(-0.23, -0.09)***
4-3 weeks prior to death/index	242 (56.6)	365 (79.5)	**0.71**	**(0.63, 0.80)***	**-0.23**	**(-0.30, -0.16)***
2 weeks prior to death/index	245 (50.2)	371 (77.1)	**0.65**	**(0.57, 0.74)***	**-0.27**	**(-0.34, -0.2)***
1 week prior to death/index	247 (45.7)	375 (76.5)	**0.60**	**(0.52, 0.69)***	**-0.30**	**(-0.38, -0.23)***

* P<0.05

A) Breast cancer cases & cancer-controls matched in ratio of 1:2.

B) Matched on tumor stage (I, II, III), age (5 year caliper) and pre-diagnostic statin use (yes/no). Pre-diagnostic statin users were also matched on the intensity of pre-diagnostic statin exposure (10% caliper).

C) Percentages for controls were weighted by the inverse number of controls matched to each case

Table 3.5: Relative risks & risk differences for continued statin use in the five years prior to colorectal cancer-specific death

	Colorectal cancer [A B]					
Statin use within exposure windows -	**Cases-N (Statin Use %)**	**Controls-N (Statin Use %) [C]**	**RR (95%CI)**		**RD (95%CI)**	
49-60 months prior to death/index	17 (94.1)	17 (76.5)	1.23	(0.97, 1.56)	0.18	(0.00, 0.36)
43-48 months prior to death/index	31 (83.9)	31 (83.9)	1.00	(0.83, 1.20)	0.00	(-0.15, 0.15)
37-42 months prior to death/index	42 (88.1)	42 (88.1)	1.00	(0.88, 1.14)	0.00	(-0.11, 0.11)
33-36 months prior to death/index	55 (87.3)	55 (85.5)	1.02	(0.90, 1.16)	0.02	(-0.09, 0.13)
29-32 months prior to death/index	64 (90.6)	64 (85.9)	1.05	(0.95, 1.17)	0.05	(-0.04, 0.14)
25-28 months prior to death/index	72 (86.1)	72 (86.1)	1.00	(0.89, 1.13)	0.00	(-0.10, 0.10)
22-24 months prior to death/index	87 (82.8)	87 (82.8)	1.00	(0.90, 1.12)	0.00	(-0.09, 0.09)
19-21 months prior to death/index	100 (84.0)	100 (82.0)	1.02	(0.93, 1.13)	0.02	(-0.06, 0.10)
16-18 months prior to death/index	106 (80.2)	106 (84.0)	0.96	(0.86, 1.06)	-0.04	(-0.12, 0.04)
13-15 months prior to death/index	118 (80.5)	118 (87.3)	0.92	(0.84, 1.02)	-0.07	(-0.15, 0.01)

Statin use within exposure windows -	Colorectal cancer [A B]							
	Cases-N (Statin Use %)		Controls-N (Statin Use %) [C]		RR (95%CI)		RD (95%CI)	
10-12 months prior to death/index	128	(78.1)	128	(86.7)	0.90	(0.81, 1.00)	-0.09	(-0.17, 0.00)
7-9 months prior to death/index	138	(76.1)	138	(81.9)	0.93	(0.83, 1.04)	-0.06	(-0.15, 0.03)
4-6 months prior to death/index	148	(75.0)	148	(83.8)	0.90	(0.80, 1.00)	-0.09	(-0.17, 0.00)
3 months prior to death/index	162	(61.1)	162	(79.0)	**0.77**	**(0.68, 0.88)***	**-0.18**	**(-0.27, -0.09)***
2 months prior to death/index	171	(56.1)	171	(79.5)	**0.71**	**(0.61, 0.81)***	**-0.23**	**(-0.32, -0.14)***
4-3 weeks prior to death/index	185	(42.7)	185	(77.8)	**0.55**	**(0.46, 0.66)***	**-0.35**	**(-0.44, -0.26)***
2 weeks prior to death/index	192	(34.9)	192	(77.1)	**0.45**	**(0.37, 0.55)***	**-0.42**	**(-0.51, -0.36)***
1 week prior to death/index	195	(30.8)	195	(77.4)	**0.40**	**(0.32, 0.49)***	**-0.47**	**(-0.55, -0.38)***

* P<0.05

A) Colorectal cancer cases & cancer-controls matched in ratio of 1:1.

B) Matched on tumor stage (I, II, III), age (5 year caliper) and pre-diagnostic statin use (yes/no). Pre-diagnostic statin users were also matched on the intensity of pre-diagnostic statin exposure (10% caliper). Colorectal cancer patients are also matched on gender (male, female)

C) Percentages for controls were weighted by the inverse number of controls matched to each case

3.4. Discussion

The probability of continuing statin use was found to be statistically significantly lower in the three months prior to death from cancer. This decline in statin use may be the result of a change in the health care priorities of the patient, and/or reduction in the pharmacotherapeutic burden [147]. In contrast, the number of patients initiating statin use did not differ between those who died of their cancer and those who did not. This suggests that a life-limiting diagnosis does not affect the prescribing of preventative medications. However, it should be noted that few people within the cohort started statin treatment in this time, peri-mortality.

Several studies have investigated statin use in those with reduced life expectancy [132,134,136,137,139]. In the time since these articles were published, a randomized study of statin discontinuation in the palliative care setting was carried out [148] and suggested that stopping statin therapy in patients with a limited life expectancy is safe and may be associated with improved quality of life [148]. The study showed no differences in time to cardiac event; 24 (6.3%) patients experienced a cardiovascular event (n=13; statin discontinuation arm, n=11; statin continuation arm) and patients who discontinued statin therapy reported an improved quality of life [148]. Although there are currently no clinical guidelines on ceasing statin treatment, this clinical trial suggests it is safe to do so in patients with limited life expectancy. In addition, Lindsay *et al*. have developed deprescribing guidelines (OncPal) for oncological palliative care in an Australian tertiary hospital setting, and they suggest statins as a potentially inappropriate medication that should be considered for discontinuation [149]. Given the lack of clinical guidelines on statin therapy discontinuation in the Irish setting, these studies may prove useful in the clinical decision making process in regards to medication received by patients who are approaching death.

The decision to discontinue statin treatment approaching the end of life may be the result of a decision to reduce pharmacotherapeutic burden on the patient. However, there may also be other clinical reasons for deciding to cease statins. Cancer cachexia is associated with changes in body composition and loss of muscle mass resulting in worsening functional performance, reduced

quality of life and reduced prognosis [150]. Cachexia affects approximately 50% of all cancer patients, and this figure increases to over 80% in the last two weeks of life [151]. Preclinical studies showed that treatment of animal models with simvastatin caused a further decrease in muscle mass and suggest that statin treatment in cachectic patients should be used with caution [152,153]. Malnutrition and cachexia in chemotherapy patients have been expressed as reasons for discontinuing lipid-modifying medications [132]. In addition, statins are metabolised in the liver by the cytochrome P450 enzyme family, which are altered by chemotherapeutic agents used in colorectal cancer, such as capecitabine and irinotecan, which may result in altered drug metabolism [154]. As well as reducing adverse physical effects on the cancer patient, prescribing doctors may be influenced by the psychological effects of ceasing a preventative medication. Physicians may choose not to discuss the stopping of preventative medications, in order to avoid prognostic estimates and emotional distress for the patient [137]. However, discussion of potential benefits and harms of continuing statin treatment may provide an opportunity for patient centred decision making [148].

Another important implication of this study is in the investigation of statin exposure and cancer outcomes. I show that poor cancer prognosis may influence the probability of remaining exposed to statin treatment. This is known as reverse causation and can lead to biased estimates of associations between post-diagnosis drug exposure and cancer outcomes [128]. As discussed previously, reverse causation has been highlighted as a threat to the validity of non-randomized studies, and should be dealt with through the inclusion of an exposure lag period, so as to exclude the exposure window prior to death [128]. Some studies investigating statin exposure and cancer outcomes do not consider the fact that a cancer diagnosis or changes in cancer prognosis may influence the probability of remaining exposed to statins [155–157]. The results from this study show that proximity to death does in fact influence statin exposure, even after matching on predictors of prognosis (age, stage). This study shows that rates of statin continuation decline prior to death from breast or colorectal cancer, when compared to matched cancer survivors. This occurs in the year prior to death, with rates of statin continuation becoming significantly lower from three

months prior to a breast or colorectal cancer. This suggests a minimum 3 month lag of statin exposure may be sufficient for reduction in reverse causation bias.

This study used prospectively collected, high quality longitudinal prescription information to compare the initiation and continuation of statin treatment in patients who died of breast or colorectal cancer, as compared to those who survived. This allowed me to differentiate between patterns of statin use in breast and colorectal cancer patients, and also in patients who are approaching the end of their lives. A limitation of this study is that prescription refill data is a proxy for medication use, and it may not represent patients who were admitted to hospice care. In addition, I did not have information on indication for statins or other medications in these patients, or reasons for ceasing treatment. Additionally, the study population is defined by eligibility for the GMS scheme, and therefore may over-represent patients who are older and of lower socioeconomic status.

To conclude, a significant proportion of breast and colorectal cancer patients will cease statin treatment as they approach a death from breast or colorectal cancer death. The decline in statin-use occurs up to 1 year prior to death, but becomes statistically significant at least 3 months prior to death. This decline in statin use may be due to different patient or clinical factors, such as a shift in treatment paradigm, or the development of contraindications [147]. To my knowledge this is first study to describe longitudinally the statin exposure in a cohort of breast or colorectal cancer patients in the time prior to death, compared to matched cancer-survivors. The results of this study have important implications for i) the statistical analyses of studies investigating post-diagnostic statin exposure and cancer outcomes; these results suggest that the inclusion of an exposure lag time is vital to account for reverse causation in these studies, and ii) for the shared decision making process at the end of life, whereby there may be an opportunity to re-evaluate medication burden in this patient group.

Chapter Four

4. De-novo post-diagnosis statin use and mortality in women with stage I-III breast cancer[2]

4.1. Introduction

As discussed in Chapter 1, randomized trials have demonstrated that statins are effective for the reduction of cholesterol and prevention of cardiovascular disease [158]. Statins inhibit the rate-limiting step of the cholesterol biosynthesis pathway, leading to reduced levels of mevalonate and its downstream products [159]. Many of these downstream products play important roles in cellular processes such as membrane integrity, protein synthesis, and cell signalling, and their inhibition by statins may have anticancer effects [160,161]. There is also some epidemiological evidence to suggest that statins could have a role in the management of breast cancer [120,155–157,162–165], with one study reporting a statistically significant reduction in recurrence for users of simvastatin, a lipophilic statin [120]. However, uncertainties over the benefit of statins in the adjuvant breast cancer setting remain, as any possible effect may be limited to reductions in locoregional recurrence [120] and to date no studies of statin use have reported a reduction in breast cancer-specific mortality [157,163,165]. Additionally, most studies have included women who initiated statin use prior to their breast cancer diagnosis, and it is unclear from their results what benefit may be attributable to the post-diagnostic initiation of statin treatment [120,155–157,163–165]. A clearer understanding of the effect of post-diagnostic statin initiation on breast cancer-specific mortality is necessary to inform the undertaking of clinical studies of statins for the adjuvant treatment of breast cancer [166].

This study aimed to measure associations between statin use initiated after a breast cancer diagnosis (de-novo), and breast cancer-specific and all-cause mortality, and to investigate whether

[2] A version of this chapter has been published in the British Journal of Cancer, PMID: 27482648

associations between statin use and mortality are modified by the solubility characteristics of statins or breast tumour characteristics.

4.2. Methods

4.2.1. Setting & data sources

This study was carried out using patient records from the National Cancer Registry Ireland (NCRI), which have been linked to individual-level prescription dispensing data from Ireland's Primary Care Reimbursement Services (PCRS) pharmacy claims database, as described in Chapter 2.

4.2.2. Cohort & exposure definitions

The study population comprised of women with a diagnosis of stage I-III breast cancer (ICD-10 C50) between 1st January 2001 and 31st December 2011. Women were included in the study population if they were aged between 50 and 80 years at diagnosis; had GMS eligibility from at least 1 year prior to diagnosis; and no history of invasive cancer, other than non-melanoma skin cancer. The study population was restricted by age because younger women are less likely to be prescribed statins and older women may be less likely to receive definitive cancer staging or treatment [167]. Prescriptions for statins dispensed in the year prior to breast cancer diagnosis were identified from the PCRS database, and women receiving statin therapy during this time were excluded from the study population.

Within the remaining cohort of women I identified de-novo post-diagnostic statin exposure from prescriptions dispensed between breast cancer diagnosis and the end of follow up (death or 31st December 2012, whichever occurred first). For each day of follow-up, I calculated statin dosing intensity based on the number of days' supply of statin received in the prior year [168]. These statin exposure histories were used to define the following time varying exposure categories: i) women were identified as exposed (yes/no) from the date they received their first statin prescription following diagnosis; ii) women were identified as having high-intensity exposure from the first date

they had taken a statin at an intensity of ≥80%, for longer than 1 year (i.e. receiving a statin at least 292 days out of 365 is considered high intensity, similar to measures of medication adherence).

4.2.3. Covariates & outcomes

The following patient, tumour and treatment characteristics were obtained from the NCRI database: age (years) at diagnosis, smoking status at diagnosis (never, past, current, unspecified), tumour stage (I, IIa, IIb, IIIa, IIIb-c), histologic tumour grade (low, intermediate, high, unspecified), oestrogen (ER), progesterone (PR) and human epidermal growth factor-2 (HER-2) receptor status (positive, negative, unspecified) and receipt of chemotherapy (yes, no) or radiotherapy (yes, no) in the year after diagnosis. Anti-oestrogen therapy started in the year after breast cancer diagnosis (yes, no) was identified using the PCRS database. The PCRS database was also used to identify other prescribed, and potentially confounding medication use in the year prior to diagnosis (exposed, unexposed); aspirin [169], anti-diabetics [169], non-steroidal anti-inflammatory drugs [170] and bisphosphonates [171]. The number of drug classes (4th level WHO-ATC classification) dispensed in the year before diagnosis was used as a proxy measure of co-morbidity [172]. Death certificates were used to determine the date and cause of death (all-cause or breast cancer-specific). Breast cancer-specific deaths were identified using SEER definitions for cancer-specific mortality (Appendix One, Table A1.2)[141].

4.2.4. Statistical analysis

All analyses were performed using SAS® v9.3 (SAS® Institute Inc, Cary, NC). The proportion of de-novo post-diagnostic statin users was tabulated for each covariate and differences in the rates of post-diagnostic statin initiation across covariates were compared using univariate Poisson regression. Results were regarded as significant at a two-sided α-level of 0.05. The length of time from diagnosis to statin initiation was calculated and the overall intensity of statin exposure while on treatment was calculated by expressing the number of days' supply received as a proportion of the number of days from initiation to last exposure.

For survival analyses, person time was calculated from the date of breast cancer diagnosis to the end of follow-up. Multivariate Cox regression models were used to estimate adjusted hazard ratios (HR) and 95% confidence intervals (CI) for associations between post-diagnosis de-novo statin use and breast cancer-specific and all-cause mortality. Patients were categorised as statin exposed (yes/no) from the time they received their first statin prescription. These exposures were lagged in analyses to reduce reverse causation bias, or the possibility that changes in breast cancer prognosis or treatment, for example a breast cancer recurrence or approaching death, influenced a patient's or prescriber's decision to initiate or continue statin therapy [128]. The exposure lag time was set at 2 years, the median survival time after a breast cancer recurrence [145], and varied in sensitivity analyses (0, 1, 3, 4 years). The previously described covariates were selected for inclusion in multivariable survival analyses, based on prior knowledge of patient and clinical characteristics associated with breast cancer-specific mortality.

I conducted the following subgroup analyses. Firstly, I stratified analyses by high/low exposure intensity, as described above (time varying, lagged by 2 years). Secondly, analyses were stratified by statin solubility: lipophilic (atorvastatin, fluvastatin, simvastatin), hydrophilic (pravastatin, rosuvastatin), both [68]. Prior studies have suggested that only lipophilic statin use is associated with improved breast cancer outcomes [120]. Thirdly, analyses were stratified by ER status (positive, negative, unspecified) as preclinical studies have reported differential effects of statins on ER positive and negative breast cancer cell lines [173,174]. The presence of effect modification was assessed with the inclusion of an interaction term in the multivariable model.

I conducted sensitivity analyses in which the high intensity statin exposure was defined as ≥80% intensity for longer than two consecutive years; and the time without pre-diagnostic statin exposure was extended from 1 to 3 years. To explore the results in further detail I conducted an analysis of lipophilic/hydrophilic statin use stratified by high/low exposure intensity.

4.3. Results

4.3.1. Cohort & exposure characteristics

I identified 4,243 women from the linked PCRS-NCRI database with stage I-III breast cancer, aged between 50 and 80, and not receiving a statin prescription prior to their diagnosis (Figure 4.1). The median post-diagnostic follow-up for these patients was 4.9 years and their characteristics are described in Table 4.1. Within this cohort, I identified 837 (19.7%) women who initiated statin use after their breast cancer diagnosis. The overall rate of de-novo statin initiation was 42.8 new users per thousand patient years. Rates of statin initiation were significantly higher in women with a history of diabetes, lower tumour stage at diagnosis and positive oestrogen receptor status. The median length of time from diagnosis to statin initiation was 2.1 years, the median duration of statin use was 6.7 years and the mean on-treatment exposure intensity was 86.3% (Table 4.2). Person time attributed to de-novo statin users and non-users was 2,426 and 12,369 years respectively.

