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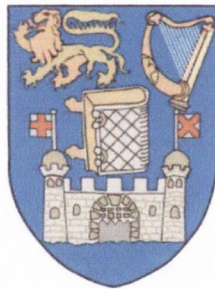
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Metformin, Aspirin, and Colorectal Cancer Outcomes

A thesis submitted to the University of Dublin, Trinity College, for the
degree of Doctor of Philosophy



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September 2013

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Declaration

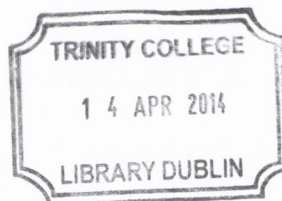
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Summary

Colorectal cancer is the second most incident cancer in men and women in Ireland and is the third greatest cause of cancer death. Metformin and aspirin, two drugs which are commonly used in type 2 diabetes and cardiovascular disease, have been identified as having a potential role in the treatment of colorectal cancer. Preclinical studies have demonstrated an inhibitory effect on colorectal tumours with each drug, and a range of potential mechanisms of action for their anti-cancer effects have been proposed. Metformin may act to inhibit colorectal tumours through direct actions on cancer cells or may have an indirect effect through insulin-related signalling pathways. Similarly, aspirin may have a direct effect by inhibiting the COX-2 enzyme in cancer cells, or may act to prevent tumour spread through mechanisms related to platelet inhibition.

Pharmacoepidemiological studies provide the opportunity to investigate the effects of drug exposures on colorectal cancer survival using existing datasets. Previous studies have found that both metformin and aspirin are associated with lower incidence of colorectal cancer and some studies have found associations between the drugs and improved cancer survival. The studies within this thesis aimed to examine associations between exposure to (i) metformin, and (ii) aspirin, and colorectal cancer survival, and also between metformin exposure and colorectal cancer dissemination.

Records from the National Cancer Registry Ireland, which had been linked to prescription claims data from the Health Service Executive Primary Care Reimbursement Service, were extracted for patients diagnosed with colorectal cancer between 2001 and 2006 inclusive. The primary study population, for examining associations between metformin/aspirin and survival, included patients with non-metastatic cancer at diagnosis. Cox proportional hazards regression was used to estimate adjusted hazard ratios (HR) with 95% confidence intervals (CI) for associations between metformin/aspirin and colorectal cancer-specific survival. Multivariate logistic regression was used to estimate adjusted odds ratios (OR) for associations between metformin exposure and tumour dissemination (presence of local or distant metastases) at diagnosis in patients with all stages of colorectal cancer. Analyses examining metformin were stratified by dosing intensity (a measure of exposure frequency) and by co-prescription with other anti-diabetic drugs. Analyses of aspirin exposure were stratified by site of colorectal tumour, and by dosing intensity and dose of aspirin dispensed.

Multivariate analyses suggested that metformin exposure (versus other anti-diabetic drugs) was associated with a lower risk of colorectal cancer-specific mortality, though not statistically significant (HR=0.61, 95% CI 0.37-1.01). Analyses stratified by exposure intensity or co-prescription with other anti-diabetic drugs produced similar results. In analyses stratified by both dosing intensity and co-prescription with other anti-diabetic drugs, high intensity, exclusive metformin use was associated with a significant survival improvement of a greater magnitude (HR=0.44, 95% CI 0.20-0.95). No effect was observed in other stratified exposure categories.

Analyses of associations between overall metformin exposure (versus other anti-diabetic drugs) and tumour dissemination at diagnosis were non-significant (OR=0.66, 95% CI 0.39-1.12). Results of analyses stratified by dosing intensity, co-prescription with other anti-diabetic drugs, or both of these factors, were also non-significant. However, the magnitude of the reduction in odds of disseminated disease was greater among patients with high intensity, exclusive metformin use (OR=0.52, 95% CI 0.25-1.10).

Multivariate analyses of associations between aspirin exposure (versus no aspirin exposure) and colorectal cancer survival did not find an overall effect of aspirin on survival among non-metastatic cancer patients (HR=0.94, 95% CI 0.80-1.09). When stratified by site, aspirin exposure was associated with a significant survival improvement among patients with proximal colon tumours (HR=0.73, 95% CI 0.54-0.99), though not among patients with distal colon or rectal tumours. Analyses of patients with overall non-metastatic colorectal cancer stratified by dosage of aspirin, dosing intensity or a combination of these factors did not suggest a survival improvement with increasing aspirin exposure.

These studies were limited by a relatively small sample size and by lack of information on some potential confounding factors. Also, only the effects of exposures prior to cancer diagnosis were examined. Strengths of the studies include the use of high-quality national-level cancer data, which enabled detailed consideration of tumour staging and survival. Also, detailed exposure data permitted the study of continuous versus infrequent drug exposure.

The overall results from these studies are broadly consistent with previous research on associations between metformin and aspirin and colorectal cancer survival. These studies also contribute novel data on how such associations may be modified by level of exposure or co-prescription with related drugs. The analyses of associations between metformin exposure and colorectal tumour dissemination represent the first study in this area. Further research into these effects, ideally in larger datasets with more detailed clinical data, is warranted.

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Dedication

To the memory of my Leaving Certificate chemistry teacher, Ms Bríd O'Connor, who passed away from cancer shortly after I completed secondary school. While a deeply religious woman, Ms O'Connor was an inspiring and energetic teacher of science and always had faith in the potential of her students to be the next great female scientists. She has never been far from my mind while completing these studies.

To Mum, Dad and Jen, and my dear friends, particularly those who have been touched by cancer. You have made this all worthwhile.

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Publications arising from this thesis

List of Publications

Publications in international peer-reviewed journals

Spillane, SC, Bennett K, Sharp L, Barron TI. A Cohort Study of Metformin Exposure and Survival in Patients with Stage I-III Colorectal Cancer. *Cancer Epidemiol Biomarkers Prev.* 2013 22(8): 1364-1373.

Spillane, SC, Bennett K, Sharp L, Barron TI. Metformin exposure and disseminated disease in patients with colorectal cancer. *Cancer Epidemiology.* 2014 (in press)

Oral presentations at international peer-reviewed conferences

“Effect of metformin versus other anti-diabetic drugs on colorectal cancer stage.”

Spillane SC, Bennett K, Sharp L, Barron TI. Presentation at 28th International Conference on Pharmacoepidemiology and Therapeutic Risk Management, Barcelona, August 2012. Session: Diabetic treatment outcomes around the world.

“Use of the anti-diabetic drug metformin and disease spread at diagnosis in colorectal cancer.”

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Poster presentations at international peer-reviewed conferences

Spillane SC, Bennett K, Sharp L, Barron TI. “Effects of metformin and sulfonylureas on overall and colorectal cancer-specific mortality.” Poster at Annual Meeting of American Society of Clinical Oncology, Chicago, June 2012. Session:

Poster presentations at national peer-reviewed conferences

Spillane SC, Bennett K, Barron TI. “The role of metformin in colorectal cancer: A review of the evidence.” Poster at 3rd National Conference on Population-Based Cancer Research in Ireland, Dublin, November 2011.

Spillane SC, Bennett K, Sharp L, Barron TI. “Patient characteristics, treatment changes, and survival: Rectal cancer patients in Ireland pre and post 2001.” Poster at 3rd National Conference on Population-Based Cancer Research in Ireland, Dublin, November 2011.