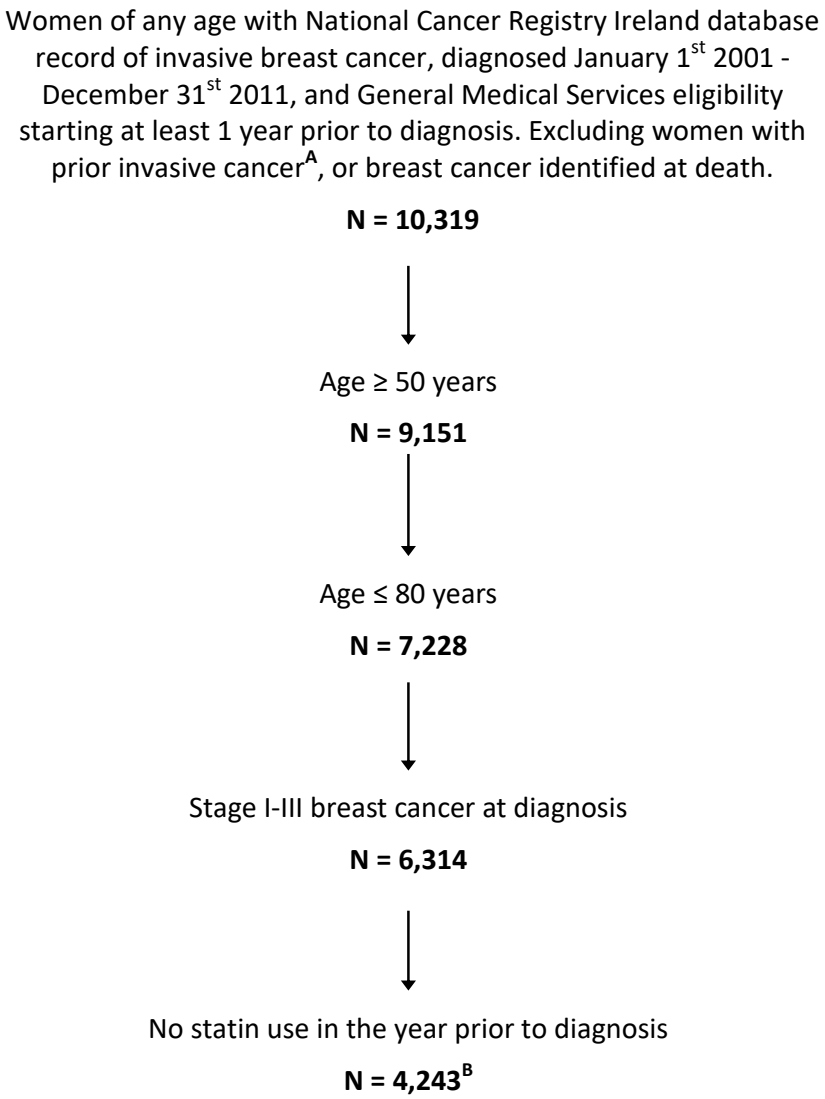

Figure 4.1: Flowchart for study cohort inclusion and exclusion criteria

A) With the exception of non-melanoma skin cancer

B) Prior to inclusion of exposure lag

4.3.2. De-novo statin use and mortality

The results from univariate and multivariate analyses of statin-use on breast cancer-specific and all-cause mortality, adjusting for patient and tumour characteristics, co-prescribed medications, and comorbidities, are shown in Table 4.2. In these I found no statistically significant association between de-novo statin initiation and breast cancer-specific mortality (HR 0.88, 95% CI 0.66, 1.17). Subgroup analyses in women taking statin at an intensity of ≥80% for longer than 12 consecutive months also yielded null associations with breast cancer-specific mortality (HR 1.04, 95% CI 0.71, 1.51). The median length of time to statin initiation in this high intensity exposure group was 2 years, the median duration of statin use was 8.5 years and the mean on-treatment exposure intensity was 89.2%. Results were unchanged in sensitivity analyses i) varying the exposure lag time from 0 to 4 years; ii) modifying the definition of high intensity exposure to ≥80% for longer than two consecutive years; and iii) increasing the pre-diagnostic period without statin exposure from one to three years (Table 4.3).

The results from subgroup analyses stratified by statin solubility characteristics (hydrophilic, lipophilic, or both) are presented in Table 4.2. I found no statistically significant associations between hydrophilic or lipophilic statin use and breast cancer-specific mortality. There appeared to be a nominal reduction in breast cancer-specific mortality for patients using lipophilic statins (HR 0.72, 95% CI 0.49, 1.04) and I explored this further in a post-hoc analysis of lipophilic/hydrophilic statin use stratified by high/low exposure intensity. In this analysis, high intensity lipophilic statin use (median duration of use 5.8 years; mean on-treatment exposure intensity was 88.2%) was not associated with a reduction in breast cancer-specific mortality (HR 1.05, 95% CI 0.67, 1.63; Table 4.2). There was no evidence of effect modification by ER status ($P_{interaction}$=0.69).

Table 4.1: Characteristics of women included in the study cohort, by post-diagnosis statin exposure

Characteristic		De-novo statin use post breast cancer diagnosis [A, B]	
		Non-user *N* = 2,759	User *N* = 837
Age in years	Median (IQR)	66 (58, 73)	65 (58, 72)
Comorbidity score [C]	Median (IQR)	6 (3, 11)	7 (3, 11)
Smoking – (%)	Current	583 (21.1)	171 (20.4)
	Past	306 (11.1)	106 (12.7)
	Never	1,324 (48.0)	422 (50.4)
	Unspecified	546 (19.8)	138 (16.5)
Aspirin – (%) [C]	Yes	432 (15.7)	153 (18.3)
	No	2327 (84.3)	684 (81.7)
NSAID – (%) [C]	Yes	1,178 (42.7)	384 (45.9)
	No	1581 (57.3)	453 (54.1)
Anti-diabetic – (%) [C] *	Yes	60 (2.2)	38 (4.5)
	No	2699 (97.8)	799 (95.5)
Bisphosphonate – (%) [C]	Yes	198 (7.2)	46 (5.5)
	No	2561 (92.8)	791 (94.5)
Tumour stage – (%) [D] *	I	917 (33.2)	297 (35.5)
	IIa	843 (30.6)	297 (35.5)
	IIb	610 (22.1)	162 (19.4)
	IIIa	166 (6.0)	40 (4.8)
	IIIb-c	223 (8.1)	41 (4.9)
Tumour grade – (%)	Low	301 (10.9)	101 (12.1)
	Intermediate	1,357 (49.2)	416 (49.7)
	High	866 (31.4)	254 (30.4)
	Unspecified	235 (8.5)	66 (7.9)
ER – (%) *	Negative	471 (17.1)	110 (13.1)
	Positive	2,028 (73.5)	610 (72.9)
	Unspecified	260 (9.4)	117 (14.0)
PR – (%)	Negative	717 (26.0)	179 (21.4)
	Positive	1,393 (50.5)	415 (49.6)
	Unspecified	649 (23.5)	243 (29.0)
HER2 – (%)	Negative	1,679 (60.9)	419 (50.1)
	Positive	339 (12.3)	99 (11.8)
	Unspecified	741 (26.9)	319 (38.1)
Chemotherapy – (%) [E]	Yes	1,123 (40.7)	344 (41.1)
	No	1636 (59.3)	493 (58.9)
Anti-Oestrogen – (%) [E]	Yes	2,065 (74.9)	642 (76.7)
	No	694 (25.1)	195 (23.3)

***Difference in statin initiation rate *P*<0.05 (Poisson regression)**

IQR: Inter-Quartile Range. **ER**: Oestrogen Receptor. **PR**: Progesterone Receptor. **HER2**: Human Epidermal Growth Factor Receptor 2. **NSAID**: Non-Steroidal Anti-Inflammatory Drug.

A) No statin use in the year prior to diagnosis and at least one statin prescription received between diagnosis and the end of follow-up, 31st December 2011.

B) Patients identified as statin users / non-users after lagging exposure by 2 years.

C) In the year prior to breast cancer diagnosis.

D) AJCC Cancer Staging Manual 6th Edition. Springer, 2002.
E) In the year post breast cancer diagnosis.

Table 4.2: Univariate and multivariate hazard ratios for associations between de-novo post-diagnostic statin use and mortality

					All-cause mortality			Breast cancer-specific mortality		
De-novo post-diagnostic statin exposure definitions	***N***	**Years on treatment (median)**	**On-treatment exposure intensity (mean %)**	**Follow-up (person years)**	**Deaths (rate) [A]**	**Univariate HR (95%CI)**	**Multivariate HR (95%CI) [B]**	**Deaths (rate) [A]**	**Univariate HR (95%CI)**	**Multivariate HR (95%CI) [B]**
Statin exposure – yes/no [C]										
Non-user	2,759	-	-	12,369	692 (55.9)	Ref -	Ref -	398 (32.2)	Ref -	Ref -
Statin user	837	6.7	86.3	2,426	128 (52.8)	0.93 (0.77, 1.14)	1.00 (0.82, 1.21)	56 (23.1)	0.79 (0.59, 1.06)	0.88 (0.66, 1.17)
Dosing intensity [C]										
Non-user	2,759	-	-	12,369	692 (55.9)	Ref -	Ref -	398 (32.2)	Ref -	Ref -
Statin user - low intensity	346	0.7	82.1	1,165	54 (46.4)	0.82 (0.62, 1.08)	0.88 (0.67, 1.17)	24 (20.6)	0.68 (0.45, 1.02)	0.76 (0.50, 1.15)
Statin user - high intensity [D]	491	8.5	89.2	1,261	74 (58.7)	1.05 (0.82, 1.35)	1.11 (0.86, 1.43)	32 (25.4)	0.92 (0.63, 1.34)	1.03 (0.71, 1.50)
Hydro/lipophilic [C]										
Non-user	2,759	-	-	12,369	692 (55.9)	Ref -	Ref -	398 (32.1)	Ref -	Ref -
Hydrophilic statin user	221	5.0	88.9	610	41 (67.2)	1.18 (0.68, 1.63)	**1.43 (1.04, 1.97)**	21 (34.4)	1.16 (0.74, 1.81)	1.35 (0.86, 2.11)
Lipophilic statin user	509	5.8	88.2	1,579	74 (46.9)	0.83 (0.65, 1.06)	0.83 (0.65, 1.06)	31 (19.6)	**0.67 (0.46, 0.97)**	0.72 (0.49, 1.04)
Both	107	7.9	71.6	236	13 (55.0)	0.98 (0.56, 1.70)	1.21 (0.69, 2.11)	4 (16.9)	0.62 (0.23, 1.66)	0.77 (0.28, 2.08)

De-novo post-diagnostic statin exposure definitions	N	Years on treatment (median)	On-treatment exposure intensity (mean %)	Follow-up (person years)	All-cause mortality: Deaths (rate) [A]	All-cause mortality: Univariate HR (95%CI)	All-cause mortality: Multivariate HR (95%CI) [B]	Breast cancer-specific mortality: Deaths (rate) [A]	Breast cancer-specific mortality: Univariate HR (95%CI)	Breast cancer-specific mortality: Multivariate HR (95%CI) [B]
Hydro/lipophilic - dosing intensity [C, E]										
Non-user	2,759	-	-	12,369	692 (55.9)	Ref -	Ref -	398 (32.1)	Ref -	Ref -
Hydrophilic statin user										
Low intensity	103	0.7	85.5	290	22 (75.9)	1.33 (0.87, 2.03)	**1.60 (1.05, 2.46)**	13 (44.8)	1.44 (0.83, 2.51)	1.68 (0.96, 2.94)
High intensity [D]	118	8.5	91.9	320	19 (59.3)	1.03 (0.65, 1.61)	1.23 (0.78, 1.92)	8 (25.0)	0.92 (0.47, 1.80)	1.07 (0.55, 2.10)
Lipophilic statin user										
Low intensity	217	0.5	85.2	805	28 (34.8)	0.62 (0.42, 0.90)	**0.63 (0.43, 0.92)**	9 (11.2)	0.37 (0.19, 0.72)	**0.39 (0.20, 0.76)**
High intensity [D]	292	8.9	90.4	774	46 (59.4)	1.07 (0.80, 1.44)	1.06 (0.79, 1.44)	22 (28.4)	0.95 (0.61, 1.48)	1.05 (0.67, 1.63)
Both	107	7.9	71.6	236	13 (55.0)	0.96 (0.48, 1.93)	1.23 (0.61, 2.48)	4 (16.9)	0.72 (0.23, 2.26)	0.91 (0.29, 2.86)

Ref: Referent Group, **HR**: Hazard Ratio, **CI**: Confidence Interval.

A) Deaths / 1,000 person years.

B) Adjusted for age at diagnosis (years); smoking status (never, past, current, unspecified); comorbidity score, tumour stage (I, IIa, IIb, IIIa, IIIb-c); tumour grade (low, intermediate, high, unspecified); ER, PR & HER2 receptor status (positive, negative, unspecified); chemotherapy in year post diagnosis (yes, no); anti-oestrogen therapy in year post diagnosis (yes, no); aspirin, bisphosphonate, NSAID & anti-diabetic medication use (yes, no).

C) Statin exposure lagged by 2 years in analysis.

D) Statin dosing intensity of ≥ 80% for ≥ 12 consecutive months defined as high dosing intensity. All other statin exposures defined as low dosing intensity

E) Analysis conducted post-hoc.

Table 4.3: Sensitivity analyses - Univariate and multivariate hazard ratios for associations between de-novo post-diagnostic statin use and mortality

					All-cause mortality			Breast cancer-specific mortality		
De-novo post-diagnostic statin exposure definitions	***N***	**Years on treatment (median)**	**On-treatment exposure intensity (mean %)**	**Follow-up (person years)**	**Deaths (rate) [A]**	**Univariate HR (95%CI)**	**Multivariate HR (95%CI) [B]**	**Deaths (rate) [A]**	**Univariate HR (95%CI)**	**Multivariate HR (95%CI) [B]**
Sensitivity analysis: yes/no exposure lagged by 0, 1, 3 & 4 years										
Statin exposure – yes/no (lag 0 years)										
Non-user	3,038	-	-	18,339	909 (49.6)	Ref -	Ref -	562 (30.7)	Ref -	Ref -
Statin user	1,205	5.7	85.6	4,496	230 (51.5)	0.94 (0.81, 1.09)	1.01 (0.87, 1.18)	107 (23.9)	0.78 (0.63, 0.97)	0.86 (0.69, 1.07)
Statin exposure – yes/no (lag 1 year)										
Non-user	3,058	-	-	15,291	804 (52.6)	Ref -	Ref -	482 (31.5)	Ref -	Ref -
Statin user	1,033	6.7	86.0	3,354	183 (54.6)	0.99 (0.84, 1.17)	1.06 (0.89, 1.25)	85 (25.3)	0.85 (0.67, 1.08)	0.94 (0.74, 1.19)
Statin exposure – yes/no (lag 3 years)										
Non-user	2,425	-	-	9,776	564 (57.7)	Ref -	Ref -	308 (31.5)	Ref -	Ref -
Statin user	640	6.1	85.9	1,686	93 (55.2)	0.99 (0.79, 1.25)	1.06 (0.84, 1.33)	40 (23.7)	0.87 (0.62, 1.22)	0.96 (0.68, 1.34)
Statin exposure – yes/no (lag 4 years)										
Non-user	2,046	-	-	7,540	427 (56.6)	Ref -	Ref -	221 (29.3)	Ref -	Ref -
Statin user	492	6.1	85.7	1,117	59 (52.8)	0.96 (0.73, 1.27)	0.99 (0.74, 1.31)	25 (22.4)	0.88 (0.57, 1.35)	0.95 (0.62, 1.46)

De-novo post-diagnostic statin exposure definitions	*N*	Years on treatment (median)	On-treatment exposure intensity (mean %)	Follow-up (person years)	All-cause mortality: Deaths (rate) [A]	All-cause mortality: Univariate HR (95%CI)	All-cause mortality: Multivariate HR (95%CI) [B]	Breast cancer-specific mortality: Deaths (rate) [A]	Breast cancer-specific mortality: Univariate HR (95%CI)	Breast cancer-specific mortality: Multivariate HR (95%CI) [B]
Sensitivity analysis: high intensity exposure ≥ 80% for ≥ 24 consecutive months [C]										
Non-user	2,759	-	-	12,369	692 (55.9)	Ref -	Ref -	398 (32.2)	Ref -	Ref -
Statin user – low intensity	480	1.6	82.8	1,613	83 (51.5)	0.91 (0.72, 1.14)	0.96 (0.76, 1.21)	37 (22.9)	0.76 (0.54, 1.06)	0.84 (0.60, 1.18)
Statin user – high intensity	357	8.5	91.0	813	45 (55.3)	1.00 (0.73, 1.36)	1.07 (0.78, 1.47)	19 (23.4)	0.88 (0.55, 1.42)	1.02 (0.63, 1.65)
Sensitivity analysis: no statin exposure in 3 years prior to diagnosis										
Statin exposure – yes/no [C]										
Non-user	2,670	-	-	12,096	677 (56.0)	Ref -	Ref -	392 (32.4)	Ref -	Ref -
Statin user	796	6.7	86.1	2,307	124 (53.8)	0.96 (0.78, 1.17)	1.03 (0.84, 1.25)	55 (23.8)	0.82 (0.61, 1.10)	0.90 (0.67, 1.21)

Ref: Referent Group, **HR**: Hazard Ratio, **CI**: Confidence Interval.

A) Deaths / 1,000 person years.

B) Adjusted for age at diagnosis (years); smoking status (never, past, current, unspecified); comorbidity score, tumour stage (I, IIa, IIb, IIIa, IIIb-c); tumour grade (low, intermediate, high, unspecified); ER, PR & HER2 receptor status (positive, negative, unspecified); chemotherapy in year post diagnosis (yes, no); anti-oestrogen therapy in year post diagnosis (yes, no); aspirin, bisphosphonate, NSAID & anti-diabetic medication use (yes, no).

C) Statin exposure lagged by 2 years in analysis.

4.4. Discussion

In this cohort of newly diagnosed breast cancer patients with stage I-III disease, I did not observe an association between de-novo post-diagnostic statin use and a reduction in breast cancer-specific mortality. The study population consisted of 4,243 women not taking a statin prior to their breast cancer diagnosis, of whom 837 initiated de-novo statin use. Within statin initiators I observed long durations of treatment, and high levels of use while on treatment, which suggests that the results are unlikely to be due to inadequate statin exposure. Additionally, in stratified analyses of high-intensity statin use (median duration >8yrs, mean treatment intensity >89%) I found consistent null or close to null estimates for all statins combined, and separately for hydrophilic or lipophilic statins. A statistically significant association with reduced breast cancer-specific mortality was observed in the low-intensity lipophilic statin subgroup. However this finding is very unlikely to be causal as the median duration of exposure in this subgroup was only six months and, as noted above, high-intensity lipophilic statin use was not associated with a reduction in breast cancer-specific mortality.

Overall, the results from this study are consistent with those from the small number of prior studies that have specifically examined de-novo post-diagnostic statin use and breast cancer-specific mortality [162,165]. In these studies, statin use initiated after diagnosis was not associated with a statistically significant improvement in breast cancer outcomes. Studies of de-novo statin use address the clinically relevant question of whether there is a benefit associated with initiating statin treatment after a breast cancer diagnosis, and their results may inform the design and conduct of clinical studies in the adjuvant setting. Several studies have also examined post-diagnostic statin use in women who initiated statin treatment prior to their breast cancer diagnosis [120,155–157,163–165] with some reporting large statistically significant reductions in breast cancer recurrence and mortality [120,155], in particular for users of lipophilic statins [120]. However, it is unclear from their results what benefit may be attributable to the post-diagnostic initiation of statin treatment.

While I observed no overall association between de-novo statin use and breast cancer-specific mortality in an unselected population, there may be specific molecular subgroups of patients for

whom statin treatment could be beneficial. In a window-of-opportunity trial by Bjarnadottir *et al*., in which women were given high dose atorvastatin (80mg/day) for two weeks between diagnosis and surgical resection of their breast tumour, statin treatment was associated with a statistically significant reduction in Ki67 proliferation index among the subgroup of women with tumours expressing HMGCR [175]. However, while the mean absolute reduction in Ki67 observed in this subgroup (4.6%) was statistically significant, it is less than that obtained with established adjuvant treatments for breast cancer, such as hormonal therapy (63.9%), and the clinical relevance of this observation is unclear [176]. Nevertheless, it would be worthwhile to evaluate tumour expression of HMGCR as a predictor of response to statin treatment in future observational studies.

Some, [120,157] but not all, [155,164] studies have suggested that associations between statin use and breast cancer outcomes may also be modified by the solubility characteristics of individual statins. However, in this study I did not observe a difference in effect between hydrophilic and lipophilic statins, overall or with high intensity use. The reasons for this between-study heterogeneity are unclear, although differences in the timing of cohort enrolment should be considered. The availability and indications for use of hydrophilic and lipophilic statins have varied considerably over time and this may result in differences between cohorts in the prescribing patterns of hydrophilic versus lipophilic statins [177,178].

This study has a number of strengths, including the use of prospectively collected breast cancer outcome and prescription refill exposure data. However, there are also some potential limitations. I could not verify whether women took the medication they received and non-compliance may have resulted in misclassification of exposure. However, I expect that women are unlikely to continue filling prescriptions for a medication they are no longer taking. I did not have information on lifestyle factors that may influence disease progression, such as obesity, and the potential for residual confounding in the analyses should be considered. Finally, when generalising the study results, it must be remembered that the study population was a subset of breast cancer cases defined by age and socioeconomic eligibility for the GMS scheme.

In conclusion, the results from this study suggest that initiating statin use after a diagnosis of stage I-III breast cancer is not associated with a reduction in breast cancer-specific mortality. I also observed no evidence of effect modification by statin solubility or hormone receptor characteristics.

Chapter Five

5. De-novo post-diagnosis statin use and mortality in people with stage I-III colorectal cancer[3]

5.1. Introduction

Two recent meta-analyses, by Gray *et al*. and Zhong *et al*., suggest that post-diagnostic statin use is associated with a non-statistically significant reduction in colorectal cancer (CRC) mortality; HR 0.84, 95% CI 0.68, 1.04 [179] and HR 0.79, 95% CI 0.58, 1.08 [180], respectively. However, these meta-analyses included only 5 studies, with varying methodological rigor; three studies did not assess statin use as a time-varying covariate [181–183], which is recommended in studies of drug-exposure and cancer outcomes [184], and none of the included studies investigate statin use only initiated after a CRC diagnosis (de-novo)[165,179,181–183], making it is difficult to determine the benefit of statin use in the adjuvant setting. Studies of de-novo statin use address the clinically relevant question of whether there is a benefit associated with initiating statin treatment after a cancer diagnosis, and their results may inform the design and conduct of clinical studies in the adjuvant setting. In a subgroup analysis by Gray *et al*., de-novo statin use after a CRC diagnosis was associated with statistically significant decrease in CRC mortality (HR 0.64, 95% CI 0.42, 0.99), however, numbers of statin-exposed individuals was small (n=24). A clearer understanding of the effect of post-diagnostic statin initiation on CRC-specific mortality is needed.

This study aimed to measure associations between statin use initiated after a CRC diagnosis (de-novo), and CRC specific and all-cause mortality, and to investigate whether associations between statin use and mortality are modified by the statin solubility or the intensity of statin use.

[3] A version of this chapter has been submitted to Cancer Epidemiology, Biomarkers and Prevention, and is currently under external review

5.2. Methods

5.2.1. Setting & data sources

This study was carried out using patient records from the National Cancer Registry Ireland (NCRI), which have been linked to individual-level prescription dispensing data from Ireland's Health Services Executive (HSE) Primary Care Reimbursement Services (PCRS) pharmacy claims database, as described in Chapter 2.

5.2.2. Cohort & exposure definitions

Adults over the age of 18 years with a new diagnosis of stage I-III colorectal cancer (ICD-10 codes: C18, C19, C20) between 1st January 2001 and 31st December 2011 were eligible for inclusion in the study population. Patients must also have had GMS eligibility from at least 1 year prior to diagnosis; and no history of invasive cancer, other than non-melanoma skin cancer. Prescriptions for statins dispensed in the year prior to colorectal cancer diagnosis were identified from the PCRS database, and patients receiving statin therapy during this time were excluded from the study population.

Within the remaining cohort, I identified de-novo post-diagnostic statin exposure from prescriptions dispensed between colorectal cancer diagnosis and the end of follow up (death or 31st December 2011, whichever occurred first). For each day of follow-up, I calculated statin dosing intensity based on the number of days' supply of statin received in the prior year [168]. These statin exposure histories were used to define the following time varying exposure categories:

i) exposed (yes/no) from the date of the first statin prescription following diagnosis;

ii) high-intensity exposure from the first date they had taken a statin at an intensity of ≥80%, for longer than 1 year)

5.2.3. Covariates & outcomes

The following patient, tumour and treatment characteristics were obtained from the NCRI database: age (years) at diagnosis, smoking status at diagnosis (never, past, current, unspecified),

socioeconomic status (1-5, least-most deprived) [100], tumour site (colon, rectum), tumour stage (I, II, III), histologic tumour grade (poorly differentiated, well/moderately differentiated, unspecified), and receipt of surgery (yes, no), chemotherapy (yes, no) or radiotherapy (yes, no) in the year after diagnosis. The number of drug classes (4th level WHO-ATC classification) dispensed in the year before diagnosis was used as a proxy measure of co-morbidity [172] - for this, the PCRS pharmacy claims database was used to identify other prescribed, and potentially confounding medication use in the year prior to diagnosis (exposed, unexposed); aspirin [169], anti-diabetics, [169] non-steroidal anti-inflammatory drugs [170] and vitamin D [185,186]. Death certificates were used to determine the date and cause of death (all-cause or colorectal cancer-specific). Colorectal cancer-specific deaths were identified using SEER definitions for cancer-specific mortality (Appendix One, Table A1.2)[141].

5.2.4. Statistical analysis

All analyses were performed using SAS® v9.3 (SAS® Institute Inc, Cary, NC). The proportion of de-novo post-diagnostic statin users was tabulated for each covariate and differences in the rates of post-diagnostic statin initiation across covariates were compared using Poisson regression. Results were regarded as significant at a two-sided α-level of 0.05. The overall intensity of statin exposure while on treatment was calculated by expressing the number of days' supply received as a proportion of the number of days from initiation to last exposure.

For survival analyses, person-time was calculated from the date of colorectal cancer diagnosis to the end of follow-up. Multivariate Cox regression models were used to estimate adjusted hazard ratios (HR) and 95% confidence intervals (CI) for associations between post-diagnosis de-novo statin use and CRC specific and all-cause mortality. Patients were categorised as statin exposed (yes/no, time-varying) from the time they received their first statin prescription. These exposures were lagged in analyses to reduce the risk of reverse causation bias [128,129], as discussed in previous chapters. The exposure lag time was set at 1 year, based on previous research [187], and varied in sensitivity analyses (0, 6 months, 2 years). The previously described covariates were

selected for inclusion in multivariable survival analyses, based on prior knowledge of patient and clinical characteristics associated with colorectal cancer-specific mortality.

I conducted the following subgroup analyses. Firstly, I stratified analyses by high/low exposure intensity, as described above (time varying, lagged by 1 year). Stratifying by high-intensity exposure is a means of determining the effect of consistent statin use, ie. at least 80% days exposed in a one-year period. Secondly, analyses were stratified by statin solubility: lipophilic (atorvastatin, fluvastatin, simvastatin), hydrophilic (pravastatin, rosuvastatin), or both [68]. Prior studies have suggested that only lipophilic statin use is associated with improved cancer outcomes [120,188,189].

I conducted sensitivity analyses in which the high intensity statin exposure was defined as ≥80% intensity for longer than two consecutive years; and the statin exposure lag was varied, as mentioned above.

5.3. Results

5.3.1. Cohort & exposure characteristics

I identified 7,544 individuals from the linked PCRS-NCRI database with stage I-III CRC, not receiving a statin prescription prior to their diagnosis (Figure 5.1). The median post-diagnostic follow-up for these patients was 2.9 years and their characteristics are described in Table 5.1. Within this cohort, I identified 828 (11%) statin initiators after CRC diagnosis. Rates of statin initiation were statistically significantly higher in individuals with a history of; aspirin, Vitamin D, NSAID and anti-diabetic medication use, and lower tumour stage and grade at diagnosis. There is also a statistically significant difference in smoking rates between statin users and non-users, but this appears to be driven by the number of people with unspecified smoking status in the exposure groups. The median duration of statin use was 1.9 years and the average on-treatment exposure intensity was 36% (Table 5.2). Person-time attributed to de-novo statin users and non-users was 3,453 and 24,012 years respectively.

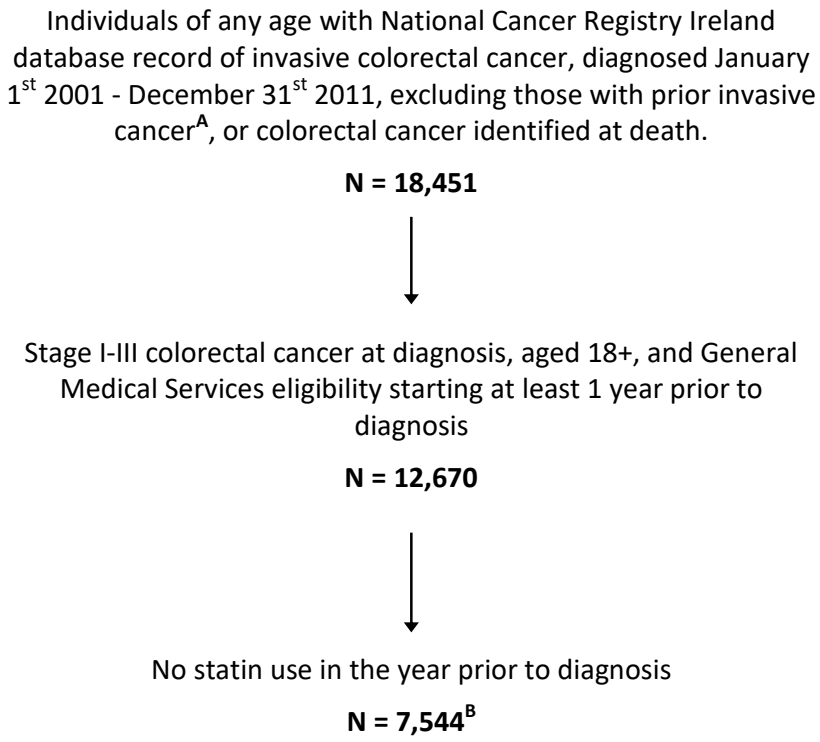

Figure 5.1: Flowchart for study cohort inclusion and exclusion criteria

A) With the exception on non-melanoma skin cancer

B) Prior to inclusion of statin exposure lag

5.3.2. De-novo statin use and mortality

The results from univariate and multivariate analyses of statin-use on colorectal cancer-specific and all-cause mortality, adjusting for patient and tumour characteristics, co-prescribed medications, and comorbidities, are shown in Table 5.2. I found no significant association between de-novo statin initiation and CRC-specific mortality in univariate and multivariate adjusted analyses; (HR 0.92, 95% CI 0.75, 1.14) and (HR 0.96, 95% CI 0.78, 1.19), respectively. Multivariate subgroup analyses in individuals taking statin at an intensity of ≥80% for longer than 12 consecutive months also yielded non-significant associations with CRC-specific mortality (HR 0.93, 95% CI 0.69, 1.25). The results remained unchanged in sensitivity analyses i) varying the exposure lag time from 0 to 2 years (Table 5.3); and ii) modifying the definition of high intensity exposure to ≥80% for longer than two consecutive years (Table 5.3).

The results from subgroup analyses stratified by statin solubility characteristics (hydrophilic, lipophilic, or both) are presented in Table 5.2. I found no statistically significant associations between hydrophilic or lipophilic statin use and CRC-specific mortality.

Table 5.1: Characteristics of people included in the study cohort, by post-diagnostic statin exposure

Characteristic [A,B]		De-novo statin use post CRC diagnosis [A, B] Non-user *N* = 5,341	User *N* = 715
Age in years	Median (IQR)	74 (68, 80)	74 (68,78)
Gender	Male (%)	2391 (44.03)	299 (41.82)
	Female (%)	3040 (55.97)	416 (58.18)
Comorbidity score [C]	Median (IQR)	0 (0,4)	8 (4, 13)
Smoking – (%)*	Current	746 (13.74)	113 (15.80)
	Past	1071 (19.72)	168 (23.50)
	Never	2188 (40.29)	314 (43.92)
	Unspecified	1426 (26.26)	120 (16.78)
Deprivation Index – (%)	1	800 (14.73)	103 (14.41)
	2	587 (10.81)	69 (9.65)
	3	724 (13.33)	99 (13.85)
	4	1000 (18.41)	133 (18.60)
	5	2000 (36.83)	269 (37.62)
	Unspecified	320 (5.89)	42 (5.87)
Aspirin – (%) [C] *	Yes	415 (7.64)	227 (31.75)
	No	5016 (92.36)	488 (68.25)
Anti-diabetic – (%) [C] *	Yes	76 (1.40)	59 (8.25)
	No	5355 (98.60)	656 (91.75)
NSAID – (%) [C] *	Yes	666 (12.26)	285 (39.86)
	No	4765 (87.74)	430 (60.14)
Vit D – (%) [C] *	Yes	76 (1.4)	59 (8.25)
	No	5355 (98.60)	656 (91.75)
Tumour stage – (%) *	I	1014 (18.67)	184 (25.73)
	II	2263 (41.67)	334 (46.71)
	III	2154 (39.66)	197 (27.55)
Tumour grade – (%)*	Low	313 (5.76)	66 (9.23)
	Intermediate	4071 (74.96)	499 (69.79)
	High	598 (11.01)	69 (9.65)
	Unspecified	449 (8.27)	81 (11.33)
Tumour site – (%)	Colon	3571 (65.75)	463 (64.76)
	Rectum	1860 (34.25)	252 (35.24)
Chemotherapy – (%) [D]	Yes	1966 (36.20)	246 (34.41)
	No	3465 (63.80)	469 (65.59)

Characteristic [A,B]		De-novo statin use post CRC diagnosis [A, B] Non-user *N* = 5,341	User *N* = 715
Radiotherapy – (%) [D]	Yes	937 (17.25)	110 (15.38)
	No	4494 (82.75)	605 (84.62)
Surgery – (%) [D]	Yes	5171 (95.21)	703 (98.32)
	No	260 (4.79)	12 (1.68)

***Difference in statin initiation rate *P*<0.05 (Poisson regression)**
IQR: Inter-Quartile Range. **NSAID**: Non-Steroidal Anti-Inflammatory Drug.
A) No statin use in the year prior to diagnosis and at least one statin prescription received between diagnosis and the end of follow-up, 31st December 2011.
B) Patients identified as statin users / non-users after lagging exposure by 1 year.
C) In the year prior to colorectal cancer diagnosis.
D) In the year post colorectal cancer diagnosis.