Abbreviations

List of Abbreviations

ACE(I)	Angiotensin converting enzyme (inhibitor)
ACF	Aberrant crypt foci
ADD	Anti-diabetic drug
ADOPT	A Diabetes Outcome Progression Trial
ADP	Adenosine diphosphate
AJCC	American Joint Committee on Cancer
AMP (K)	Adenosine monophosphate (kinase)
ARB	Angiotensin receptor blocker
ATC	Anatomical Therapeutic Classification
ATP	Adenosine triphosphate
BMI	Body Mass Index
BRAF	Proto-oncogene B-Raf
CCB	Calcium channel blocker
CI	Confidence Interval
COX	Cyclooxygenase
CRC	Colorectal cancer
CSO	Central Statistics Office
CPRD	Clinical Practice Research Database
DPP4	Dipeptidyl peptidase 4
EMA	European Medicines Agency
FAP	Familial Adenomatous Polyposis
FDA	Food and Drugs Administration
GI	Gastrointestinal
GLP 1	Glucagon-like peptide 1
GMS	General Medical Services (Scheme)
HbA _{1c}	Haemoglobin A _{1c} (glycated haemoglobin)
HER2+	Human Epidermal Growth Factor Receptor 2 (positive)
HIPE	Hospital Inpatient Enquiry (System)
HNPCC	Hereditary Non-polyposis Colorectal Cancer (Lynch syndrome)
HR	Hazard Ratio

HSE-PCRS	Health Service Executive – Primary Care Reimbursement Services
ICD-10	International Classification of Diseases, 10 th edition
ICD-9	International Classification of Diseases, 9 th edition
IGF (R)	Insulin-like growth factor (receptor)
IQR	Interquartile range
KRAS	Kirsten rat sarcoma viral oncogene homolog
LKB1	Liver kinase B1
LTI	Long Term Illness (Scheme)
MeSH	Medical Subject Headings
MSI	Microsatellite instability
mTOR	Mammalian target of rapamycin protein
NCRI	National Cancer Registry Ireland
NSAID	Non-steroidal anti-inflammatory drug
OCT	Organic cation transporter
OHA	Oral hypoglycaemic agent
OR	Odds Ratio
PDC	Proportion of days covered (measure of dosage intensity)
PG	Prostaglandin
PI3K	Phosphatidylinositide 3-kinase
PIK3CA	Catalytic subunit alpha of PI3K
RCT	Randomised Controlled Trial
RR	Relative Risk
Rx	Prescription
SD	Standard deviation
SEER	Surveillance Epidemiology and End Results (database)
SGLT ₂	Sodium glucose co-transporter 2
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
THIN	The Health Improvement Network (database)
TNM	Tumour, Node, Metastasis Classification system
UK	United Kingdom
US	United States (of America)
WHO	World Health Organisation

1. Introduction

1.1. Colorectal Cancer

1.1.1. Burden of disease in Ireland

Colorectal cancer (also known as bowel cancer) is the third most incident cancer after cancers of the breast and prostate (excluding non-melanoma skin cancer).¹ It represents the second most incident cancer when men and women are considered separately, accounting for 14% and 11% of all incident cancers in men and women in Ireland, respectively.¹ The epidemiology of colorectal cancer in Ireland has most recently been described in a report by the National Cancer Registry of Ireland examining colorectal cancer trends between 1994 and 2010.² Approximately 850 female cases and 1,100 male cases were diagnosed on average each year between 1994 and 2010; the age-standardised rate of incidence was not observed to change during this time and was found to be similar to the European average.² Between 2010 and 2020, colorectal cancer cases are expected to increase by 34% in women and 45% in men.²

Previous research from the National Cancer Registry Ireland using data collected prior to 2002 found that Ireland had one of the highest colorectal cancer mortality rates in the developed world.³ However, mortality rates have shown a steady decline in recent years and are now in line with the European average.² Significant improvements in survival have also been noted and are attributed to greater uptake of treatment and earlier diagnosis.² However, colorectal cancer remains the third leading cause of cancer death in women and the second leading cause of cancer death in men. Currently there are, on average, 400 female and 552 male deaths from colorectal cancer per year in Ireland.²

In addition to physical, emotional and financial consequences of a diagnosis of colorectal cancer for the individual patient and their family, the associated losses of productivity and economic costs that accompany colorectal cancer are a high societal burden, as seen in studies conducted in Ireland.^{4, 5} The average direct medical cost (from the healthcare payer perspective) of treating a case of colorectal cancer in Ireland has recently been estimated at €39,607.⁶

1.1.2. Risk factors associated with colorectal cancer

The greatest risk factor for sporadic colorectal cancer is increasing age, followed by family history.⁷ A recent study performed a series of meta-analyses examining family history and other potential risk factors in order to quantify their influence on cancer incidence; factors examined included inflammatory bowel disease, postmenopausal hormone therapy, aspirin/non-steroidal anti-inflammatory (NSAID) use, cigarette smoking, body mass index (BMI), physical activity, diet, and alcohol intake.⁸ Inflammatory bowel disease (Crohn's disease or Ulcerative Colitis) (RR=2.93, 94% CI 1.79-4.81) and family history in a first-degree relative (RR=1.80, 95% CI 1.61-2.02) were found to considerably increase an individual's risk of cancer. The following were associated with moderate increases in risk of colorectal cancer: increased BMI, low physical activity, cigarette smoking, consumption of red meat, low consumption of fruit and/or vegetables. Trends towards increased risk were observed for alcohol and processed meat but were not statistically significant. Postmenopausal hormone therapy and aspirin/NSAID use showed a trend towards a protective effect, but were also non-significant.⁸

Cigarette smoking, low physical activity and consumption of processed and red meat have all been significantly associated with shorter colorectal cancer survival.⁹⁻¹² Combined hormonal therapy in postmenopausal women has not been found to confer a survival benefit.¹³ Establishing a causal relationship between obesity and colorectal cancer survival is complex (see section 5.5.5) but pre-diagnostic BMI has been associated with poorer colorectal cancer survival.¹⁴ Recently, much attention has been given to the relationship between type 2 diabetes and colorectal cancer survival; this will be discussed in Chapter Three. The relationship between aspirin/NSAID use and colorectal cancer survival will be discussed in detail in Chapter Four.

1.1.3. Anatomy, pathology and staging

Colorectal cancer consists of a malignant solid tumour of the digestive tract (Figure 1.1) located in the colon, rectum or rectosigmoid junction (Figure 1.2). The commonest symptoms of the disease include abdominal pain, change in bowel habit, and rectal bleeding or anaemia.⁷
¹⁵ Initial evaluation of these symptoms can include faecal occult blood testing and diagnosis is usually confirmed following referral for colonoscopy, the gold standard method of diagnosis.⁷

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Approximately 5-6% of colorectal tumours have a clear genetic basis, arising due to hereditary conditions such as Familial Adenomatous Polyposis (FAP) or Hereditary Nonpolyposis Colorectal Cancer¹⁶, but the majority of tumours develop sporadically as adenocarcinomas,

that is, they develop from pre-cancerous adenomatous polyps which acquire the characteristics necessary for malignancy.¹⁷ Transformation to a malignant tumour occurs in a step-wise manner over a time period of between 10 and 15 years; sequential molecular abnormalities occur, resulting from the accumulation of activated oncogenes and inactivated tumour-suppressor genes.¹⁶

Tumours of the colon and rectum are genetically indistinguishable in the majority of cases, though genetic mutations are more common in the ascending, or proximal, colon.¹⁸ However, the anatomical differences between colon and rectal tumours have implications for surgical and radio-therapeutic management, and may affect prognosis.¹⁸



Figure 1.1: Human digestive tract¹

¹ Figure 1.1 produced in part using Servier Medical Art (www.servier.com)

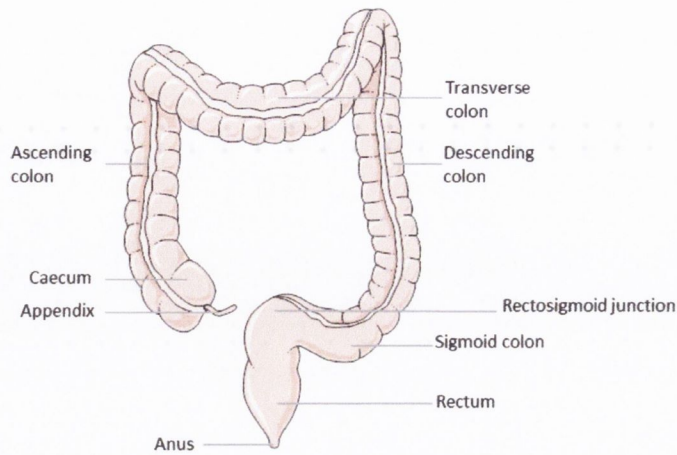


Figure 1.2: Colon and Rectum²

Treatment of colorectal cancer (see section 1.1.4) is primarily determined by the stage of disease. Clinical staging, based on physical examination, radiologic findings and endoscopy, and pathologic staging, based on surgical approaches, are often combined to determine the TNM stage for the patient.¹⁹ The TNM stage system of the American Joint Committee on Cancer describes: (i) the extent of spread of the tumour through the colorectal wall (T), (ii) whether or not, and the extent to which, the cancer has spread to nearby lymph nodes (N), and (iii) whether or not the cancer has spread (metastasised) to other organs or distant lymph nodes (M). The TNM stage is then grouped to produce a summary stage of cancer expressed as stage I, II, III or IV disease (Figure 1.3). Stage I tumours are the least advanced; such tumours have invaded the inner layers of the colorectal wall but have not spread beyond this. Stage II tumours have invaded the outermost layers of the intestinal wall but have not spread to the lymph nodes or distant sites. Stage III tumours have invaded the outermost walls of the colorectal wall and cancer cells may have entered the nearby lymph nodes. Stage IV tumours have invaded to the point that they have spread to distant organs or distant lymph nodes.¹⁹

² Figure 1.2 produced in part using Servier Medical Art (www.servier.com)

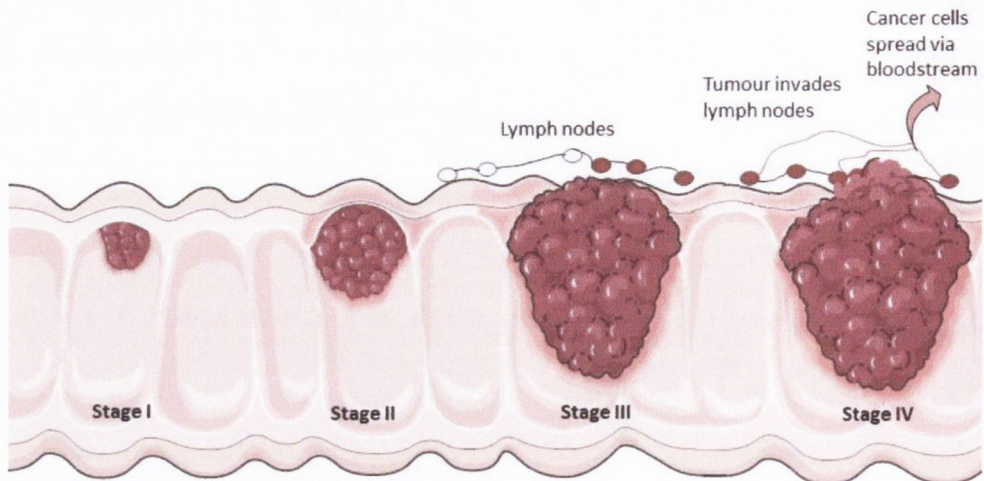


Figure 1.3: Staging of Colorectal Cancer ³

Spreading of the primary tumour to distant organs (e.g. liver and lung) or lymph nodes is the process known as metastasis. This is a complex and poorly understood phenomenon but is thought to occur by a process called epithelial to mesenchymal transition (EMT) and by the interaction of this process with cancer stem cells.^{20, 21} EMT involves the acquisition by a tumour cell of characteristics that enable it to invade and migrate to other sites. Cancer stem cells are a recently identified subdivision of tumour cells which have the ability to self-renew by asymmetric division, to differentiate into diverse phenotypes, to initiate tumours from small numbers of cells, and which exhibit high chemoresistance.²⁰ These cell types have been identified in colorectal tumours in a number of studies;^{22, 23} research is carried out in this area as agents that could control the growth of these cells could be utilised for cancer prevention and treatment.²⁴

1.1.4. Treatment

The primary basis of treatment decision-making for patients with colorectal cancer is the TNM stage.²⁵ Surgical resection for localised colorectal cancer is the only likely curative treatment for colorectal cancer. Adjuvant chemotherapy is administered to colorectal cancer patients with high risk of local recurrence following surgery; options include 5-fluorouracil and folinic acid, or this combination with additional oxaliplatin.² In rectal cancer patients with locally advanced tumours (stage II-III), pre-operative (neoadjuvant) radiotherapy with synchronous chemotherapy may be administered in order to reduce the size of the tumour prior to surgery.²⁶ While this will result in some level of tumour regression in most patients, some will

³ Figure 1.3 produced in part using Servier Medical Art (www.servier.com)

be found to exhibit a 'pathologic complete response', i.e., by the time of surgery there is found to be a complete lack of viable tumour cells in the surgical specimen.²⁶ This response occurs in approximately 15-30% of patients and is correlated with improved survival.^{27, 28}

Patients with advanced colorectal cancer (metastatic) are offered palliative surgery and/or palliative chemotherapy where curative resection of the disease is not expected to be possible.² Recent advances in the chemotherapy of colorectal cancer include the use of biologic agents such as bevacizumab, cetuximab and panitumumab. However, these agents, which come at great expense, have led to only modest improvements in quality of life and overall survival, reinforcing the need for additional novel therapies.^{6, 29}

1.1.5. Reducing the burden of Colorectal Cancer

Screening, with the aim of enabling earlier detection of colorectal tumours, is an effective method to reduce the burden of colorectal cancer.³⁰ At the time of writing, the first phase of 'BowelScreen', Ireland's first national population-based colorectal cancer screening programme, was being rolled out.³¹ The implementation of this programme was announced in January 2010, promising the introduction of screening on a two-yearly basis for the 55- to 74-year age-group by means of the faecal immunochemical (FIT) home test as the primary screening tool.⁶ In line with international colorectal cancer screening programmes, this initiative is expected to be highly cost-effective in reducing mortality and improving quality of life as a result of earlier detection of tumours.³²

There is also extensive evidence in favour of increasing primary prevention efforts to reduce the incidence of colorectal cancer. Research suggests that the most successful strategy for colorectal cancer prevention will include combined approaches of screening and lifestyle modification, i.e. minimising the risk factors of obesity, physical inactivity, cigarette smoking, and intake of red meat, processed meat and alcohol.³³ In addition to lifestyle modification, the field of cancer research is increasingly considering the concept of primary prevention via preventive medicine, that is, chemoprevention.³⁴

1.2. Chemoprevention of colorectal cancer

The concept of preventive medicine has been widely applied in the cardiovascular disease setting; regimens aimed at reducing cholesterol, blood pressure and platelet aggregation have achieved significant decreases in mortality from myocardial infarction and stroke.^{35, 36} Cancer chemoprevention is a similar approach and involves pharmacological intervention with the aim of arresting or reversing the process of carcinogenesis at early stages of tumour development.

³⁷ This field holds much promise and much research is taking place to explore cancer chemoprevention possibilities, but the safety-related practicalities of administering a cancer-preventing drug to otherwise healthy patients remain an obstacle to progress.³⁵ As described in a recent review of colon cancer chemoprevention:

*“The ideal chemopreventive agent is one that has proven to be effective, has a convenient dosing schedule, has minimal side effects or a low but acceptable toxicity profile in high-risk populations, and should be inexpensive”.*³⁸

The utility of chemoprevention in colorectal cancer is currently most widely considered in individuals with intermediate to high risk of the disease due to genetic predisposition, family history or inflammatory bowel disease.³⁹ Evaluation of the prospect of chemoprevention has found it to be potentially cost-effective in such groups, particularly following removal of polyps.³⁹

One example of attempts at colorectal cancer chemoprevention in a group at greater risk for colorectal cancer involves the use of 5-aminosalicylate drugs in patients with ulcerative colitis. These drugs are used to control the inflammation of ulcerative colitis that gives rise to the symptoms of the disease, and studies among patients exposed to these agents originally also suggested a potential protective effect against the development of colorectal cancer. However, following review of the accumulated evidence on this subject, current opinion is that these drugs are of little chemopreventive value.⁴⁰ Another drug which was specifically licensed as a chemopreventive agent for colorectal cancer was celecoxib, a COX-2 inhibitor which was licensed by the FDA for reduction of polyp growth in patients with FAP. However, concerns about serious cardiovascular events attributed to the drug have led to its withdrawal as a recommended chemopreventive agent.⁴¹

A large number of pharmaceutical agents and natural substances have been evaluated for their chemopreventive potential in colorectal cancer, including NSAIDs, HMG-CoA reductase inhibitors (statins), postmenopausal hormonal therapy, calcium, and the plant extract curcumin.³⁸ In recent years, due to the publication of a number of studies in large patient cohorts, particular focus has been drawn towards the potential of the drugs aspirin and metformin in colorectal cancer. The cancer literature examining these two agents, which are the subject of this thesis, will be discussed in detail in Chapters Three and Four.