Table 5.2: Univariate and multivariate hazard ratios for associations between de-novo post-diagnostic statin use and mortality

					All-cause mortality			Colorectal cancer-specific mortality		
De-novo post-diagnostic statin exposure definitions	***N***	**Years on treatment (median)**	**On-treatment exposure intensity (mean %)**	**Follow-up (person years)**	**Deaths (rate) [A]**	**Univariate HR (95%CI)**	**Multivariate HR (95%CI) [B]**	**Deaths (rate) [A]**	**Univariate HR (95%CI)**	**Multivariate HR (95%CI) [B]**
Statin exposure – yes/no [C]										
Non-user	5431	-	-	18141	1936 (106.7)	Ref -	Ref -	1244 (68.6)	Ref -	Ref -
Statin user	715	1.9	36%	2682	256 (95.4)	1.06 (0.93, 1.22)	1.04 (0.91, 1.20)	107 (39.9)	0.92 (0.75, 1.14)	0.96 (0.78, 1.19)
Dosing intensity [C]										
Non-user	5431	-	-	18141	1936 (106.7)	Ref -	Ref -	1244 (68.6)	Ref -	Ref -
Statin user - low intensity	266	0.2	10%	1196	122 (101.9)	1.09 (0.90, 1.31)	1.05 (0.87, 1.27)	56 (46.8)	0.94 (0.71, 1.23)	0.93 (0.71, 1.22)
Statin user - high intensity [D]	449	3.3	51%	1486	134 (90.2)	1.04 (0.87, 1.26)	1.04 (0.86, 1.26)	51 (34.3)	0.90 (0.67, 1.21)	0.93 (0.69, 1.25)
Hydro/lipophilic [C]										
Non-user	5431	-	-	18141	1936 (106.7)	Ref -	Ref -	1244 (68.6)	Ref -	Ref -
Hydrophilic statin user	165	1.4	32%	679	64 (94.1)	1.03 (0.80, 1.33)	1.04 (0.80, 1.34)	27 (39.7)	0.88 (0.60, 1.29)	0.92 (0.63, 1.40)
Lipophilic statin user	460	1.7	35%	1684	173 (102.7)	1.14 (0.97, 1.34)	1.08 (0.92, 1.28)	69 (41.0)	0.94 (0.73, 1.20)	0.95 (0.74, 1.23)
Both	90	2.9	45%	318	19 (59.7)	0.69 (0.44, 1.09)	0.80 (0.50, 1.26)	11 (34.6)	0.93 (0.51, 1.70)	1.16 (0.64, 2.13)

Ref: Referent Group, **HR**: Hazard Ratio, **CI**: Confidence Interval.

A) Deaths / 1,000 person years.

B) Adjusted for age at diagnosis (years); smoking status, socioeconomic status, comorbidity score, tumour stage, tumour grade, chemotherapy/radiotherapy/surgery in year post diagnosis (yes, no), Vitamin D, aspirin, NSAID & anti-diabetic medication use (yes, no).

C) Statin exposure lagged by 1 year in analysis.

D) Statin dosing intensity of ≥ 80% for ≥ 12 consecutive months defined as high dosing intensity. All other statin exposures defined as low dosing intensity

Table 5.3: Sensitivity analyses - Univariate and multivariate hazard ratios for associations between de-novo post-diagnostic statin use and mortality

					All-cause mortality			Colorectal cancer-specific mortality		
De-novo post-diagnostic statin exposure definitions	***N***	**Years on treatment (median)**	**On-treatment exposure intensity (mean %)**	**Follow-up (person years)**	**Deaths (rate) [A]**	**Univariate HR (95%CI)**	**Multivariate HR (95%CI) [B]**	**Deaths (rate) [A]**	**Univariate HR (95%CI)**	**Multivariate HR (95%CI) [B]**
Sensitivity analysis: vary statin exposure lag										
Statin exposure – yes/no (lag 0 years)										
Non-user	6716	-	-	24012	3249 (135.3)	Ref -	Ref -	2227 (92.7)	Ref -	Ref -
Statin user	828	1.9	36%	3453	341 (98.7)	1.04 (0.92, 1.17)	0.99 (0.88, 1.12)	156 (45.2)	0.91 (0.76, 1.08)	0.89 (0.75, 1.06)
Statin exposure – yes/no (lag 6 months)										
Non-user	5809	-	-	20952	2331 (111.3)	Ref -	Ref -	1555 (74.2)	Ref -	Ref -
Statin user	772	2.5	36%	3053	296 (97.0)	1.06 (0.93, 1.21)	1.04 (0.91, 1.19)	131 (42.9)	0.93 (0.77, 1.12)	0.97 (0.80, 1.17)
Statin exposure – yes/no (lag 2 years)										
Non-user	4190	-	-	13339	1326 (99.4)	Ref -	Ref -	754 (56.5)	Ref -	Ref -
Statin user	608	1.7	36%	2028	194 (65.7)	1.12 (0.95, 1.31)	1.11 (0.94, 1.30)	76 (37.5)	1.00 (0.78, 1.28)	1.07 (0.83, 1.39)
Sensitivity analysis: high intensity exposure ≥ 80% for ≥ 24 consecutive months [C]										
Non-user	5431	-	-	18141	1936 (106.7)	Ref -	Ref -	1244 (68.6)	Ref -	Ref -
Statin user – low intensity	358	0.4	15%	1659	160 (96.4)	1.04 (0.88, 1.23)	1.02 (0.86, 1.20)	73 (44.0)	0.91 (0.71, 1.16)	0.91 (0.71, 1.16)
Statin user – high intensity	357	3.8	57%	1022	96 (93.9)	1.12 (0.89, 1.39)	1.10 (0.86, 1.38)	34 (33.2)	0.95 (0.67, 1.37)	0.98 (0.68, 1.40)

Ref: Referent Group, **HR**: Hazard Ratio, **CI**: Confidence Interval.

A) Deaths / 1,000 person years.

B) Adjusted for age at diagnosis (years); smoking status, socioeconomic status, comorbidity score, tumour stage, tumour grade, chemotherapy/radiotherapy/surgery in year post diagnosis (yes, no), Vitamin D, aspirin, NSAID & anti-diabetic medication use (yes, no).

C) Statin exposure lagged by 1 year in analysis.

5.4. Discussion

In this cohort of newly diagnosed CRC patients, I did not observe an association between de-novo post-diagnostic statin use and a reduction in CRC-specific or all-cause mortality. Results remained unchanged when stratified by i) intensity of statin use, and ii) type of statin received (Table 5.2).

As mentioned, two recent meta-analyses, by Gray *et al*. and Zhong *et al*., suggest that post-diagnostic statin use is associated with a non-statistically significant reduction in CRC mortality (HR 0.84, 95% CI 0.68, 1.04)(5) and (HR 0.79, 95% CI 0.58, 1.08)(6), respectively. These meta-analyses included 5 studies, with varying methodological rigor; three studies did not assess statin use as a time-varying covariate (7–9), which is recommended in studies of drug-exposure and cancer outcomes, as time-fixed analyses can often lead to an overestimation of the treatment effect (10). None of the included studies investigate statin use only initiated after a CRC diagnosis as a main analysis (de-novo)(5,7–9,11), making it is difficult to determine the benefit of statin use in the adjuvant setting, as a proportion of the benefit may be due to pre-diagnostic exposure. The largest of the included studies, by Gray *et al*., observed similar results to the current study, i.e. no significant association with CRC mortality in post-diagnostic statin users compared to non-users (HR 0.90, 95% CI 0.77, 1.05)[179]. When they restricted analyses to those with de-novo statin use (new initiators) post diagnosis, the effect was much stronger (HR 0.64, 95% CI 0.42, 0.99), although it should be noted, the estimate is much less precise due to reduced sample size (exposed n=24, unexposed n=434) [179].

The proposed mechanisms through which statins can affect colorectal cancer are accumulating. Inhibition of HMGCR by statins leads to a decrease in cholesterol synthesis, but also to reduced generation of other intermediates of the mevalonate pathway, including non-sterol isoprenoids, farnesyl pyrophosphate (FPP) and geranyl pyrophosphate (GPP) [68]. FFP and GPP are strongly implicated in carcinogenesis [190]. HMGCR-independent pathways have also been implicated, including effects on inflammation [191], and angiogenesis [192].

While I observed no overall association between de-novo statin use and CRC mortality in this population, it remains unclear as to whether there may be specific molecular subgroups of patients for whom statin treatment might be beneficial. In a cohort study investigating statin use and colon cancer-specific survival, Gray *et al* found no association between perioperative statin use and CRC survival when stratified by HMGCR expression (high/low) or KRAS mutation status. However, statin-users were more likely to have high-HMGCR expression [193]. In a study by Bengtsson *et al*, CRC patients with high expression of HMGCR were less likely to have distant metastases and vascular invasion at diagnosis, but no significant improvement in CRC-specific survival [194]. Despite these conflicting findings, it would be worthwhile to evaluate the relationship between statin use, tumour expression of HMGCR, and CRC cancer outcomes in future studies.

This study has a number of strengths, including the use of prospectively collected CRC outcome and statin exposure data from pharmacy claims. However, there are also some potential limitations. I could not verify whether participants took the medication they received and non-compliance may have resulted in misclassification of exposure. However, I expect that statin users are unlikely to continue filling prescriptions for a medication they are no longer taking. In addition, the follow-up period was relatively short (2.9 years) and may not be sufficient to determine the potential beneficial effects of statin use in this setting. There were some statistically significant differences between statin users and non-users, and although I adjusted for many important patient and tumour characteristics in analyses, and the potential for residual confounding in the analyses should be considered. The study population was a subset of CRC cases defined by age and socioeconomic eligibility for the GMS scheme; therefore, generalisability of the findings needs to be considered.

In conclusion, the results from this study suggest that initiating statin use after a diagnosis of stage I-III CRC is not significantly associated with in CRC-specific, or all-cause mortality. I also observed no evidence of effect modification by statin solubility or intensity of statin use.

Chapter Six

6. Pre-diagnostic statin use, lymph node status, and mortality in women with stage I-III breast cancer[4]

6.1. Introduction

As discussed, statins are widely used for the prevention of cardiovascular disease and it is estimated that up to 30% of Americans over the age of 40 years of age receive statins, with utilization similar across Europe [73,195]. Potentially anti-cancer effects of statins involve the reduction of downstream products in the cholesterol pathway, which play important roles in cellular processes such as membrane integrity, protein synthesis, and cell signalling [160,161]. In addition, a recent study suggests that statin treatment may have breast tumour anti-proliferative properties due to effects on cell cycle regulators P21 and P27 [196]. A window-of-opportunity trial has shown that treatment of breast cancer patients with short duration, high-dose atorvastatin (80mg/day) results in decreased tumour proliferation and an increase in tumour HMGCR expression [175]. Interestingly, Brennan *et al*. found that breast cancer patients with high tumour HMGCR expression were more likely to have smaller, node negative cancer [197]. However, this study did not record information on prescribed medications in these patients, and could not assess the potential effect of statin use.

A recent meta-analysis suggests that pre-diagnostic statin use is associated with significantly improved cancer-specific survival (HR 0.73, 95% CI 0.61, 0.89) in women with breast cancer [180]. In a study by Ahern *et al*., statin use was associated with reduced breast cancer recurrence; this benefit was observed only in women with ER+ tumours (HR 0.69, 95% CI 0.55, 0.88) and not in women with ER- tumours (HR 0.75, 95% 0.47, 1.2)[120]. This effect modification by ER status has not yet been observed in studies investigating statin exposure and breast cancer-specific survival [198].

[4] A version of this chapter has been published in the British Journal of Cancer, PMID: 28720842

In this study, I investigate associations between pre-diagnostic statin use and: i) lymph node status at diagnosis, and ii) breast cancer-specific and all-cause mortality, and (iii) whether any associations with were modified by estrogen receptor status

6.2. Methods

6.2.1. Setting & data sources

This cohort study was carried out using records from the National Cancer Registry Ireland (NCRI), which are linked to individual-level prescription dispensing data from Ireland's Primary Care Reimbursement Services (PCRS) pharmacy claims database, as described in Chapter 2.

6.2.2. Cohort & exposure definitions

The study population comprised of women diagnosed with stage I-III breast cancer (ICD-10 C50) between 1st January 2001 and 31st December 2011. Women were included in the study population if they were aged 50-80 years at diagnosis; had GMS coverage from at least 1 year prior to diagnosis; and no history of invasive cancer, other than non-melanoma skin cancer. As in Chapter 4, the study population was restricted by age because younger women are less likely to be prescribed statins and older women may be less likely to receive definitive cancer staging and treatment [167].

I identified pre-diagnostic statin prescriptions dispensed to the women in the study cohort from the PCRS database using WHO-ATC classifications (Appendix One, Table A1.1). For each day of follow-up, I calculated statin dosing intensity from the number of days' supply of statin received in the prior year [168]. These statin exposure histories were used to define the following time-varying exposure categories: i) women were identified as exposed (yes/no) from the date they received their first statin prescription; ii) women were identified as having high-intensity exposure once they had taken a statin at an intensity of ≥80%, for at least 1 year (eg., a statin supply for at least 292 out of a 365 day period was considered high intensity). The overall intensity of statin exposure while on treatment was calculated by expressing the number of days' supply received as a proportion of the number of days from initiation to last exposure. Patients with de-novo post-diagnostic statin

use were excluded from analyses, so as to determine the effect of statin use in patients with pre-diagnostic use.

6.2.3. Covariates & outcomes

The NCRI database was used to identify lymph node status at diagnosis (positive, negative). Women were lymph node positive if they had a nodal status of N1/2/3. The following information was also obtained from the NCRI database: age (years) at diagnosis, smoking status at diagnosis (never, past, current, unspecified), tumour presentation (organised screening, opportunistic screening, incidental, symptomatic, unknown), tumour size (T1, T2, T3, T4), tumour stage (I, IIa, IIb, IIIa, IIIb-c), histologic tumour grade (low, intermediate, high, unspecified), oestrogen (ER), progesterone (PR), human epidermal growth factor-2 (HER-2) receptor status (positive, negative, unspecified) and receipt of chemotherapy (yes, no) in the year after diagnosis. Anti-oestrogen therapy started in the year after breast cancer diagnosis (yes, no) was identified using the PCRS database (Appendix One, Table A1.2). The PCRS database was also used to identify other potentially confounding medication use in the year prior to diagnosis (exposed, unexposed); aspirin [169], anti-diabetics [169], non-steroidal anti-inflammatory drugs [170], and bisphosphonates [171]. The number of drug classes (4th level WHO-ATC classification) dispensed in the year before diagnosis was used as a proxy measure of co-morbidity [172]. Death certificates were used to determine the date and cause of death. Breast cancer-specific deaths were identified using SEER definitions (Appendix One, Table A1.2)[141].

6.2.4. Statistical analysis

The proportion of statin-users and non-users was tabulated for each covariate and differences in the rates of statin use across covariates were compared using univariate Poisson regression. Univariate and multivariate log-binomial models were used to estimate relative risks (RR) and 95% confidence intervals (CI's) for associations between pre-diagnostic statin use and lymph node negative breast cancer at diagnosis.

In survival analyses, multivariate Cox proportional hazards models were used to estimate adjusted hazard ratios (HR) and 95% CI's for associations between pre-diagnostic statin use and breast cancer-specific and all-cause mortality. Women were categorised as statin exposed (yes/no) from the time they received their first statin prescription. These exposures were lagged by 1 year in survival analyses to reduce reverse causation bias, as previously discussed [128,187].

The following pre-planned subgroup analyses were applied to both lymph-node status analyses and survival analyses. Firstly, analyses were stratified by ER status (positive, negative, unspecified). In survival analyses, the presence of effect modification by ER status was assessed with the inclusion of an interaction term in the multivariable model. Secondly, as prior studies have suggested that only lipophilic statin use is associated with improved breast cancer outcomes [120] analyses were also stratified by statin solubility: lipophilic (atorvastatin, fluvastatin, simvastatin), hydrophilic (pravastatin, rosuvastatin), both [68]. Finally, I stratified analyses by high/low exposure intensity. The previously described covariates were selected for inclusion in multivariable analyses, based on prior knowledge of patient and clinical characteristics associated with breast cancer-specific mortality.

I conducted the following sensitivity analyses; i) associations between pre-diagnostic statin use and lymph node status, all-cause and cancer-specific mortality were assessed with stratification by mode of tumour presentation, ii) in survival analyses, high intensity statin exposure was defined as ≥80% intensity for longer than two consecutive years, and iii) in survival analyses, statin exposure lag time was varied (0, 6 months, 2 years) to account for possible reverse causation bias, as mentioned above. All analyses were performed using SAS® v9.3 (SAS® Institute Inc, Cary, NC). Results were regarded as significant at a two-sided α-level of 0.05.

6.3. Results

6.3.1. Cohort and exposure characteristics

I identified 6,314 women eligible for inclusion in the study (Figure 6.1). The characteristics of pre-diagnostic statin users (n=2,082) and nonusers (n=4,232) are presented in Table 6.1. There were some statistically significant differences in the baseline characteristics between users and non-users; statin users were significantly older and had a significantly higher comorbidity score than non-users. Statin users were also significantly more likely to be prescribed aspirin, NSAIDs, anti-diabetics, and bisphosphonates.

6.3.2. Pre-diagnostic statin use and lymph node status

Relative risks for associations between pre-diagnostic statin use and lymph node negative breast cancer are presented in Table 6.2. The proportion of women with node-negative status in the statin user and non-user groups was 54% and 53%, respectively. No significant association was found between pre-diagnostic statin use and lymph node negative status at diagnosis, in both univariate (RR 1.01, 95% CI 0.96, 1.06) and multivariate adjusted analyses (RR 1.00, 95% CI 0.98, 1.03) (Table 6.2). Analyses stratified by; high intensity statin use, duration of statin use, and type of statin received, also yielded null findings (Table 6.2). No effect modification was observed by ER status, or mode of tumour presentation (Table 6.2). In univariate analyses, statin-users with breast cancers diagnosed through mammography screening were significantly more likely to be lymph node negative (RR 1.32, 95% CI 1.23, 1.43); however, this effect was non-significant in multivariate adjusted analyses (RR 1.01, 95% CI 0.95, 1.08) (Table 6.2).