1.3. Drug repurposing: Aspirin and metformin

The discovery of anti-cancer activity among drugs such as aspirin and metformin, which are commonly used for other clinical indications, is an exciting prospect. Both of these drugs are off-patent and relatively inexpensive (see Chapters Two and Four), in contrast to agents considered in conventional cancer drug discovery.⁴² This reason, amongst others, has led to encouragement for the potential repurposing of these drugs as anti-cancer chemopreventive agents and/or treatments.⁴²⁻⁴⁵

Drug repurposing (also known as repositioning, redirecting, or reprofiling) is the investigation of existing drugs, such as aspirin or metformin, for application outside the scope of their original therapeutic indications.⁴⁶ This concept represents a substantially faster route of drug development than what would conventionally be expected and examples of its application include the repurposing of thalidomide for erythema nodosum laprosium and multiple myeloma, or the repurposing of sildenafil as a treatment for erectile dysfunction.⁴⁶ Several phases of the process can be bypassed as the drug candidate will often have undergone these testing procedures for their original indication; the pharmacokinetic, pharmacodynamics and toxicity profiles of the drug candidates are already well established, meaning their progression to phase II and III clinical studies may be expedited.^{42, 46} This is particularly relevant in the field of cancer therapeutics where toxicity and expense of new therapies limit their application. Repurposing may also involve the consideration of drugs such as aspirin or metformin as lead compounds; identification of anti-cancer effects of these drugs results in new drug development efforts to improve upon the original chemicals.⁴⁷

1.4. Pharmacoepidemiological studies as a source of evidence

In considering the repurposing of an existing drug towards a new therapeutic indication, it is important to establish a strong evidence base to support testing of the drug in clinical trials. Evidence may be generated from preclinical studies (i.e. laboratory studies of in vitro or in vivo effects) or through analyses of data from patients who have already been taking the drug for other indications. 'Pharmacoepidemiology' is the branch of research which studies the utilisation and effects of drugs in large numbers of people; it represents the application of epidemiological methods to pharmacological questions.⁴⁸

The randomised controlled clinical trial (RCT) is viewed as the gold standard in evidence-based medicine.⁴⁹ However, clinical trials may not always be the most feasible method of study due to ethical concerns where the potential harm associated with the intervention is unknown.⁵⁰ Clinical trials are also costly and time-consuming and may therefore be inappropriate where

there is a relatively urgent need for information regarding a treatment's effect.⁵¹ Additionally, they represent an idealised treatment situation (as subject inclusion criteria and drug exposures are controlled or restricted) that may not reflect the real-life treatment adherence of patients, or the eventual population prescribed the drug.⁵¹

Pharmacoepidemiological studies, or observational studies, are primarily distinguished from clinical trials by the method of allocation of the drug of interest to the groups under study.⁵² In the case of RCTs, the investigator randomly assigns (often in a blinded manner) patients to study groups. In contrast, observational studies are non-interventional; the investigator has no control over which patients are exposed or unexposed.⁵² This may result in the presence of bias and/or confounding, as described below.

1.4.1. Bias and confounding in pharmacoepidemiological studies

Bias is a distortion of study results which may occur when the study groups under investigation (exposed and unexposed) are identified, treated or evaluated differently to each other.⁵³ Forms of bias include selection bias, where exposed and unexposed patients are selected for study due to reasons which may influence the outcomes, and information bias, where data is collected in an unbalanced manner between exposed and unexposed groups.⁵³ Confounding is where the results of a study are not due to a true association between exposure and outcome, but may in fact be explained by another factor, e.g. age, which is associated with both the exposure and the outcome.⁵³

In RCTs, the random assignment of a large number of participants into either the treatment or control group is expected to result in a balance of known and unknown risk factors/confounders for the outcome among the two groups. However, in observational studies, exposure to the drug under investigation occurs in a non-random manner.⁵⁰ A patient may receive a certain drug for a variety of reasons known or unknown to the researcher. For example, the age of a patient or the cost of a treatment may be a factor in deciding whether a particular drug is suitable for a patient. If these factors are also associated with the outcome under investigation, the exposure/outcome relationship is likely to be confounded. Various methods have been suggested to reduce confounding in observational studies and include both study design and analysis approaches.⁵⁴ Study design approaches include matching exposed and control patients by a common potential confounding factor, or restriction of the analysis to only one level of a particular confounding factor. Common approaches in dealing with confounding at the analysis stage include stratification and multivariate analysis.⁵⁴

1.4.2. Pharmacoepidemiological study designs

Observational studies in pharmacoepidemiology include a variety of study types, most commonly cohort and case-control studies.⁵²

Cohort studies involve following a group of exposed subjects and a group of unexposed subjects over time; these subjects may enter and exit the cohort at different moments in time but entry to the cohort (time zero) must be defined by a fixed episode, e.g. diagnosis of cancer.⁵⁵ A cohort study may be performed prospectively, where the study is designed before the patients are followed over time, or retrospectively, where the outcomes of the study have already occurred before the data for the study is collected.⁵² Cohort studies allow the establishment of a temporal relationship between an exposure and an outcome.⁵⁶

Case-control studies involve first identifying individuals with the disease/outcome of interest (cases) and without (controls) and then determining the prevalence of the exposure within each group in order to examine associations between the exposure and the disease.⁵⁰

1.4.3. Pharmacoepidemiological databases

Observational studies examining candidates for potential drug repurposing commonly involve obtaining patient data from administrative healthcare databases.^{57, 58} Examples of healthcare databases include pharmacy dispensing records, health insurance claims databases, general practice research databases, electronic hospital records, and disease registries; such databases may be linked in order to provide access to particular information, e.g. the linkage of pharmacy claims databases to disease registries in order to determine particular medications received by patients with a condition of interest.⁵⁸

The use of linked administrative data for cancer research was recently described.⁵⁹ Cancer registries are a particularly rich source of information in cancer research and usually include detailed tumour information such as stage, grade and morphology. They may also include details of cancer treatment and may include accurate outcome data. Medical record linkage of a cancer registry to pharmacy dispensing records can provide information on the type of regular medications patients were receiving, thus permitting the evaluation of associations between the drugs and tumour characteristics and/or cancer outcomes. This type of linked database represents a valuable resource as it comprises independently collected prospective data, obviating concerns regarding recall bias which may affect other studies of cancer patients.⁶⁰ However, as these databases contain previously collected data, they often lack information on potential confounding factors relevant to the study being performed.^{61, 62} In

spite of this limitation, these types of data sources have the advantages of providing 'real-world' data, and a longitudinal perspective as they often contain observations of health care utilisation before, during and after a cancer diagnosis.⁵⁹ They therefore allow rapid assessment of associations between drugs and cancer outcomes, often at a population level.

Within Ireland, researchers in the field of pharmacoepidemiology have recently linked data from the National Cancer Registry Ireland to pharmacy dispensing records from the General Medical Services (GMS) scheme operated by the Health Service Executive Primary Care Reimbursement Service (HSE-PCRS). These data sources are described in detail in section 5.1. They have already proven a rich resource for cancer pharmacoepidemiology research examining drug repurposing candidates, and have given rise to several publications.^{63, 64} The studies described later in this thesis utilise this linked data in order to examine associations between metformin, and aspirin, and colorectal cancer outcomes.

1.5. Research Aims and Objectives

1.5.1. Research aims

This program of research aims to use pharmacoepidemiological methods to examine the commonly used drugs metformin and aspirin as potential candidates in the treatment of colorectal cancer. Research efforts will seek to add to the knowledge base by investigating causal associations between (i) metformin, and (ii) aspirin, and outcomes in patients with diagnosed colorectal cancer.

1.5.2. Research objectives

- 1)** Summarise the existing literature which investigates metformin and aspirin as potential therapeutic agents in colorectal cancer.
- 2)** Determine and apply appropriate methodology for examining, in a national-level dataset, associations between (i) metformin, and (ii), aspirin, and colorectal cancer outcomes.
- 3)** Examine associations between metformin exposure and colorectal cancer survival in a national-level dataset.
- 4)** Examine associations between metformin exposure and dissemination of colorectal cancer at diagnosis in a national-level dataset.
- 5)** Examine associations between aspirin exposure and colorectal cancer survival in a national-level dataset.

1.6. Chapter Outline

Chapter Two (Metformin: Use as an anti-diabetic agent) describes the background information on the drug metformin and its current clinical uses as an anti-diabetic agent. The clinical application, safety and mechanism of action of metformin will be described, followed by presentation of statistics on the prevalence of metformin usage in Ireland.

Chapter Three (Metformin and Colorectal Cancer) summarises the existing literature on associations between metformin and colorectal cancer. The wider discussion of associations between type 2 diabetes and cancer will first be introduced, followed by a synopsis of the preclinical studies, observational evidence and clinical trials that have addressed the topic of metformin and colorectal cancer. Potential mechanisms of action of the drug in cancer will be discussed, followed by consideration of the main questions in relation to the potential of this drug as a therapy in colorectal cancer.

Chapter Four (Aspirin: Current uses and potential applications in colorectal cancer), in a similar format to Chapters Two and Three, will discuss the current therapeutic indications for aspirin as well as the evidence base for prompting the consideration of aspirin as an anti-cancer drug.

Chapter Five (Patients and methods) describes the methodologies applied in the successive three chapters which comprise the studies in this thesis. The overall data source will be described in detail, followed by the methods for determination of drug exposure, disease outcomes and covariates of interest. Approaches to avoid bias and confounding common to the three studies of the thesis are also discussed.