6.3.3. Pre-diagnostic statin use and mortality

After lagging statin exposure by 1 year, I identified 2,024 women with pre-diagnostic statin use. In multivariate adjusted survival analyses, pre-diagnostic statin use was associated with a significant, 19% reduction in breast cancer-specific mortality (HR 0.81, 95% CI 0.68, 0.96) and a significant 22% reduction in all-cause mortality (HR 0.78, 95% CI 0.69, 0.89)(Table 6.3). This cancer-specific survival benefit was observed in women with high intensity use (HR 0.70, 95% CI 0.52, 0.94) but not in those

with low intensity statin use (HR 0.90, 95% CI 0.67, 1.2). In analyses stratified by type of statin received (hydrophilic, lipophilic, both), survival benefit was significant in women who received a lipophilic statin (HR 0.76, 95% CI 0.61, 0.95) but not hydrophilic statin.

In multivariate survival analyses stratified by mode of tumour presentation, a similar effect on all-cause (HR 0.78, 95% CI 0.68, 0.90) and breast cancer-specific (HR 0.83, 95% CI 0.68, 1.00) mortality was seen in those with tumours diagnosed through symptomatic presentation. This effect was not seen in women with tumours diagnosed through organised screening; however, this may be due to fewer numbers of women in this subgroup (Table 6.3).

In analyses of effect-modification by ER status, pre-diagnostic statin use was associated with a more marked, statistically significant, 31% reduction in breast cancer-specific mortality in patients with ER+ tumours (HR 0.69, 95% CI 0.55, 0.85) ($P_{interaction} < 0.01$) (Table 15). This survival benefit was not observed in women with ER- tumours (HR 1.10, 95% CI 0.81, 1.10) (Table 6.3).

In sensitivity analyses, a similar reduction in breast cancer-specific mortality was observed when high intensity exposure window was increased to 2 years (HR 0.67, 95% CI 0.47, 0.94) (Table 6.4). Again, a similar effect was seen when varying the statin exposure lag time in survival analyses (Table 6.4).

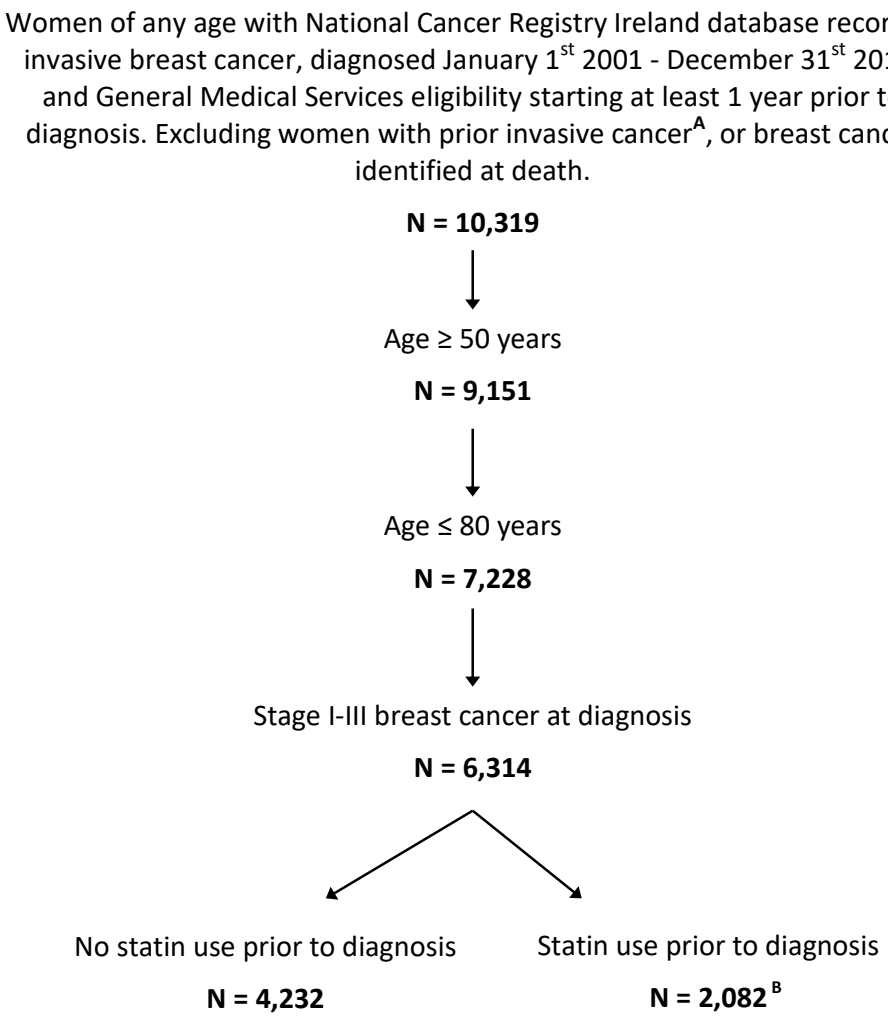

Figure 6.1: Flowchart for study cohort inclusion and exclusion criteria.

A) With the exception of non-melanoma skin cancer.

B) Prior to inclusion of statin exposure lag

Table 6.1: Characteristics of women selected for inclusion in study cohort

Characteristic		Statin use prior to diagnosis	
		Non-user *N* = 4,232	User *N* = 2,082
Age in years*	Median (IQR)	67 (58, 74)	71 (63, 75)
Comorbidity score *	Median (IQR)	7 (3, 11)	11 (7, 16)
Smoking – (%)	Current	885 (20.9)	381 (18.3)
	Past	490 (11.6)	262 (12.6)
	Never	2009 (47.5)	994 (47.7)
	Unspecified	848 (20.0)	445 (21.4)
Tumour presentation (%)	Screening; organized	750 (17.7)	324 (15.6)
	Screening; opportunistic	51 (1.2)	28 (1.3)
	Screening; unspecified	151 (3.8)	86 (4.1)
	Incidental	87 (2.1)	46 (2.2)
	Symptomatic	2990 (70.7)	1476 (70.9)
	Unspecified	203 (4.8)	122 (5.9)
Tumour Morphology (%)	Lobular	527 (12.5)	273 (13.1)
	Ductal	3098 (73.2)	1543 (74.1)
	Other	607 (14.3)	266 (12.8)
Aspirin – (%)*	Yes	713 (16.9)	1061 (51.0)
	No	3519 (83.1)	1021 (49.0)
NSAID – (%)*	Yes	1848 (43.7)	988 (47.5)
	No	2384 (56.3)	1094 (52.5)
Anti-diabetic – (%)*	Yes	143 (3.4)	330 (15.9)
	No	4089 (96.6)	1752 (84.1)
Chemotherapy – (%)[a]*	Yes	1685 (39.8)	718 (34.5)
	No	2547 (60.2)	1364 (65.5)
Anti-estrogen – (%)[a]*	Yes	3131 (74.0)	1630 (78.3)
	No	1101 (26.0)	452 (21.7)
Bisphosphonate– (%)*	Yes	326 (7.7)	283 (13.6)
	No	3906 (92.3)	1799 (86.4)
Nodal status (%)	Positive	1756 (41.7)	847 (40.7)
	Negative	2261 (53.4)	1125 (54.0)
	Unspecified	215 (5.1)	110 (5.3)
Tumour size – (%)	T0	31 (0.7)	18 (0.9)
	T1	1796 (42.4)	907 (43.6)
	T2	1850 (43.7)	919 (44.1)
	T3	262 (6.2)	134 (6.4)
	T4	283 (6.7)	98 (4.7)
	Unspecified	10 (0.2)	6 (0.3)
Tumour stage – (%)*	I	1366 (32.3)	687 (33.0)
	IIa	1333 (31.5)	675 (32.4)
	IIb	882 (20.8)	428 (20.6)
	IIIa	263 (6.2)	140 (6.7)
	IIIb-c	388 (9.2)	152 (7.3)

Characteristic		Statin use prior to diagnosis	
		Non-user ***N* = 4,232**	**User** ***N* = 2,082**
Tumour grade – (%)*	Low	454 (10.7)	201 (9.7)
	Intermediate	2079 (49.1)	1087 (52.2)
	High	1352 (32.0)	673 (32.3)
	Unspecified	347 (8.2)	121 (5.8)
ER – (%)*	Negative	720 (17.0)	326 (15.7)
	Positive	3066 (72.5)	1605 (77.1)
	Unspecified	446 (10.5)	151 (7.3)
PR – (%)*	Negative	1109 (26.2)	534 (25.7)
	Positive	2108 (49.8)	1170 (56.2)
	Unspecified	1015 (24.0)	378 (18.2)
HER2 – (%)*	Negative	2511 (59.3)	1460 (70.1)
	Positive	530 (12.5)	246 (11.8)
	Unspecified	1191 (28.1)	376 (18.1)

***** = Difference in statin use $P < 0.05$ (Poisson regression)
ER: oestrogen receptor,
PR: progesterone receptor,
HER2: human epidermal growth factor receptor 2,
IQR: interquartile range,
NSAID: non-steroidal anti-inflammatory drug.
a: In the year after diagnosis

Table 6.2: Univariate and multivariate relative risks for associations between pre-diagnostic statin use and lymph node negative breast cancer

					Node Negative Breast Cancer			
	Node +	(%)	Node -	(%)	Univariate RR (95% CI)		Multivariate RR (95% CI) [A]	
Statin exposure								
Non-user	1,756	41.5	2,261	53.4	Ref	-	Ref	-
Pre-diagnostic statin user	847	40.7	1,125	54.0	1.01	0.96, 1.06	1.00	0.98, 1.03
Hydro/lipophilic								
Non-user	1,756	41.5	2,261	53.4	Ref	-	Ref	-
Hydrophilic statin user	216	36.9	335	57.2	1.07	1.00, 1.16	1.00	0.97, 1.04
Lipophilic statin user	444	41.9	562	53.0	0.99	0.93, 1.05	1.00	0.97, 1.03
Both	186	43.0	226	52.2	0.97	0.89, 1.07	1.01	0.97, 1.05
Dosing Intensity								
Non-user	1,756	41.5	2,261	53.4	Ref	-	Ref	-
Low-intensity user	163	41.3	204	51.7	0.96	0.87, 1.06	0.98	0.94, 1.02
High-intensity user	684	40.6	921	54.6	1.03	0.97, 1.09	1.01	0.99, 1.04
Effect Modification								
ER +								
Non-user	1,756	41.5	2,261	53.4	Ref	-	Ref	-
Pre-diagnostic statin user	636	39.6	883	55.0	1.04	0.98, 1.09	1.01	0.97, 1.06
Symptomatic presentation								
Non-user	1,756	41.5	2,261	53.4	Ref	-	Ref	-
Pre-diagnostic statin user	659	44.7	735	49.8	**0.91**	**0.86, 0.96**	1.00	0.97, 1.03
Screening presentation								
Non-user	1,756	41.5	2,261	53.4	Ref	-	Ref	-
Pre-diagnostic statin user	95	29.3	227	70.1	**1.32**	**1.23, 1.43**	1.01	0.95, 1.08

Ref: Referent Group, **HR**: Hazard Ratio, **CI**: Confidence Interval **ER:** estrogen receptor.
A) Adjusted for age at diagnosis (years); smoking status (never, past, current, unspecified); comorbidity score, tumour stage (I, IIa, IIb, IIIa, IIIb-c); tumour grade (low, intermediate, high, unspecified); ER, PR & HER2 receptor status (positive, negative, unspecified); chemotherapy in year post diagnosis (yes, no); anti-oestrogen therapy in year post diagnosis (yes, no); aspirin, bisphosphonate, NSAID & anti-diabetic medication use (yes, no), mode of tumour presentation (screening, incidental, symptomatic, unspecified), tumour morphology (ductal, lobular, other).

Table 6.3: Univariate and multivariate hazard ratios for associations between pre-diagnostic statin use and mortality

		All-cause mortality			Breast cancer-specific mortality		
Statin exposure definitions	***N***	**Deaths (rate) [A]**	**Univariate HR (95%CI)**	**Multivariate HR (95%CI) [B]**	**Deaths (rate) [A]**	**Univariate HR (95%CI)**	**Multivariate HR (95%CI) [B]**
Statin exposure – yes/no [C]							
Non-user	4,069	1002 55.0	Ref -	Ref -	575 31.5	Ref -	Ref -
Statin user	2,024	379 49.6	1.01 (0.90, 1.13)	**0.78 (0.69, 0.89)**	198 25.9	0.88 (0.75, 1.03)	**0.81 (0.68, 0.96)**
Dosing intensity [C]							
Non-user	4,069	1002 55.0	Ref -	Ref -	575 31.5	Ref -	Ref -
Statin user - low intensity	166	34 8.9	1.10 (0.95, 1.27)	0.78 (0.66, 0.92)	20 5.2	0.92 (0.75, 1.12)	0.90 (0.67, 1.20)
Statin user - high intensity [D]	1,858	345 30.7	0.94 (0.81, 1.09)	**0.79 (0.67, 0.92)**	178 15.8	0.85 (0.70, 1.04)	**0.70 (0.52, 0.94)**
Hydro/lipophilic [C]							
Non-user	4,069	1002 55.0	Ref -	Ref -	575 31.5	Ref -	Ref -
Hydrophilic statin user	572	114 48.1	0.92 (0.83, 1.19)	**0.79 (0.65, 0.95)**	56 23.6	0.85 (0.66, 1.09)	0.79 (0.61, 1.03)
Lipophilic statin user	1,031	181 46.2	0.96 (0.83, 1.12)	**0.73 (0.63, 0.86)**	102 26.0	0.87 (0.71, 1.07)	**0.76 (0.61, 0.95)**
Both	421	84 61.9	1.19 (0.96, 1.48)	0.84 (0.67, 1.05)	40 29.5	0.97 (0.72, 1.32)	0.82 (0.60, 1.13)
Symptomatic presentation [C]							
Non-user	2,859	854 65.1	Ref -	Ref -	503 38.4	Ref -	Ref -
Statin user	1,422	304 55.2	0.99 (0.87, 1.12)	**0.78 (0.68, 0.90)**	167 30.3	0.88 (0.74, 1.04)	0.83 (0.68, 1.00)
Screening presentation [C]							
Non-user	746	40 13.6	Ref -	Ref -	19 6.5	Ref -	Ref -
Statin user	320	21 18.6	1.48 (0.87, 2.51)	0.64 (0.32, 1.27)	10 8.8	1.41 (0.65, 3.07)	0.65 (0.23, 1.81)

		All-cause mortality			Breast cancer-specific mortality		
Statin exposure definitions	***N***	**Deaths (rate) [A]**	**Univariate HR (95%CI)**	**Multivariate HR (95%CI) [B]**	**Deaths (rate) [A]**	**Univariate HR (95%CI)**	**Multivariate HR (95%CI) [B]**
Effect modification – ER status [C]							
ER+	1,573						**0.69 (0.55, 0.85)**
ER-	303						1.10 (0.81, 1.49)
ER unspecified	148						0.96 (0.61, 1.53)

Ref: Referent Group, **HR**: Hazard Ratio, **CI**: Confidence Interval. Bold text indicates significant results at p<0.05.
A) Deaths / 1,000 person years.
B) Adjusted for age at diagnosis (years); smoking status (never, past, current, unspecified); comorbidity score, tumour stage (I, IIa, IIb, IIIa, IIIb-c); tumour grade (low, intermediate, high, unspecified); ER, PR & HER2 receptor status (positive, negative, unspecified); chemotherapy in year post diagnosis (yes, no); anti-oestrogen therapy in year post diagnosis (yes, no); aspirin, bisphosphonate, NSAID & anti-diabetic medication use (yes, no), mode of tumour presentation (screening, incidental, symptomatic, unspecified), tumour morphology (ductal, lobular, other).
C) Statin exposure lagged by 1 year in analysis.
D) Statin dosing intensity of ≥ 80% for ≥ 12 consecutive months defined as high dosing intensity. All other statin exposures defined as low dosing intensity

Table 6.4: Sensitivity analyses - Univariate and multivariate hazard ratios for associations between statin use and mortality

		All-cause mortality			Breast cancer-specific mortality		
Statin exposure definitions	***N***	**Deaths (rate) [A]**	**Univariate HR (95%CI)**	**Multivariate HR (95%CI) [B]**	**Deaths (rate) [A]**	**Univariate HR (95%CI)**	**Multivariate HR (95%CI) [B]**
Sensitivity analysis: varied exposure lag times							
Statin exposure – yes/no (lag 0 years)							
Non-user	4,232	1165 48.1	Ref -	Ref -	682 28.2	Ref -	Ref -
Statin user	2,082	437 55.5	1.01 (0.92, 1.13)	**0.77 (0.68, 0.87)**	230 29.2	0.87 (0.75, 1.00)	**0.77 (0.66, 0.90)**
Statin exposure – yes/no (lag 6 months)							
Non-user	4,149	1082 51.1	Ref -	Ref -	630 29.7	Ref -	Ref -
Statin user	2,052	407 52.5	1.00 (0.90, 1.12)	**0.77 (0.68, 0.88)**	217 28.0	0.88 (0.76, 1.03)	**0.80 (0.68, 0.95)**
Statin exposure – yes/no (lag 2 years)							
Non-user	3,566	832 58.8	Ref -	Ref -	462 32.6	Ref -	Ref -
Statin user	1,701	301 50.0	1.03 (0.90, 1.17)	**0.81 (0.70, 0.93)**	148 24.5	0.87 (0.73, 1.04)	**0.81 (0.66, 0.98)**
Sensitivity analysis: high intensity exposure ≥ 80% for ≥ 24 consecutive months [C]							
Non-user	4,069	1002	Ref -	Ref -	575 53.1	Ref -	Ref -
Statin user – low intensity	302	64	1.08 (0.95, 1.23	**0.81 (0.70, 0.94)**	35	0.92 (0.77, 1.11)	0.87 (0.67, 1.13)
Statin user – high intensity	1,722	315	0.89 (0.74, 1.07)	**0.73 (0.61, 0.89)**	163	0.80 (0.63, 1.03)	**0.67 (0.47, 0.94)**

Ref: Referent Group, **HR**: Hazard Ratio, **CI**: Confidence Interval. Bold text indicates significant results at p<0.05.
A) Deaths / 1,000 person years.
B) Adjusted for age at diagnosis (years); smoking status (never, past, current, unspecified); comorbidity score, tumour stage (I, IIa, IIb, IIIa, IIIb-c); tumour grade (low, intermediate, high, unspecified); ER, PR & HER2 receptor status (positive, negative, unspecified); chemotherapy in year post diagnosis (yes, no); anti-oestrogen therapy in year post diagnosis (yes, no); aspirin, bisphosphonate, NSAID & anti-diabetic medication use (yes, no), mode of tumour presentation (screening, incidental, symptomatic, unspecified), tumour morphology (ductal, lobular, other).
C) Statin exposure lagged by 1 year in analysis.

6.4. Discussion

In this study of 6,314 women with stage I-III breast cancer, pre-diagnostic statin use was not significantly associated with lymph node status at diagnosis but was associated with a statistically significant reduction in all-cause and breast cancer-specific mortality, even when adjusting for major prognostic factors. The survival benefit was even more pronounced in women with ER+ tumours.

The survival benefit observed is similar to findings from a meta-analysis of studies investigating statin use and breast cancer-specific mortality by Zhong *et al*. (HR 0.73, 95% CI 0.62, 0.86)[180], and another by Mansourian *et al*. (HR 0.85, 95% CI 0.83, 0.87)[199]. This study showed cancer-specific survival benefit was strongest among women receiving lipophilic statins, and in those with high intensity statin exposure. The exact cause of reductions in breast cancer mortality is still largely unknown. However, possible mechanisms have been suggested; pre-clinical studies have shown effects on cell signalling through stabilization of cyclin dependant kinase inhibitors p21 and p27 [200]. Statins have also been shown to exhibit immunomodulatory properties; cerivastatin was shown to enhance tumour CD8+ T cell infiltration and induced tumour associated macrophages to an M1-like phenotype; creating an anti-tumour environment [201].

A number of studies have been published investigating associations between pre and/or post-diagnostic statin exposure and breast cancer outcomes [120,155–157,162,163,165,180,199,202–204]. To my knowledge, this is the first study investigating associations between pre-diagnostic statin use and lymph node status at diagnosis. In this study, pre-diagnostic statin use was not associated with lymph node negativity in multivariate adjusted analyses. Relative risks remained unchanged after stratification by statin type and statin intensity. Statin-users with breast cancer detected through mammography screening were more likely to be lymph node negative at diagnosis (RR 1.32, 95% CI 1.23, 1.43), however, this significant association was not observed after adjustment for important variables. As mentioned in Chapter 4, in a clinical trial in which breast cancer patients were administered short-term high-dose (80 mg/day) atorvastatin; post-treatment tumour biopsies had significantly increased expression of HMGCR, the target enzyme for statins

[175]. Interestingly, moderate/strong HMGCR expression in breast tumour biopsies has been shown to be associated with a less aggressive tumour phenotype; lymph node negativity, lower grade and ER/PR positivity [205]. Although I did not observe an association between pre-diagnostic statin exposure and lymph node negativity in this study, it is possible that there may be specific subgroups of patients, for example; those with tumour expression of HMGCR, for whom statin treatment may be beneficial.

I found a more marked reduction in breast cancer mortality for users of lipophilic statins (HR 0.76, 95% CI 0.61, 0.95), which is in keeping with previous studies [120,165,173,206]. However, it should be noted that the numbers of patients receiving a hydrophilic statin were much lower than lipophilic, and any association may be under-powered. Studies have shown that lipophilic statins can inhibit breast cancer cell survival and cell proliferation through effects on p-MEK1/2 and NF-κB [173]. Lipophilic statins have been shown to inhibit anti-apoptotic Bcl-XL expression and induce the expression of pro-apoptotic/anti-proliferative PTEN [206]. In addition, lipophilic statin use was associated with reduced breast cancer recurrence in a Danish cohort of women with breast cancer [120]. A possible explanation for the differential effect by statin structure is due to lipophilic statins being more widely distributed throughout the body and their ability to penetrate the plasma membrane passively [64]. Hydrophilic statins, however, require uptake by the OATP1B1 transporter which is mainly found in the liver [64].

A 30% risk reduction in breast cancer mortality was observed in women with high intensity statin exposure. When the minimum period with high intensity exposure was extended to two years in a sensitivity analysis (ie. receiving a statin for at least 584 days in a 730 day period), the cancer-specific survival benefit was even greater (HR 0.67, 95% CI 0.47, 0.94). This suggests a possible dose-response relationship between statin exposure and improved breast cancer survival. However, it should be noted that over 85% of statin-users were high intensity users.

To my knowledge, this is the first study to report a significant reduction in breast cancer mortality, stratified by ER status. In a study investigating statin use and breast cancer stage, lipophilic statin-

users were significantly less likely to present with late-stage breast cancer at diagnosis (HR 0.80), and this was more marked in those with ER+ tumours (HR 0.72) [157]. Ahern *et al*. found that significant reductions in breast cancer recurrence in lipophilic statin-users were confined to ER+ patients (HR 0.69) [120]. Unfortunately, I did not have access to recurrence information and cannot determine whether reductions in breast cancer mortality in this study are due to reduced recurrence in statin-users. It is known that 27-hydroxycholesterol (27HC) is cholesterol metabolite and a selective estrogen receptor modulator (SERM) capable of promoting proliferation in ER+ cells [207]. As statins decrease the level of cholesterol in the circulation, and subsequent level of 27HC, it is possible that this leads to a decrease in ER+ tumour cell proliferation [208]. As mentioned, tumour expression of HMGCR may play an important role in the anticancer properties of statins. Interestingly, in studies investigating the prognostic role of breast tumour HMGCR expression, a combination of both HMGCR and ER positivity was associated with improved response to tamoxifen [197], breast cancer-specific survival and recurrence free survival [209]. The complex interplay between statin exposure, HMGCR expression, ER status, and subsequent cancer outcomes warrant further investigation.

This study also found a statistically significant, 22% reduction in all-cause mortality in statin-users (HR 0.78, 95% CI 0.69, 0.89), which is similar to that of other studies in the literature [210]. Although this study may be underpowered to detect difference in rates of cardiac events, it could be postulated that these reductions in all-cause mortality are due to the CVD preventative effects of statins.

To conclude, the results from this study suggest that pre-diagnostic statin use in women with stage I-III breast cancer is associated with a significant reduction in both breast cancer-specific and all-cause mortality, particularly in those with ER+ breast cancer, but is not significantly associated with lymph node status at diagnosis. In future studies, I suggest that the association between statin exposure, tumour HMGCR expression, and breast cancer outcomes be explored further.

Chapter Seven

7. Pre-diagnostic statin use, lymph node status, and mortality in people with stage I-III colorectal cancer

7.1. Introduction

A recent meta-analysis of statin use and colorectal cancer mortality suggests that pre-diagnostic statin use is associated with an 18% reduction colorectal cancer-specific mortality (HR 0.82, 95% CI 0.79, 0.86) [179]. A study by Armstrong *et al*. investigated the predictors of pathological complete response (pCR) to neoadjuvant chemoradiation in rectal cancer, and found that statin users at the time of surgical consultation were significantly more likely to have a pCR (OR 0.72, 95% CI 1.02, 2.92)[211]. While this is a relatively small study, it poses an interesting suggestion that statin use may have a role in improving cancer prognosis. It is well established that lymph node metastasis has an important role in colorectal cancer staging, as patients with node-negative disease have 5-year survival rates of 70%-80% in contrast to 30%-60% in those with node-positive disease [212].

In this study, I investigate associations between pre-diagnostic statin use and: i) lymph node status at diagnosis, ii) colorectal cancer-specific and all-cause mortality, and iii) whether associations are modified by intensity of pre-diagnostic statin use or type of statin received.

7.2. Methods

7.2.1. Setting & data sources

This cohort study was carried out using records from the National Cancer Registry Ireland (NCRI), which are linked to individual-level prescription dispensing data from Ireland's Primary Care Reimbursement Services (PCRS) pharmacy claims database, as described previously[95].

7.2.2. Cohort & exposure definitions

The study population comprised of individuals diagnosed with stage I-III colorectal cancer (ICD-10 C18, 19, 20) between 1st January 2001 and 31st December 2011. Participants were included in the study population if they were aged 18 years or over at diagnosis; had GMS coverage from at least 1 year prior to diagnosis; and no history of invasive cancer, other than non-melanoma skin cancer.

I identified pre-diagnostic statin prescriptions dispensed to the people in the study cohort from the PCRS database using WHO-ATC classifications (Appendix One, Table A1.1). For each day of follow-up, I calculated statin dosing intensity from the number of days' supply of statin received in the prior year[168]. These statin exposure histories were used to define the following time-varying exposure categories: i) exposed (yes/no) from the date they received their first statin prescription; ii) high-intensity exposure once they had taken a statin at an intensity of ≥80%, for at least 1 year (eg., a statin supply for at least 292 out of a 365 day period was considered high intensity). The overall intensity of statin exposure while on treatment was calculated by expressing the number of days' supply received as a proportion of the number of days from initiation to last exposure. Patients with de-novo post-diagnostic statin use were excluded from analyses, so as to determine the effect of statin use in patients with pre-diagnostic use.

7.2.3. Covariates & outcomes

The following patient, tumour and treatment characteristics were obtained from the NCRI database: age (years) at diagnosis, smoking status at diagnosis (never, past, current, unspecified), socioeconomic status (1-5, least-most deprived)[100], tumour site (colon, rectum), tumour stage (I, II, III), histologic tumour grade (poorly differentiated, well/moderately differentiated, unspecified), and receipt of surgery (yes, no), chemotherapy (yes, no) or radiotherapy (yes, no) in the year after diagnosis. The number of drug classes (4th level WHO-ATC classification) dispensed in the year before diagnosis was used as a proxy measure of co-morbidity (Appendix, Table A1.2)[172] for this the PCRS pharmacy claims database was used to identify other prescribed, and potentially confounding medication use in the year prior to diagnosis (exposed, unexposed); aspirin [169], anti-

diabetics, [169] non-steroidal anti-inflammatory drugs [170] and vitamin D [185,186]. Death certificates were used to determine the date and cause of death (all-cause or colorectal cancer-specific).

7.2.4. Statistical analysis

The proportion of statin-users and non-users was tabulated for each covariate and differences in the rates of statin use across covariates were compared using univariate Poisson regression. Univariate and multivariate log-binomial models were used to estimate relative risks (RR) and 95% confidence intervals (CI's) for associations between pre-diagnostic statin use and lymph node negative CRC at diagnosis.

In survival analyses, multivariate Cox proportional hazards models were used to estimate adjusted hazard ratios (HR) and 95% CI's for associations between pre-diagnostic statin use and colorectal cancer-specific and all-cause mortality. Individuals were categorised as statin exposed (time varying, yes/no) from the time they received their first statin prescription. These exposures were lagged by 1 year in survival analyses to reduce the possibility that changes in CRC prognosis or treatment, for example a cancer recurrence or approaching death, influenced a patient's or prescriber's decision to initiate or continue statin therapy [128,187].

The following pre-planned subgroup analyses were applied to both lymph-node status analyses and survival analyses. Firstly, as prior studies have suggested that only lipophilic statin use is associated with improved cancer outcomes [120] analyses were also stratified by statin solubility: lipophilic (atorvastatin, fluvastatin, simvastatin), hydrophilic (pravastatin, rosuvastatin), both[68]. Secondly, I stratified analyses by high/low exposure intensity. The previously described covariates were selected for inclusion in multivariable analyses, based on prior knowledge of patient and clinical characteristics associated with CRC-specific mortality.

I conducted the following sensitivity analyses; i) in survival analyses, high intensity statin exposure was defined as ≥80% intensity for longer than two consecutive years, and ii) in survival

analyses, statin exposure lag time was varied (0, 6 months, 2 years) to account for possible reverse causation bias, as mentioned above. All analyses were performed using SAS® v9.3 (SAS® Institute Inc, Cary, NC). Results were regarded as significant at a two-sided α-level of 0.05.

7.3. Results

7.3.1. Cohort and exposure characteristics

I identified 8,521 individuals eligible for inclusion in the study (Figure 7.1). The characteristics of pre-diagnostic statin users (n=929) and nonusers (n=6,017) included in the study are presented in Table 7.1. Statin users more likely to be former smokers, had a significantly higher comorbidity score than non-users, and were more likely to have received surgical treatment. Statin users were also significantly more likely to be prescribed aspirin, NSAIDs, vitamin D, and anti-diabetics.

7.3.2. Pre-diagnostic statin use and lymph node status

RRs for associations between pre-diagnostic statin use and lymph node negative colorectal cancer are presented in Table 7.2. The proportion of individuals with node-negative status in the statin user and non-user groups was 53% and 55%, respectively. No association was found between pre-diagnostic statin use and lymph node negative status at diagnosis, in univariate analysis (RR 0.97, 95% CI 0.91, 1.03) or multivariate adjusted analyses (RR 0.99, 95% 0.96, 1.02) (Table 7.2). Analyses stratified by; high intensity statin use, duration of statin use, and type of statin received, also yielded null findings (Table 7.2).

7.3.3. Pre-diagnostic statin use and mortality

After lagging statin exposure by 1 year, I identified 929 individuals with pre-diagnostic statin use. In multivariate adjusted survival analyses, pre-diagnostic statin use was associated with a non-significant, 14% reduction in colorectal cancer-specific mortality (HR 0.86, 95% CI 0.73, 1.00) and a non-significant 11% reduction in all-cause mortality (HR 0.89, 95% CI 0.79, 1.00) (Table 7.3). In analyses stratified by dosing intensity, low-intensity use was associated with a significant reduction in colorectal cancer specific mortality, in multivariate analyses (HR 0.74, 95% CI 0.55, 0.99); however

this benefit was not observed in those with high intensity use (HR 0.78, 95% CI 0.60, 1.03). In analyses stratified by type of statin received (hydrophilic, lipophilic, both), survival benefit was significant in those who received a lipophilic statin (HR 0.75, 95% CI 0.61, 0.93) but not hydrophilic statin. In sensitivity analyses, a reduction in colorectal cancer-specific mortality was observed when varying the statin exposure lag time in survival analyses from 0 years to 6 months (Table 7.4). No survival benefit was observed when the period of high-intensity use was extended from 1 to 2 years.

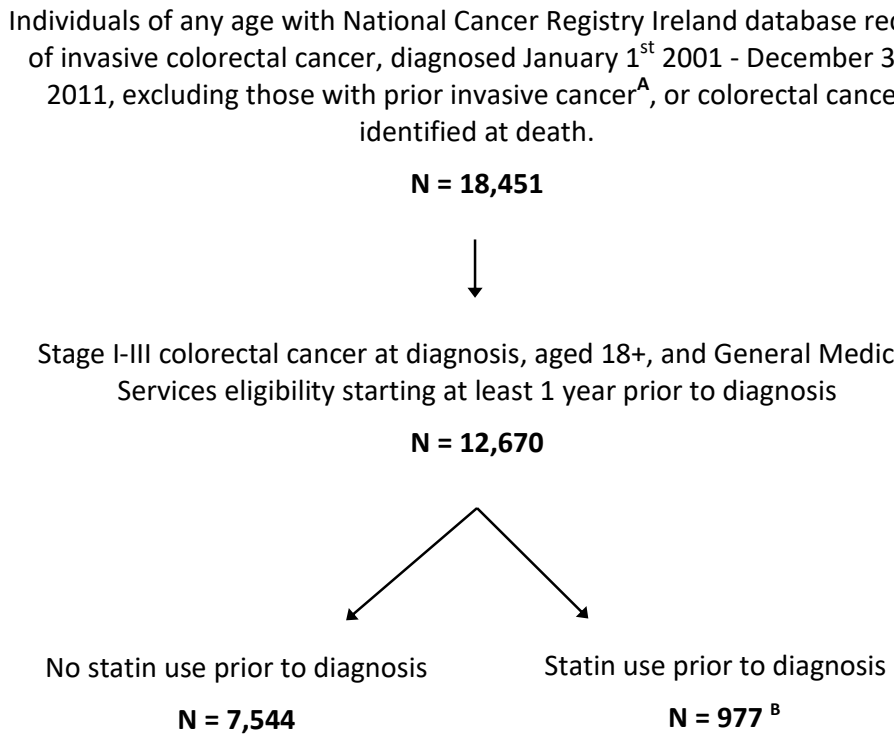

Figure 7.1: Flowchart for study cohort inclusion and exclusion criteria.

A) With the exception of non-melanoma skin cancer.