Chapter Six (Metformin exposure and survival in patients with non-metastatic cancer) investigates associations between pre-diagnostic metformin exposure and colorectal cancer-specific survival among diabetic patients, focusing on a non-metastatic cancer population. Results are presented stratified by exposure dosing intensity, that is, a measure of frequency of exposure to the drug in the year prior to diagnosis, and by co-prescription with other anti-diabetic drugs. Secondary analyses presented include non-diabetic patients as the reference group.

Chapter Seven (Metformin exposure and risk of lymph node positive or metastatic disease in patients with colorectal cancer) examines associations between pre-diagnostic metformin exposure and disseminated colorectal cancer at the time of diagnosis. As in Chapter Six, results are stratified by exposure dosing intensity and by co-prescription with other anti-diabetic

drugs, and secondary analyses are presented with non-diabetic patients as the reference group.

Chapter Eight (Aspirin exposure and survival in patients with non-metastatic colorectal cancer) investigates associations between pre-diagnostic aspirin exposure and colorectal cancer-specific survival, focusing on a non-metastatic cancer population. Results are presented stratified by site of colorectal cancer, and by aspirin dosage and dosing intensity.

Chapter Nine (Conclusion) first summarises the outcomes of the studies described in the three preceding chapters. The contribution of these studies to the literature and the implications of the research studies are evaluated. Possible areas for future research are discussed and conclusions are presented on the anti-cancer potential of metformin and aspirin.

2. Metformin: Use as an anti-diabetic agent

2.1. Diabetes Mellitus

Diabetes mellitus, including Type 1 diabetes and Type 2 diabetes, is a disorder of metabolism characterised by chronic hyperglycaemia due to relative insulin deficiency, insulin resistance or both.⁶⁵ The current world prevalence of diabetes mellitus (including type 1 and type 2 diabetes) has been estimated at approximately 6.4% and is projected to increase to 7.7% (439 million adults) by 2030.⁶⁶ Estimates from 2010 suggest that over 8.9% of Irish adults in the 45+ years age-group have diabetes (6.2% clinically diagnosed, 2.7% undiagnosed)⁶⁷. The rate of clinically diagnosed diabetes for all adults aged 18+ years was 3.2% (106,000 people)⁶⁷.

Type 1 diabetes comprises 5-10% of patients with diabetes mellitus.⁶⁸ This form of the disorder is immune-mediated and involves destruction of pancreatic beta-cells, which leads to insulin deficiency.⁶⁵ Type 2 diabetes comprises 90-95% of diabetes mellitus and involves patients who have insulin resistance and, usually, a relative insulin deficiency.⁶⁸ Most patients with type 2 diabetes are obese or have high central adiposity.⁶⁸

The chronic hyperglycaemia of diabetes mellitus is associated with long-term damage to various organs, and patients with diabetes have a higher risk of cardiovascular, arterial and cerebrovascular disease.⁶⁸ The goal of anti-diabetic treatment is to achieve adequate glycaemic control to avoid such complications. Patients with type 1 diabetes are treated with insulin injections and careful dietary management to maintain normal blood glucose levels.⁶⁵ In type 2 diabetes, weight reduction, exercise and oral glucose-lowering agents (hypoglycaemic drugs), including metformin and other anti-diabetic drugs, are the primary approaches to achieving glycaemic control.⁶⁸ Current treatment guidelines recommend metformin as the first line drug therapy in type 2 diabetes (Figure 2.1).^{69, 70} If glycaemic control is not reached following treatment with metformin, an additional agent may be added, such as a sulfonylurea or insulin. Intensive insulin therapy is usually only required after failure of treatment with oral anti-diabetic drugs (Figure 2.1).⁶⁸ Blood glucose control may be estimated for type 2 diabetes patients using the parameter HbA_{1c}; this is a form of haemoglobin which serves as a marker of the average blood glucose concentration over a three month period.⁷¹ HbA_{1c} measurement may also be used to estimate adherence to anti-diabetic therapy.⁷¹

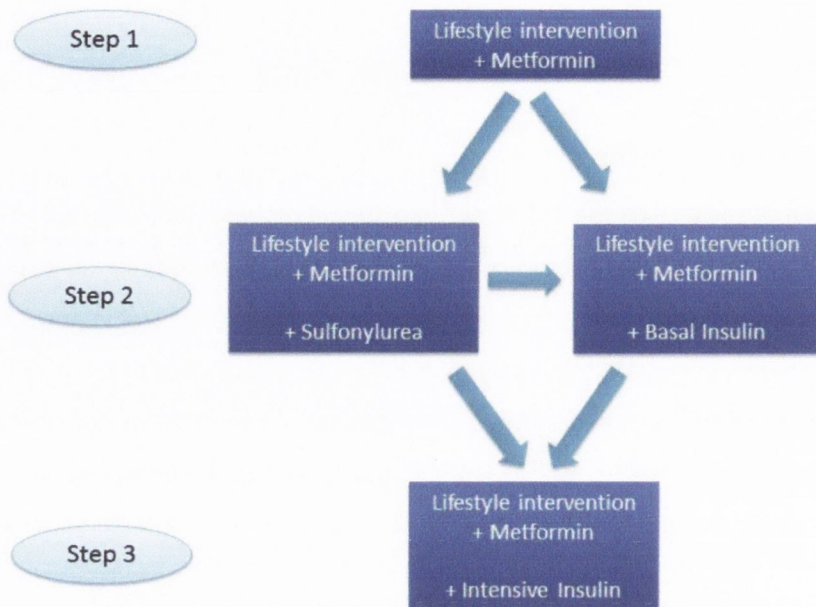


Figure 2.1: Steps in the management of Type 2 diabetes ^{69, 70}

Lifestyle intervention and metformin treatment are the first steps in the management of newly diagnosed type 2 diabetes. If these measures fail to achieve glycaemic targets, another medication may be added (step 2). If glycaemic control is still not reached, insulin may be added (or intensified) to the treatment regimen.⁶⁹

2.2. Metformin in Diabetes

The modern oral hypoglycaemic drug metformin is a derivative of a natural product, guanidine, which was isolated from *Galega officinalis* (Goat's rue, French lilac). This herb was used in medieval Europe to relieve excessive urination accompanying the condition which is now known as diabetes mellitus.^{72, 73} Guanidine in its isolated form was later shown to produce hypoglycaemic effects but was not applied clinically for the treatment of diabetes due to its high toxicity; drug development efforts subsequently led to the biguanide class of drugs including phenformin (Figure 2.2) and metformin (Figure 2.3). The initial synthesis of metformin (1,1-dimethylbiguanide) was performed in Trinity College Dublin in 1922 by the chemists Werner and Bell.⁷⁴ Today metformin is the main oral drug used in the treatment of type 2 diabetes. While the use of metformin in type 1 diabetes has been investigated in some clinical trials, no clear benefit has been observed with its use and it is not part of standard

therapy.⁷⁵ Due to safety concerns (section 2.2.2), metformin is the only member of the biguanide class of oral hypoglycaemic drugs that is available for use today.

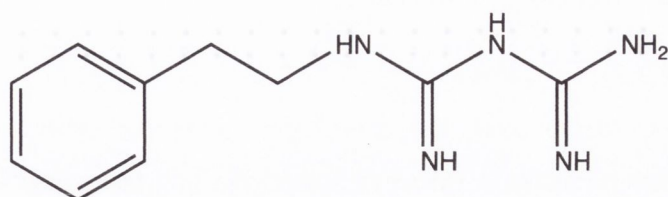


Figure 2.2: Chemical structure of phenformin

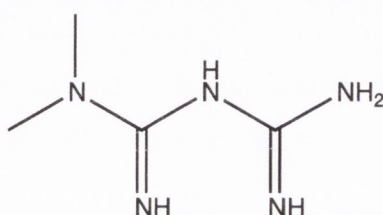


Figure 2.3: Chemical structure of metformin

2.2.1. Clinical usage

Metformin was first approved in the UK for the treatment of hyperglycemia in 1958⁷⁶ and was licensed in Ireland in 1988 (www.imb.ie) but did not receive approval for use in the US until 1995.⁷⁶ Currently, it is the eight most prescribed pharmaceutical product in the US with over 61 million prescriptions being dispensed for the drug in 2012.⁷⁷ In Ireland, metformin was the eighteenth most commonly dispensed drug within the GMS scheme in the same year.⁷⁸ The patent on metformin in the US expired in 2002 and generic versions of the drug are now available globally at low cost; an 84-tablet pack of generic 500mg metformin tablets currently carries a cost price of approximately £0.81 (British pound).^{79, 80} Metformin is licensed for the treatment of type 2 diabetes mellitus, particularly in overweight patients, when dietary management and exercise alone is not successful in achieving adequate blood glucose control.⁸¹ It is currently the first-line treatment for type 2 diabetes owing to its record of effectiveness and safety relative to other treatments.^{69, 70} Metformin is effective as monotherapy but may be combined with other oral anti-diabetic agents or with insulin also; combination metformin products are increasingly available.⁸²⁻⁸⁴ Metformin is usually administered in tablet form; the recommended starting dose for adults is 500mg or 850mg taken two or three times daily with food.⁸⁰ Dosage is adjusted based on blood glucose measurements up to a maximum recommended daily dose of 3g.⁸⁰ A slow increase of dose is

recommended in order to minimise gastrointestinal side effects (see below).⁸¹ Metformin has also been used for other indications, for example, in polycystic ovary syndrome, despite not being licensed for this use in any country.^{80, 85}