B) Prior to inclusion of a statin exposure lag

Table 7.1: Characteristics of participants selected for inclusion in the study cohort

Characteristic [A,B]		Statin use prior to diagnosis	
		Non-user *N* = 6,017	User *N* = 929
Age in years	Median (IQR)	74 (68, 79)	74 (69, 78)
Gender	Male (%)	3376 (56.1)	493 (53.1)
	Female (%)	2641 (43.9)	436 (46.9)
Comorbidity score [C]*	Median (IQR)	0 (0, 6)	12 (8, 17)
Smoking – (%)*	Current	842 (14.0)	119 (12.8)
	Past	1215 (20.2)	220 (45.6)
	Never	2431 (40.4)	424 (23.7)
	Unspecified	1529 (25.4)	166 (17.9)
Deprivation Index – (%)	1	884 (14.7)	129 (13.9)
	2	641 (10.7)	104 (11.2)
	3	809 (13.5)	116 (12.5)
	4	1110 (18.5)	166 (17.9)
	5	2219 (36.9)	350 (37.7)
	Unspecified	354 (5.7)	64 (6.8)
Aspirin – (%) [C]*	Yes	594 (9.9)	582 (62.7)
	No	5423 (90.1)	347 (37.3)
Anti-diabetic – (%) [C]*	Yes	124 (2.1)	136 (14.6)
	No	5893 (97.9)	793 (85.4)
NSAID – (%) [C]*	Yes	906 (15.1)	386 (41.6)
	No	5111 (84.9)	543 (58.4)
Vit D – (%) [C]*	Yes	143 (2.4)	103 (11.1)
	No	5874 (97.6)	826 (88.9)
Tumour stage – (%) *	I	1162 (19.3)	222 (23.9)
	II	2550 (42.4)	366 (39.4)
	III	2305 (38.3)	341 (36.7)
Tumour grade – (%)	Low	369 (6.1)	76 (8.2)
	Intermediate	4474 (74.4)	681 (73.2)
	High	655 (10.9)	86 (9.3)
	Unspecified	519 (8.6)	86 (9.3)
Tumour site – (%)	Colon	3946 (65.6)	612 (65.9)
	Rectum	2017 (34.4)	317 (34.1)
Chemotherapy – (%) [D]	Yes	2168 (36.0)	330 (35.5)
	No	3849 (64.0)	599 (64.5)
Radiotherapy – (%) [D]	Yes	1033 (17.2)	146 (15.7)
	No	4984 (82.8)	783 (84.3)
Surgery – (%) [D]*	Yes	5748 (95.5)	905 (97.4)
	No	269 (4.5)	24 (2.6)

*Difference in statin initiation rate $P<0.05$ (Poisson regression)
IQR: Inter-Quartile Range. **NSAID**: Non-Steroidal Anti-Inflammatory Drug.
A) No statin use initiated after diagnosis
B) Patients identified as statin users / non-users after lagging exposure by 1 year.
C) In the year prior to colorectal cancer diagnosis.
D) In the year post colorectal cancer diagnosis

Table 7.2: Univariate and multivariate relative risks for associations between pre-diagnostic statin use and lymph node negative CRC

							Node Negative CRC			
	Node +	(%)	Node -	(%)	Node Unspecified	(%)	Univariate RR (95% CI)		Multivariate RR (95% CI) [A]	
Statin exposure										
Non-user	2913	38.6	4122	54.6	509	6.8	Ref	-	Ref	-
Pre-diagnostic statin user	380	38.9	516	52.8	81	8.3	0.97	(0.91, 1.03)	0.99	(0.96, 1.02)
Hydro/lipophilic										
Non-user	2913	38.6	4122	54.6	509	6.8	Ref	-	Ref	-
Hydrophilic statin user	111	35.4	182	58.0	21	6.6	1.07	(0.97, 1.18)	1.02	(0.96, 1.08)
Lipophilic statin user	183	41.4	222	50.2	37	8.4	0.92	(0.84, 1.01)	0.98	(0.94, 1.03)
Both	62	41.3	77	51.3	11	7.3	0.94	(0.81, 1.10)	0.98	(0.91, 1.06)
Dosing Intensity										
Non-user	2913	38.6	4122	54.6	509	6.8	Ref	-	Ref	-
Low-intensity user	67	30.3	142	64.3	12	5.4	1.03	(0.95, 1.14)	1.00	(0.96, 1.04)
High-intensity user	136	35.5	216	56.4	31	8.1	0.93	(0.85, 1.00)	0.99	(0.95, 1.03)

Ref: Referent Group, **RR**: Relative Risk, **CI**: Confidence Interval

A) Adjusted for age at diagnosis (years), gender, smoking status, socioeconomic status, comorbidity score, tumour stage, tumour grade, chemotherapy/radiotherapy/surgery in year post diagnosis (yes, no), Vitamin D, aspirin, NSAID & anti-diabetic medication use (yes, no).

Table 7.3: Univariate and multivariate hazard ratios for associations between pre-diagnostic statin use and all cause and CRC-specific mortality

		All-cause mortality			CRC-specific mortality		
Statin exposure definitions	***N***	**Deaths (rate) [A]**	**Univariate HR (95%CI)**	**Multivariate HR (95%CI) [B]**	**Deaths (rate) [A]**	**Univariate HR (95%CI)**	**Multivariate HR (95%CI) [B]**
Statin exposure – yes/no [C]							
Non-user	6017	2123 (94.9)	Ref -	Ref -	1319 (59.0)	Ref -	Ref -
Statin user	929	467 (190.1)	1.00 (0.91, 1.11)	0.89 (0.79, 1.00)	249 (101.4)	0.92 (0.80, 1.05)	0.86 (0.73, 1.00)
Dosing intensity [C]							
Non-user	6017	2123 (94.9)	Ref -	Ref -	1319 (59.0)	Ref -	Ref -
Statin user - low intensity	363	188 (149.7)	0.99 (0.85, 1.15)	0.87 (0.75, 1.02)	92 (73.3)	0.84 (0.68, 1.04)	**0.74 (0.55, 0.99)**
Statin user - high intensity [D]	566	279 (232.4)	1.01 (0.89, 1.14)	0.90 (0.79, 1.04)	157 (130.8)	0.97 (0.82, 1.14)	0.78 0.60, 1.03)
Hydro/lipophilic [C]							
Non-user	6017	2123 (94.9)	Ref -	Ref -	1319 (59.0)	Ref -	Ref -
Hydrophilic statin user	329	178 (180.4)	1.09 (0.93, 1.27)	1.02 (0.87, 1.20)	97 (98.3)	1.01 (0.83, 1.23)	1.02 (0.82, 1.27)
Lipophilic statin user	455	209 (176.7)	0.89 (0.77, 1.03)	**0.79 (0.68, 0.92)**	111 (93.8)	0.82 (0.67, 0.99)	**0.75 (0.61, 0.93)**
Both	145	80 (279.0)	1.18 (0.94, 1.47)	0.94 (0.75, 1.19)	41 (143.0)	1.02 (0.75, 1.40)	0.86 (0.62, 1.19)

Ref: Referent Group, **HR**: Hazard Ratio, **CI**: Confidence Interval. Bold text indicates significant results at $p<0.05$.
A) Deaths / 1,000 person years.
B) Adjusted for age at diagnosis (years), gender, smoking status, socioeconomic status, comorbidity score, tumour stage, tumour grade, chemotherapy/radiotherapy/surgery in year post diagnosis (yes, no), Vitamin D, aspirin, NSAID & anti-diabetic medication use (yes, no).
C) Statin exposure lagged by 1 year in analysis.
D) Statin dosing intensity of ≥ 80% for ≥ 12 consecutive months defined as high dosing intensity. All other statin exposures defined as low dosing intensity

Table 7.4: Sensitivity analyses - Univariate and multivariate hazard ratios for associations between pre-diagnostic statin use and all cause and CRC specific mortality

		All-cause mortality			CRC-specific mortality		
Statin exposure definitions	***N***	**Deaths (rate) [A]**	**Univariate HR (95%CI)**	**Multivariate HR (95%CI) [B]**	**Deaths (rate) [A]**	**Univariate HR (95%CI)**	**Multivariate HR (95%CI) [B]**
Sensitivity analysis: varied exposure lag times							
Statin exposure – yes/no (lag 0 years)							
Non-user	7391	3497 (119.3)	Ref -	Ref -	2334 (79.6)	Ref -	Ref -
Statin user	1130	668 (222.1)	0.99 (0.91, 1.07)	**0.86 (0.78, 0.95)**	390 (129.7)	0.91 (0.82, 1.02)	**0.82 (0.72, 0.92)**
Statin exposure – yes/no (lag 6 months)							
Non-user	6445	2551 (98.9)	-	Ref -	1648 (63.9)	Ref -	Ref -
Statin user	986	524 (199.6)	0.98 (0.90, 1.08)	**0.88 (0.79, 0.98)**	294 (112.0)	0.91 (0.80, 1.03)	**0.84 (0.73, 0.97)**
Statin exposure – yes/no (lag 2 years)							
Non-user	4682	1464 (89.0)	Ref -	Ref -	805 (49.0)	Ref -	Ref -
Statin user	825	368 (170.1)	1.03 (0.92, 1.16)	0.92 (0.91, 1.05)	182 (84.1)	0.98 (0.83, 1.15)	0.93 (0.78, 1.13)
Sensitivity analysis: high intensity exposure ≥ 80% for ≥ 24 consecutive months [C]							
Non-user	4682	1464 (89.0)	Ref -	Ref -	805 (48.9)	Ref -	Ref -
Statin user – low intensity	485	225 (147.7)	1.03 (0.90, 1.19)	0.94 (0.81, 1.10)	107 (70.2)	0.95 (0.78, 1.17)	0.83 (0.62, 1.12)
Statin user – high intensity	340	143 (223.6)	1.04 (0.87, 1.23)	0.89 (0.74, 1.07)	75 (117.3)	1.01 (0.80, 1.28)	0.77 (0.52, 1.14)

Ref: Referent Group, **HR**: Hazard Ratio, **CI**: Confidence Interval. Bold text indicates significant results at p<0.05.
A) Deaths / 1,000 person years.
B) Adjusted for age at diagnosis (years), gender, smoking status, socioeconomic status, comorbidity score, tumour stage, tumour grade, chemotherapy/radiotherapy/surgery in year post diagnosis (yes, no), Vitamin D, aspirin, NSAID & anti-diabetic medication use (yes, no).
C) Statin exposure lagged by 1 year in analysis.

7.4. Discussion

In this study of 8,521 individuals with colorectal cancer, pre-diagnostic statin use was not significantly associated with lymph node status at diagnosis in multivariable analyses. Pre-diagnostic statin use was associated with a, non-statistically significant, 14% reduction in colorectal cancer-specific mortality (HR 0.86, 95% CI 0.73, 1.00) and 11% reduction in all-cause mortality (HR 0.89, 95% CI 0.79, 1.00). This survival benefit was also observed in sensitivity analyses, whereby the exposure lag time was varied to 0 and 6 months, as shown in Table 20.

The survival benefit observed, albeit non-statistically significant, is similar to findings from recent meta-analyses of pre-diagnostic statin use and colorectal cancer survival by Zhong *et al*. (HR 0.82, 95% CI 0.73, 0.91) and a larger meta-analysis by Gray *et al*. (HR 0.82, 95% CI 0.79, 0.86) [179,180]. This study showed that colorectal cancer-specific survival benefit was strongest in those with pre-diagnostic lipophilic statin use (HR 0.75, 95% CI 0.61, 0.93), and this had not yet been shown in the literature. Gray *et al*. carried out a subgroup analysis of simvastatin (a lipophilic statin) users in the year prior to colorectal cancer diagnosis, and no survival benefit was observed (HR 0.97, 95% CI 0.88, 1.06) [179]. However, Cardwell *et al*. carried out subgroup analyses of post-diagnostic statin use by statin received, and only post-diagnostic simvastatin users had colorectal cancer survival benefit (HR 0.72, 95% CI 0.61, 0.85) [213]. Interestingly, a meta-analysis of statin use and colorectal cancer risk showed a significant association between lipophilic statin use and CRC risk (RR 0.88, 95% CI 0.85, 0.93) and the same risk reduction was not seen in those with hydrophilic statin use (RR 0.88, 95% CI 0.76, 1.02)[214]. It is hypothesized that lipophilic statins may possess a greater chemoprotective effect than hydrophilic statins due to greater lipid solubility and membrane permeability [66]. In a pre-clinical study, treatment of colorectal cancer cells with lovastatin or simvastatin significantly increased expression of the cell cycle regulator, p21. Increased levels of p21 resulted in a reduction in cell proliferation [215]. This study also showed that simvastatin could induce apoptosis in the colorectal cancer cell line by regulating the p38-MAPK-p53-survivin signalling pathway [215].

A statistically significant, 26% reduction in colorectal cancer-specific mortality was observed in low-intensity statin users. However, this finding is unlikely to be causal as the same benefit was not observed in the high intensity user group, and it is well known that a dose-response is a key factor supporting a causal relationship between an exposure and outcome, as highlighted by Bradford-Hill [216]. When expanding the window of high intensity exposure from one to two years, no survival benefit was observed for low or high intensity users.

To my knowledge, this is the first study to investigate the association between pre-diagnostic statin use and lymph node status at CRC diagnosis. Here, pre-diagnostic statin use was not associated with lymph node negativity in univariate and multivariate models, or in subgroup analyses. I did not have information on the mode of presentation, but as Bowel Screen was introduced after this data was collected, it would not have affected these results.

As discussed in previous studies, the target for statins, HMGCR, may play an interesting role in the association between statins and cancer outcomes. Bengtsson *et al*. found that colorectal tumours that were positive for HMGCR were less likely to have distant metastasis at diagnosis [194]. Lipkin *et al*. carried out a study to investigate the effects of genetic variation in HMGCR, and it was found that the a particular HMGCR genotype was associated with reduced risk of colorectal cancer, and increased reductions in serum cholesterol [217]. However, a recent study investigated the effect of statin use and colorectal cancer survival, when stratified by HMGCR status [193]. While no survival benefit was found, the complex interplay between statin use, HMGCR status, and cancer outcomes remains an interesting topic.

This study has a number of strengths, including the use of prospectively collected CRC outcome and statin exposure data from pharmacy claims. However, as discussed in previous chapters, there are also some potential limitations. I could not verify whether participants took the medication they received and non-compliance may have resulted in misclassification of exposure. However, I expect that statin users are unlikely to continue filling prescriptions for a medication they are no longer taking. There were some statistically significant differences between statin users and non-users,

and although I adjusted for many important patient and tumour characteristics in analyses, and the potential for residual confounding in the analyses should be considered. The study population was a subset of CRC cases defined by age and socioeconomic eligibility for the GMS scheme; therefore, generalisability of the findings needs to be considered.

In conclusion, the results from this study suggest that pre-diagnostic statin use is not significantly associated with lymph node status at presentation in stage I-III colorectal cancer. However, statin use is associated with a non-statistically significant 14% reduction in colorectal cancer-specific mortality and an 11% reduction in all-cause mortality.

Chapter Eight

8. Conclusion

8.1. Introduction

There has been a dramatic increase in the understanding of the biological mechanisms of cancer in the last number of years [218], leading to the development of many novel therapies and treatment options. However, despite advances in both cancer prevention and treatment, cancer remains one of the leading causes of mortality worldwide. Statins have been identified through preclinical, clinical, and epidemiologic research as having a potential role in the prevention and/or treatment of cancer [160,166]. The studies described in this thesis have sought to further elucidate the effects of statins in patients with breast or colorectal cancer in an Irish population. These studies used reliable data sources and rigorous methodologies to investigate the associations between statin exposures and cancer outcomes. This thesis contains five pharmacoepidemiological studies using the linked NCRI-PCRS database, as described in Chapter 2. The original thesis aims were to examine:

1) Patterns of statin use in patients with breast or colorectal cancer, towards end-of-life
2) Associations between de-novo statin use and breast cancer survival
3) Associations between de-novo statin use and colorectal cancer survival
4) Associations between pre-diagnostic statin use and LN status and breast cancer survival
5) Associations between pre-diagnostic statin use and LN status and colorectal cancer survival

8.2. Summary of research findings

Analyses of the patterns of statin use in the months and years prior to death from breast or colorectal cancer showed that the probability of continuing statin use was significantly lower in the three to six months prior to death from these cancers. This decline in statin use may be the result of a change in the health care priorities of the patient, and/or reduction in the pharmacotherapeutic

burden [147]. In contrast, the number of patients initiating statins did not differ between those who died of their cancer and those who did not. This suggests that a life-limiting diagnosis does not affect the prescribing of preventative medications. The results suggest that it is important to account for this peri-mortality change in statin exposure in pharmacoepidemiological studies through the use of an exposure lag time.

In analyses of de-novo statin use and breast cancer-specific mortality, I found no association between de-novo statin initiation and breast cancer-specific mortality after adjusting for important covariates (HR 0.88, 95% CI 0.66, 1.17). Subgroup analyses in women taking statin at an intensity of ≥80% for longer than 12 consecutive months also yielded null associations with breast cancer-specific mortality (HR 1.04, 95% CI 0.71, 1.51).

Similar to Chapter 4, analyses of statin use on colorectal cancer-specific mortality found no significant association between de-novo statin use and colorectal cancer-specific mortality in multivariate adjusted analyses (HR 0.96, 95% CI 0.78, 1.19). Multivariate subgroup analyses in individuals taking statin at an intensity of ≥80% for longer than 12 consecutive months also yielded non-significant associations with CRC-specific mortality (HR 0.93, 95% CI 0.69, 1.25). These studies suggest there may be limited benefit for statins in an adjuvant setting for an unselected population.

In Chapter 6, I found no significant association between pre-diagnostic statin use and lymph node status at breast cancer diagnosis. However, pre-diagnostic statin use was associated with a significant, 19% reduction in breast cancer-specific mortality (HR 0.81, 95% CI 0.68, 0.96). This cancer-specific survival benefit was observed in women with high intensity use (HR 0.70, 95% CI 0.52, 0.94) and in those who received a lipophilic statin (HR 0.76, 95% CI 0.61, 0.95). Interestingly, pre-diagnostic statin use was associated with a more marked, statistically significant, 31% reduction in breast cancer-specific mortality in patients with ER+ tumours (HR 0.69, 95% CI 0.55, 0.85).