2.2.2. Safety

Metformin is considered a relatively safe drug as, unlike most other anti-diabetic drugs, it does not cause hypoglycaemia.⁸¹ However, metformin has been associated with vitamin B12 deficiency and gastrointestinal effects including diarrhoea.⁸⁶⁻⁸⁸ Most gastrointestinal effects appear to lessen over time and can be minimised by starting with low doses and gradually titrating upwards towards the target dose over a number of weeks.⁸⁹ However, metformin-associated diarrhoea is of concern in relation to tolerability of the drug and associated adherence.⁹⁰ Patients transferred from immediate release metformin to sustained release formulations have been found to be more adherent following the formulation switch (increasing from 62% to 81% adherence), which may reflect improved gastrointestinal tolerability of sustained release formulations or the effect of reduced dosing frequency.⁹¹

As a member of the biguanide class of oral anti-diabetic drugs, the risk of lactic acidosis has often been the chief concern in the prescribing of metformin.⁹² Lactic acidosis is a metabolic disorder associated with a poor prognosis.⁹³ The precursor to metformin, phenformin, was widely prescribed as an anti-diabetic drug until its discontinuation in the late 1970s due to its association with lactic acidosis.⁹⁴ However, phenformin has been found to be up to 150-fold more potent than metformin in inducing lactic acidosis *in vivo*.^{95, 96} There is no evidence to date that metformin, when prescribed according to treatment guidelines, is associated with an increased risk of lactic acidosis compared with other anti-hyperglycaemic agents⁹⁷ and the overall safety of metformin has been confirmed in numerous studies.⁹⁸⁻¹⁰⁰ Metformin is still contraindicated in patients with renal failure or where patients have conditions which may cause tissue hypoxia as these may potentially lead to the precipitation of lactic acidosis. However, some authors have suggested that the benefits of metformin outweigh the harms even in patients for whom the drug has been contra-indicated.^{101, 102}

2.2.3. Mechanism of Action

The main actions of metformin in diabetes are to lower blood glucose by reducing hepatic glucose production and increasing peripheral glucose uptake. Metformin also reduces lipogenesis and enhances insulin sensitivity.¹⁰³ In patients with polycystic ovary syndrome, metformin has been found to produce reductions in insulin levels, particularly among non-

obese women, thereby reducing the over production of androgens and relieving symptoms associated with the syndrome.¹⁰⁴

Metformin is thought to exert its effects by activation of AMP-dependent protein kinase (AMPK) via LKB1-dependent phosphorylation in a dose-dependent manner (Figure 2.4).^{105, 106} AMPK is a cellular sensor of nutrients and energy which plays a key role in energy homeostasis. The role of the AMPK-signalling pathway is thought to be to respond to energy-stress caused by glucose starvation.¹⁰⁷ It has also been found to play a part in the extension of life span that has been observed in some preclinical studies studying the effects of caloric restriction, or indeed, metformin.¹⁰⁷ The enzyme is activated physiologically under conditions of energy stress but is also switched on by xenobiotic compounds (such as metformin) which cause a cellular energy imbalance, detected as increases in the ratios of ADP:ATP and AMP:ATP. Metformin is thought to produce such an energy imbalance by acting as a mild inhibitor of Complex I of the mitochondrial respiratory chain, thereby inhibiting ATP synthesis and thus increasing cellular ADP and AMP.¹⁰⁸ It has therefore been described as a 'mitochondrial poison'.¹⁰⁹ The activation of AMPK results in further activation of a wide variety of downstream targets associated with regulating metabolism; such targets have been described previously.¹⁰⁸ Catabolic pathways are switched on resulting in the uptake and metabolism of glucose and fatty acids while biosynthetic pathways (including synthesis of glucose, glycogen and lipids) are switched off. The action of metformin could therefore be explained as the activation of AMPK promoting muscle glucose uptake and metabolism, and inhibition of hepatic glucose production (gluconeogenesis). Also, as fatty acid and triglyceride synthesis is associated with insulin resistance, the switching off of production of these molecules following AMPK activation would also explain the effect of metformin in improving insulin sensitivity.¹⁰⁸ While the activation of AMPK by metformin provides a plausible mechanism of action for metformin's effects as an anti-diabetic agent, it has recently been argued that this pathway is unlikely to be solely responsible for the actions of the drug.¹¹⁰ Rather, it has been suggested that inhibition of hepatic glucose production is brought about initially by the rise in AMP levels resulting from the above-mentioned mitochondrial respiratory chain inhibition. This rise in AMP in turn causes the AMPK-independent inhibition of adenylate cyclase and/or fructose-1,6-bisphosphatase, which signals to prevent gluconeogenesis.^{110, 111}

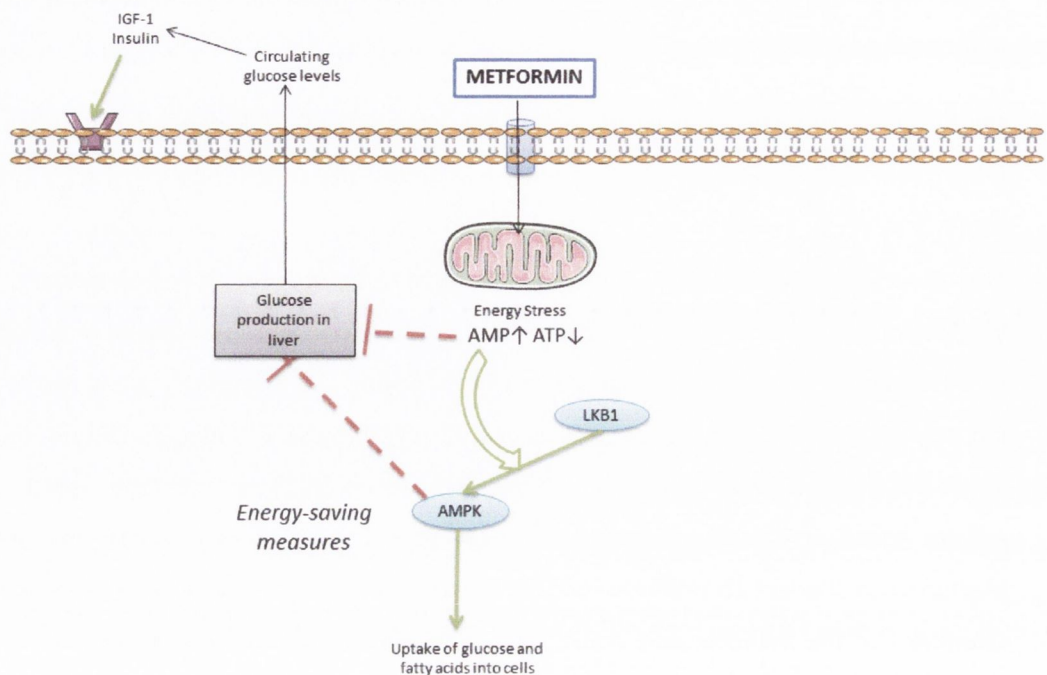


Figure 2.4: Cellular mechanism of action of metformin in type 2 diabetes

Following transport into the cell the target of metformin is thought to be the mitochondrion. Metformin inhibits ATP synthesis in the mitochondrion, causing an increase in the ratio of AMP:ATP. This, via LKB1, activates AMPK, which results in the activation of energy-saving pathways, which include uptake of glucose into the cell, and reduction in synthesis of glucose by the liver.¹⁰⁸ Reduction in hepatic glucose production following increases in AMP may possibly also occur in an AMPK-independent manner.¹⁰⁸ (Broken lines represent a series of steps in a pathway. Green lines represent activating pathways while red lines represent inhibition.)