Finally, in analyses of pre-diagnostic statin use and lymph node status in colorectal cancer, no association was found in multivariate adjusted analyses. However, pre-diagnostic statin use was associated with a non-significant, 14% reduction in colorectal cancer-specific mortality (HR 0.86,

95% CI 0.73, 1.00). In analyses stratified by dosing intensity, low-intensity use was associated with a significant reduction in colorectal cancer specific mortality, in multivariate analyses (HR 0.74, 95% CI 0.55, 0.99); however this benefit was not observed in those with high intensity use (HR 0.78, 95% CI 0.60, 1.03). In analyses stratified by type of statin received (hydrophilic, lipophilic, both), colorectal cancer survival benefit was significant in those who received a lipophilic statin (HR 0.75, 95% CI 0.61, 0.93) but not hydrophilic statin.

8.3. Contribution of findings to the existing literature

As detailed in the individual chapters, the overall results of the studies investigating patterns of statin use, and associations between statin use and cancer outcomes, are broadly consistent with the existing literature.

Chapter 3 used prospectively collected, high quality longitudinal prescription information to compare the initiation and continuation of statin treatment in patients who died of breast or colorectal cancer, as compared to those who survived. This is in contrast to the largely cross-sectional study designs of previous studies describing statin use at the time of death in similar patient cohorts [132,134,135]. There are two main implications from this study, and these are statistical and clinical. This is the first study, to my knowledge, to describe the timing of statin cessation as breast or colorectal patients approach end-of-life. This has relevance for pharmacoepidemiological studies investigating statin use and cancer outcomes, which should employ a statin exposure lag to account for reverse causation. This study provides evidence that these pharmacoepidemiological studies should use an exposure lag of approximately 6 months to 1 year. In terms of clinical importance, this study showed that many cancer patients are still receiving a statin prescription as they approach the end of their life. It has been highlighted in the literature that this may not be appropriate, and the discussion whether to continue with statin treatment may actually provide clinicians and patients with the opportunity for shared decision making and patient autonomy at the end of life [149].

Results from Chapters 4 and 5, which investigated the associations between de-novo statin use and breast and colorectal cancer-specific mortality respectively, yielded null findings. As discussed in these chapters, this is largely in keeping with results of similar studies. The studies presented in Chapters 4 and 5 use more robust methodologies than previous literature; excluding prevalent users to determine the benefit of new-use of statins in the adjuvant setting, and employing an exposure lag to reduce reverse causation bias. While these studies suggest that there is not evidence to support the use of statins in an adjuvant treatment setting, it must be noted that these were unselected patient groups. It is possible that there are subgroups of patients for which adjuvant statin treatment may be beneficial. The role of the statin target, HMGCR, has been researched in the breast cancer setting, yielding interesting results. Borgquist *et al*. have shown that tumour HMGCR expression is associated with favourable breast cancer characteristics such as: low grade, small tumour size, ER positivity, and improved recurrence free survival [209]. Similar results were found by Gustbee *et al*., which showed that tumour HMGCR expression was associated with lower grade, ER positivity and less lymph node involvement [205]. Brennan *et al*. showed that HMGCR expression was an independent predictor of improved recurrence free survival in a cohort stage II breast cancer patients [197]. Further, Bjarnadottir *et al*. showed that high-dose atorvastatin (80mg/day) administered for two weeks prior to breast cancer surgery resulted in statistically significantly increased tumour expression of HMGCR. Together, these findings suggest that there may be a complex interplay between statin use, HMGCR expression, and cancer outcomes.

Results of studies investigating the role of HMGCR in colorectal cancer are more conflicting. Bengtsson *et al*. have shown that tumour HMGCR expression in colorectal cancer was associated with reduced distant metastasis, and reduced vascular invasion at diagnosis, but no improvement in survival after adjusting cliniopathological characteristics was observed [194]. In addition, no survival benefit was observed in statin users when stratified by HMGCR expression status in a study by Gray *et al*. [193]. However, one study has identified a specific genetic variant of HMGCR which modifies the relationship between statin use and reduced risk of developing colorectal cancer [217].

Study participants with this genetic variant also had reduced levels of LDL cholesterol, suggesting enhanced drug efficiency in this subgroup, which warrents further investigation.

Chapters 6 and 7 investigate the associations between pre-diagnostic statin use, LN status and breast and colorectal cancer specific mortality, respectively. In both studies, pre-diagnostic statin use was not associated with LN status at diagnosis. Given the evidence presented above for the role of HMGCR and cancer outcomes, the lack of associations in Chapters 6 and 7 between pre-diagnostic statin use and LN status may be due to these unselected patient groups. Pre-diagnostic statin use was associated with reductions in breast and colorectal cancer-specific mortality (HR 0.81, 95% CI 0.68, 0.96 and HR 0.86, 95% CI 0.73, 1.00, respectively), even after adjusting for important clinical and patient characteristics. These studies also confirm, using robust methodologies, what has been seen in the literature regarding the benefit for lipophilic statins; both breast and colorectal cancer-specific mortality was reduced even more significantly in lipophilic statin users. A recent meta-analysis has shown that lipophilic statin use is associated with significantly reduced breast cancer mortality (HR 0.57, 95% CI 0.46, 0.70) [219]. A meta-analysis of statin use and risk of developing colorectal cancer has also shown benefit for lipophilic statin use (RR 0.88, 95% CI 0.85, 0.93) [214]. As discussed previously, this apparent benefit for lipophilic statin users may be due to the due to greater lipid solubility and membrane permeability [66].

Chapter 6 also shows, for the first time in literature, the further reduced breast cancer-specific mortality in those with ER positive tumours. As discussed in Section 6.4, previous studies have shown lipophilic statin-users with ER positive tumours were significantly less likely to present with late-stage breast cancer at diagnosis [157]. In addition, Ahern *et al*. found that significant reductions in breast cancer recurrence in lipophilic statin-users were confined to ER positive patients [120]. As mentioned previously, tumour expression of HMGCR may play an important role in the effects statins. Interestingly, in studies investigating the prognostic role of breast tumour HMGCR expression, a combination of both HMGCR and ER positivity was associated with improved response to tamoxifen [197], breast cancer-specific survival and recurrence free survival [209].

The studies presented in this thesis are the first pharmacoepidemiological analyses of statin use and breast and colorectal cancer outcomes, using the linked NCRI-PCRS dataset. These studies not only show the potential role for statins in a chemopreventative setting, but also the great potential of this dataset in pharmacoepidemiological research.

8.4. Study limitations

The limitations of the data used are introduced in Chapter 2, and the limitations of the individual studies are described in each discussion. However, there are some common limitations to all studies. While the NCRI data may be fully representative of the population, the information regarding prescription medication use is limited to only those with eligibility for the GMS Scheme. The GMS population is approximately 40% of the population, but those who are socio-economically disadvantaged and those aged ≥70 years are over-represented; therefore, there may be some limitations as regards generalisability in pharmacoepidemiological studies [94]. For those aged ≥70 years, the database is representative of the Irish population at the same age; as of 2013, 90% of men and 94% of women aged ≥70 years were eligible. However, over 30% of the Irish population aged >45 years of age are covered by the GMS scheme [94].

Data for some covariates, e.g. smoking and grade, were missing for a proportion of the population, as recorded in the patient characteristics tables of the individual studies. This may have introduced bias if there were differences in the status of these characteristics between the exposed and unexposed groups of patients for whom data was missing.

As with all pharmacoepidemiological research, there is the potential for residual confounding. The data available for this thesis lacked some important information such as obesity status and tumour recurrence. A meta-analysis by Protani *et al*. reported a statistically significant increase in breast cancer-specific mortality in obese versus non-obese women (HR 1.33, 95% CI 1.19, 1.50), regardless of whether obesity was characterized by BMI or 'waist to hip' ratio, study design, menopausal status, and date of study [220]. A less pronounced, but statistically significant increase in colorectal cancer-specific mortality was observed in a meta-analysis of BMI and colorectal cancer outcomes

(RR 1.14, 95% CI 1.05, 1.24) [221]. Additionally, in a survey of statin users, a high proportion (80%) of people reported a BMI of ≥25 kg/m^2 which is classified as overweight [222]. In addition, diagnostic codes were not available, and the exact indication for initiating or ceasing prescribed drugs is unknown.

I could not verify whether individuals took the medication they were dispensed and non-compliance may have resulted in exposure misclassification. However, I expect that people are unlikely to continue filling prescriptions for medication they no longer take. It is important to consider that statins may be preferentially prescribed for, and taken by, patients who engage in healthier behaviours and have superior health outcomes [106,107,223]. This is known as healthy-user bias and may cause an overestimation of any beneficial effect of statins [111]. In Chapter 6, the rates of breast cancers detected through mammography screening were similar in statin-users and non-users, suggesting that healthy-user bias in this cohort may be minimal [107]. I did not have information on all lifestyle factors that may influence disease progression, eg. BMI, and the potential for residual confounding in the analyses should be considered. Up to 28% of women have an unspecified HER2 status; these women may have been diagnosed prior to the introduction of the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) HER2 testing guidelines in 2007 [224].

8.5. Potential for future research

Future studies with access to tumour recurrence data could provide more insight into the mechanisms through which pre-diagnostic statin use are associated with reduced cancer-specific mortality; there is the potential that statin use is associated with reduced tumour recurrence and subsequent reduced cancer-specific mortality, but it was not possible to study this using the current linked datasets. Also, the NCRI-PCRS linked dataset did not include molecular information would hugely increase the impact of this research given the potential associations between tumour expression of HMGCR and cancer outcomes, as discussed previously. The potential for this type of molecular epidemiological research has been recognised by the Irish Cancer Society, and in 2013 they funded 'Breast-Predict', the first of the Irish Cancer Collaborative Research Centres. Breast-

Predict funding is contributing towards the prospective collection of patient tumour samples and study questionnaires to help capture both molecular information, such as HMGCR status, and lifestyle information such as exercise and obesity, thus increasing the capacity for this type of research. This information was not fully available for inclusion in the thesis.

It would also be of benefit to link the NCRI-PCRS dataset used in the present studies to further databases, such other HSE Schemes as described in Chapter 2. The inclusion of prescription information from patients registered on these schemes would provide a more generalisable patient population, as well as increasing the overall number of patients available for analysis. Additional opportunities for expanding the data available include collaboration with other research groups who have access to similar datasets. Pooling of data such as this, using meta-analytical approaches, could increase the statistical power for identifying associations between prescribed medications and cancer outcomes.

8.6. Impact

The results of this thesis can have an impact in a number of areas. The health research impact framework devised by Kuruvilla *et al*. has been used to classify the potential impacts of this thesis across four main domains relevant for health-related research: Research, Policy, Service and Societal [225].

8.6.1. Research impact

This thesis has contributed to the field of pharmacoepidemiology by increasing the evidence-base on the association between pre and post-diagnostic statin use and breast and colorectal cancer outcomes, in an Irish setting. It has also provided, for the first time, a recommendation for a statin exposure lag time in pharmacoepidemiologic studies of statin use and cancer outcomes.

To date, three peer-reviewed publications have arisen from this thesis (Chapters 3, 4 and 6), as well as one publication currently under-review (Chapter 5) and one being prepared for publication (Chapter 7). This research has also been presented as poster and oral presentations at several

national and international academic conferences. I also have been awarded travel scholarships to attend the largest pharmacoepidemiological research conference (International Conference on Pharmacoepidemiology & Therapeutic Risk Management) in 2017 and 2018. Beyond traditional forms of dissemination, other activities undertaken include: presenting work from Chapters 3, 4 and 6 to the international scientific advisory board (SAB) at Breast-Predict annual review meetings, discussing my research with the public at the launch of the Irish Cancer Society's Daffodil Day, and writing a lay blog post on research as part of the Irish Cancer Society's 'Researcher in Focus'.

8.6.2. Policy impact

While it takes a large body of strong evidence to influence policy and practice, there are potential implications resulting from Chapter 3. This study showed that one month prior to death, over 50% of breast cancer patients and over 40% of colorectal cancer patients were still receiving a statin prescription. It has been highlighted in the literature that along with the many advantages of statins, there are diminishing benefits and in some cases harm, when used in patients-with limited life expectancy [226]. Although there are currently no clinical guidelines on ceasing statin treatment, a recent clinical trial suggests that it is safe to do so in patients with limited life expectancy [148]. The results from Chapter 3 highlight, in an Irish setting, the need for clear clinical guidelines for medication deprescribing, when clinicians are presented with such a scenario. The policy impact of the other studies included in this thesis is perhaps less clear, due the uncertainty around the exact benefit for statins as a chemotherapeutic agent. However, as the body of pre-clinical, clinical and epidemiological evidence continues to grow, it is possible that statins may be recommended in a chemotherapeutic role, as in the case of bisphosphonates and aspirin (as discussed in section 1.3).

8.6.3. Societal impact

Societal impact encompasses changes in knowledge, attitude, and behaviour for example. While difficult to determine, lay presentation of findings from this thesis (in poster, oral, and unstructured form) may have raised awareness of pharmacoepidemiology in the public. As mentioned in Section

8.6.2, research disseminated from this thesis could alter clinician's decision making processes at the end of life in these patient cohorts, which may lead to behavioural change in (de)prescribing.

Recently, there has been increased research into the many personal, healthcare and economic costs associated with cancer. There are costs associated with the healthcare system due to screening, diagnostic tests, and treatments, and personal and financial costs incurred by the patient, as well as economic costs due to time taken off work to attend treatments, etc. As such, The Irish Cancer Society has published a report on the 'Real Cost of Cancer' in Ireland. The research behind this report revealed over half (56%) of the cancer patients surveyed were spending approximately €150 a month on medication, with 80% of people experiencing treatment side effects and purchasing over-the-counter (OTC) medication [227]. However, the majority of financial strain came from costs not associated directly with the disease. These costs included: travel to appointments, hospital parking and eating out. The report revealed that the overall average cost associated with attending appointments is €287 per month per person [227]. A large international study by de Boer *et al*. also showed that cancer survivors were significantly less likely to be employed than healthy control participants [228]. In economic terms, cancer-related productivity losses are the result work absences, workforce departure, and premature mortality [5]. Should statins go on to be recommended in chemotherapeutic setting, this would reduce some of the costs outlined above; statins are a low-cost medication, reducing the cost to the health system, and they have minimal side effects, reducing the need for out of pocket payments for treatment of side effects.

8.7. Overall conclusion

The association between statins and cancer has been the focus of research in the last number of years, with publications of 'statin' and 'cancer' on PubMed Central®, a free full-text archive of biomedical and life sciences journal literature at the U.S. National Institutes of Health's National Library of Medicine, increasing from 33 publications in 2000, to 253 publications in 2017.

The studies within this thesis contribute to the evidence-base on patterns of statin use in those with specific cancers, and also the association between statin use and breast and colorectal cancer

outcomes. These studies demonstrate the potential for using Irish data to perform further pharmacoepidemiological outcomes research in the area of breast and colorectal cancer. Future research efforts would benefit from the incorporation of additional relevant clinical information and molecular tumour details; these will hopefully be achieved with on-going and potential future data linkage projects. Overall, the studies described support the continued investigation of the potential clinical uses of statins in breast and colorectal cancer.

Appendix

Appendix

Table A 1.2: WHO-ATC drug classifications

Drug Exposures	WHO ATC Drug Codes
Hydrophilic Statin	
Pravastatin	C10AA03
Rosuvastatin	C10AA07
Pravastatin and Acetylsalicylic acid	C10BX02
Lipophilic Statin	
Simvastatin	C10AA01
Lovastatin	C10AA02
Fluvastatin	C10AA04
Cerivastatin	C10AA06
HMG-CoAR inhibitors in combination with other lipid-modifying agents	C10BA
Simvastatin and Acetylsalicylic acid	C10BX01
Atorvastatin	C10AA05
Atorvastatin and Amlodipine	C10BX03

Table A 1.3: Study outcome/covariate definitions

Study Covariate / Outcome	Definition
Drug Exposures	**WHO ATC Drug Codes**
Aspirin	B01AC06, M01BA03, N02BA01, N02BA51, N02BA71
Anti-diabetic	A10
Bisphosphonate	M05BA, M05BB
Hydrophilic Statin	C10AA03, C10AA07, C10BX02,
Lipophilic Statin	C10AA01, C10AA02, C10AA04, C10AA06, C10BA, C10BX01, C10AA05, C10BX03
Other NSAID	M01A
Hormonal Therapy	L02BA01, L02BA02, L02BA03, L02BG03, L02BG04, L02BG06
Vitamin D	A11CC, A12AX
Tumor Receptor Status	**NCRI Coding Definition**
ER, PR	Estrogen and progesterone receptor activity was defined as positive if recorded by the NCRI database as unclear/possibly, some receptor activity or positive/strong.
HER2	HER2 receptor activity was defined as positive by immunohistochemistry if recorded by the NCRI database as score 2+, weak/strong positive or weak/strong complete membrane staining in >10% of tumor cells. HER2 receptor activity was defined as positive by fluorescence in-situ hybridization if recorded by the NCRI database as weak/strong positive or some/strong amplification. Where IHC & FISH results were recorded, FISH results were used.
Breast cancer-specific mortality	**ICD10 Codes**
From Howlader *et al.* [229]	B201-B219; C50, D05, D24, D486 C445, D225, D485 C000–C444, C446–C499, C510–D049, D060–D224, D226–D239, D250–D484, D487–D489 N610–N649
Colorectal cancer-specific mortality	**ICD10 Codes**
From Howlader *et al.* [229]	B210-B219 C18-20, C785 D010-D012, D12, D374-D375 C17, C21, C26, D371–D373, D376–D379 C000–C169, C220–C259, C270–C784, C786–D009, D013–D119, D130–D370, D380–D489 K200–K319, K350–K389, K510–K579, K620–K639, K650–K669, K920–K929

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