2.3. Other anti-diabetic drugs

Other drug treatments for type 2 diabetes comprise insulin and non-metformin oral anti-diabetic drug classes, which include sulfonylureas, meglitinides, thiazolidinediones, dipeptidyl peptidase-4 (DPP-4) inhibitors, glucagon-like peptide 1 (GLP-1) agonists, acarbose and the novel agent dapagliflozin (a sodium glucose co-transporter 2 (SGLT2) inhibitor), which is not yet licensed.⁸⁰ Table 2.1 provides a comparison of anti-diabetic agents in terms of mechanisms of action and adverse effects.

Sulfonylureas, meglitinides, DPP-4 inhibitors and GLP-1 agonists all act, through various mechanisms, as secretagogues stimulating insulin release.⁸⁹ Unlike the above anti-diabetic drugs, but similar to metformin, thiazolidinediones do not act to release insulin.

Thiazolidinediones instead produce the effects of enhanced insulin sensitivity and reduction of free fatty acids. These actions result from activation of the peroxisome proliferation activation receptor γ (PPAR γ) family of nuclear receptors, which regulate genes related to glucose and lipid metabolism.⁸⁹ In addition to the side effects listed in Table 2.1, rosiglitazone (a thiazolidinedione drug) has been linked to an increased risk of myocardial infarction in recent years and was withdrawn from the Irish market in 2010.^{112, 113} Acarbose acts to delay absorption of carbohydrates from the intestine while SGLT2 inhibitors increase urinary clearance of glucose.⁸⁹

Insulin preparations used in type 2 diabetes have various durations of action, including short (e.g. insulin aspart), intermediate (e.g. isophane insulin) and long-acting (e.g. insulin glargine) products.⁸⁰ Administration is by subcutaneous injection.

Table 2.1: Comparison of anti-diabetic drug treatments

Anti-diabetic drug class	Examples	Therapeutic mode of action ⁸⁹			Potential effects on weight ^{89, 114}	Important adverse effects ¹¹⁴
		Insulin-related Actions	Non-insulin related Actions	Additional actions ⁸⁹		
Biguanides	Metformin	Improve insulin sensitivity	Reduce hepatic glucose production, increase glucose uptake	May improve cholesterol profile	Neutral / weight loss	Rarely, lactic acidosis among patients with renal insufficiency. Commonly, gastrointestinal side effects. Possible vitamin B12 deficiency.
Thiazolidinediones	Pioglitazone	Improve insulin sensitivity	Reduce hepatic glucose production, increase glucose uptake	Lipid metabolism: Reduction of free fatty acids, Stimulation of adiponectin. May improve cholesterol profile.	Weight gain	Oedema. Heart Failure. Fracture risk.
Insulin products	Long-acting, short-acting, intermediate-acting.	Exogenous insulin	n/a	-	Weight gain	Hypoglycaemia
Sulfonylureas	Gliclazide, Glimepiride	Insulin secretagogue	-	-	Weight gain	Hypoglycaemia
Meglitinides	Nateglinide, repaglinide	Insulin secretagogue	-	-	Neutral	Hypoglycaemia
DPP-4 Inhibitors	Sitagliptin, Vildagliptin	Insulin secretagogue	-	-	Neutral	Hypoglycaemia
GLP-1 agonists	Exenatide	Insulin secretagogue	-	-	Weight loss	Hypoglycaemia
Alpha-glucosidase inhibitors	Acarbose	n/a	Reduce gastrointestinal glucose absorption	-	Neutral	Flatulence, diarrhoea
SGLT2 inhibitors	Dapaglifozin	n/a	Increase urinary clearance of glucose	-	Weight loss	Genitourinary infection. Diuresis, dehydration. ¹¹⁵

DPP-4: Dipeptidyl peptidase 4; **GLP-1:** Glucagon-like peptide; **SGLT2:** Sodium glucose co-transporter 2

2.4. Anti-diabetic drug prescribing trends in Ireland

Availability and prescribing patterns of anti-diabetic agents have changed significantly in recent years. Insulin, metformin, sulfonylureas, meglitinides, acarbose and thiazolidinediones were all approved for use in Europe prior to the year 2000. Exenatide, the first in class of the GLP-1 agonists, and sitagliptin, the first in class of the DPP-4 inhibitors, were licensed for use in Ireland in 2006 and 2007 respectively (www.imb.ie). Regarding thiazolidinediones, pioglitazone was approved for use in Ireland in 2000 (www.imb.ie) and as of 2010 is the only member of this drug class licensed for use. This followed the withdrawal of the previously licensed drug, rosiglitazone, in 2010 due to accumulating evidence concerning its cardiovascular safety.¹¹⁶ Of the range of anti-diabetic drugs available for prescription, the prevalence of prescribing of metformin in the United Kingdom and Ireland has increased substantially in the past decade; some studies have shown an almost linear yearly increase in metformin prescribing while prescribing of sulfonylureas has declined.¹¹⁶⁻¹¹⁸ This is in line with international clinical guidelines recommending metformin as first-line therapy.⁶⁹

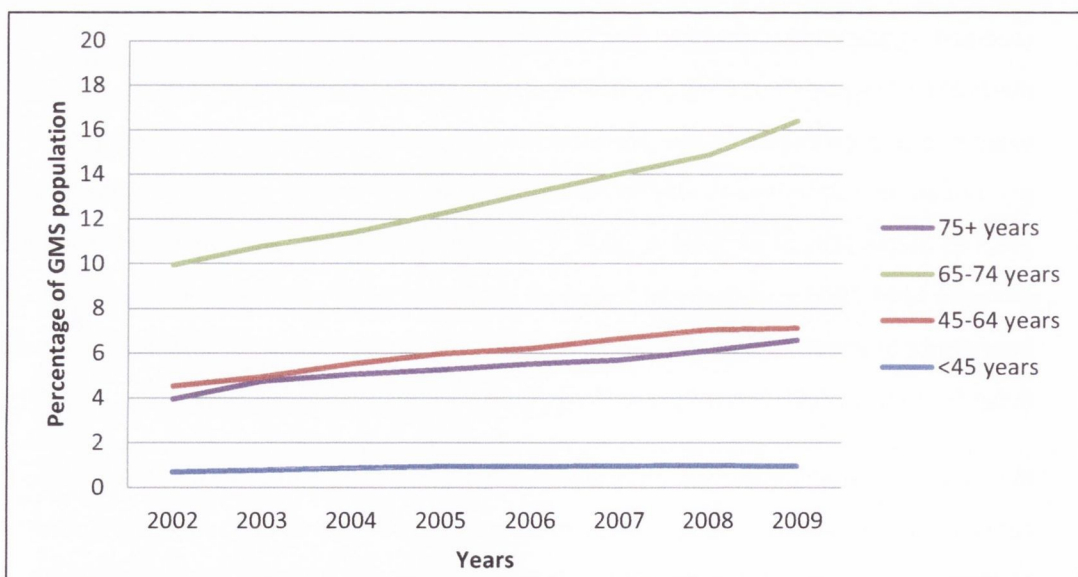
Previous work carried out in Ireland has examined anti-diabetic drug prescribing trends using both data from the GMS scheme (as mentioned in section 1.4.3 and described in section 5.1.2) and data from the Long Term Illness scheme, an additional pharmacy claims database operated by the HSE-PCRS.¹¹⁸ Both of these schemes provided prescription medications free of charge to eligible patients during the time period in which the study was carried out. Among patients with type 1 or type 2 diabetes identified in these schemes, approximately two thirds received medications through the GMS scheme while one third of patients received medications through the Long Term Illness scheme.¹¹⁹ Claims for the period 2003-2007 were examined and estimates of the prevalence of anti-diabetic drug use were calculated.¹¹⁸ The prescribing of sulfonylureas was found to fall from almost 70% of patients with diabetes in 2003 to below 60% of patients in 2007.¹¹⁸ Overall thiazolidinedione prescribing was found to increase until 2005 and declined between 2005 and 2007, but did not reach a prescribing prevalence of above 10% of all patients with type 2 diabetes. All other non-insulin anti-diabetic drugs had a low prevalence of prescribing (<10%).

In order to provide context for the studies within this thesis, further descriptive analyses were carried out to examine the prevalence of anti-diabetic therapies in Ireland specifically in relation to patients within the GMS scheme and for the years 2000-2009, and trends in metformin versus non-metformin drug usage during this time. These results are presented in order to provide information on the prevalence of anti-diabetic treatments in the GMS

population, which is used for the studies within this thesis. Age-specific prevalence estimates are presented to provide a perspective on anti-diabetic drug use within population subgroups. The studies within this thesis used HSE-PCRS GMS data for patients exposed to medications between 2001 and 2006 inclusive (section 5.1.2); trends in anti-diabetic therapy use are presented here also for the years 2000-2009 to provide information on more recent trends in drug use.

The GMS population denominator consisted of the number of persons eligible for the GMS scheme for each particular year as of December of that year, according to official PCRS reports.¹²⁰ Age-specific prevalence estimates were not calculated for the years 2000 or 2001 as GMS population denominator information was not available broken down by the detailed age-groups. Due to the absence of diagnostic information, type 1 diabetic patients could not be distinguished from type 2 diabetes patients in these analyses. However, such patients are low in number, representing less than 10% of treated diabetes patients.⁶⁸

Age-specific prevalence estimates for receipt of anti-diabetic therapies in the period 2002-2009 are presented in . Usage of anti-diabetic therapies was relatively stable over this time period in patients under the age of 45. In other age-groups there was a marked rise in prevalence of anti-diabetic therapy use. Patients in the 65-74 year age-group had the highest anti-diabetic therapy use and the highest rise in usage over the time period, increasing from 10% in 2002 to 16% in 2009.



GMS: General Medical Services scheme

Figure 2.5: Age-specific prevalence of anti-diabetic therapy usage in the GMS population for the years 2002-2009.

Therapy-specific prevalence estimates are presented in Figure 2.6. The increase in receipt of metformin over the timescale 2000-2009 is very apparent and is in line with previous studies of anti-diabetic drug pharmacoepidemiology.¹¹⁶⁻¹¹⁸ As described in section 2.2.1, metformin was the 18th most frequently dispensed drug in the GMS scheme in 2011. The drug was dispensed over 600,000 times, representing 1.06% of the total scheme's dispensing records but only 0.2% of the scheme's cost.⁷⁸

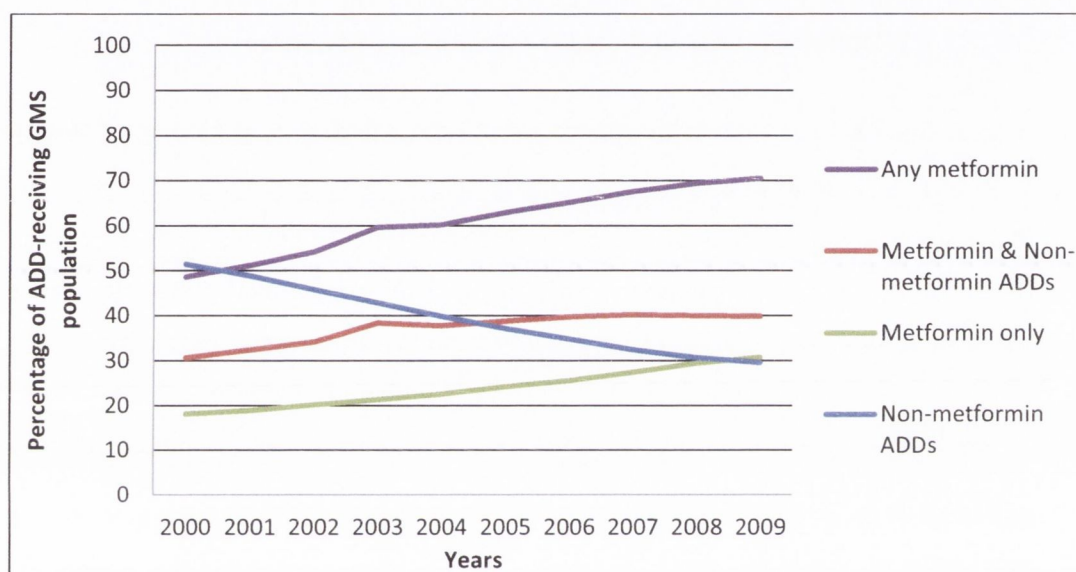


Figure 2.6: Prevalence of usage of metformin and non-metformin anti-diabetic therapies within the GMS treated diabetic population for the years 2000-2009.

ADD: anti-diabetic drug. GMS: General Medical Services scheme

In summary, the prevalence of anti-diabetic drug prescribing in the GMS population in Ireland has increased during the period 2000-2009, particularly within the 65-74 year age group. Metformin prescribing has markedly increased and in 2009 over 70% of treated diabetic patients were receiving metformin. While the above analyses did not incorporate a breakdown of individual anti-diabetic therapies other than metformin, previous work has shown that the majority of patients receiving non-metformin anti-diabetic drugs were in receipt of sulfonylurea drugs and between 8 and 12% of patients received insulin.¹¹⁸

3. Metformin and Colorectal Cancer

The evidence regarding the association between metformin and cancer is introduced in this chapter within the context of the overall association between type 2 diabetes and cancer, as follows. Preclinical, mechanistic and observational studies of the effects of metformin in colorectal cancer are discussed thereafter.

3.1. Diabetes, anti-diabetic treatments, and cancer

3.1.1. Associations between type 2 diabetes and cancer incidence and outcomes

There exists a well-established association between type 2 diabetes mellitus and increased incidence of cancer, as compared with the general population.¹²¹ The strength of this association, however, appears to be dependent on cancer site; consistent associations have been observed in endometrial, breast, colorectal, bladder, non-Hodgkin lymphoma and kidney cancer.¹²¹ In the case of colorectal cancer, a risk increase of approximately 30% has been observed in several meta-analyses.¹²²⁻¹²⁴

The effect of diabetes mellitus on cancer outcomes has also been the subject of much research.¹²⁵ Diabetes has been associated with reduced survival from cancer in general, and individually for breast cancer and prostate cancer.¹²⁵ Decreased short-term survival among diabetic patients has also been observed for oesophageal cancer, while mixed results have been found for pancreatic cancer and lung cancer.¹²⁵ In colorectal cancer, diabetes has been associated with poorer colorectal cancer-specific survival in patients with non-metastatic cancer¹²⁶, particularly in rectal cancer.¹²⁷ In patients with all stages of colorectal cancer, one study reported a significant association between diabetes and poorer cancer-specific survival¹²⁸ while two other studies found no such association.^{129, 130}

Caution should be exerted when interpreting studies of associations between diabetes and cancer incidence and outcomes due to the potential for confounding. The Diabetes and Cancer Research Consortium, established in 2011, has described several key differences which may arise between diabetic and non-diabetic patients in the cancer pathway; these may affect cancer incidence and mortality, confounding associations between diabetes and cancer.¹²¹ The differences described by the Consortium include uptake of cancer screening, cancer treatment

selection, cancer treatment complications, peri-treatment mortality and competing risks for long-term mortality (e.g. cardiovascular disease).¹²¹ The consortium has reviewed methodological issues relating to the study of links between diabetes and cancer, and has recently proposed approaches to be adopted in the design aspects of observational studies in order to minimise bias and confounding.^{121, 131}

3.1.2. Possible biological rationale for links between diabetes and tumour development

Various biological mechanisms have been put forward to explain potential associations between diabetes and tumour development, but the main hypotheses broadly relate to hyperglycaemia, or hyperinsulinaemia and enhanced activation of the insulin-like growth factor (IGF) receptor system.¹³² Hyperglycaemia has been considered a logical rationale as cancer cells are known to have high glucose requirements and a hyperglycaemic state could therefore confer a growth advantage.¹³² However, in laboratory studies which simulated insulin deficiency, hyperglycaemia was not observed to provide a growth advantage to tumours, suggesting that activation of insulin-related pathways in the cell is likely to be more relevant in determining tumour growth.¹³²

Insulin receptors and the IGF-1 receptor, in addition to their metabolic functions, are capable of stimulating tumour cell proliferation and metastasis.¹³² The hyperinsulinaemia of type 2 diabetes, either endogenous due to insulin resistance or exogenous due to anti-diabetic treatments (administered insulin or insulin secretagogues such as sulfonylureas), may result in tumour proliferation due to over-activation of insulin/IGF receptors. Additionally, high insulin levels in turn increase levels of the IGF-1 protein, which binds to insulin and IGF receptors resulting in mitogenic effects.^{132, 133} This pathway has been noted to be of particular importance in tumours where IGF receptors are overexpressed, which include colorectal tumours.^{134, 135}

Patients with type 1 diabetes have previously been found to have elevated risks of cancers of the stomach, cervix and endometrium.¹³⁶ However, no link was found between type 1 diabetes and colorectal cancer, or other cancers which have been associated with type 2 diabetes.^{121, 136} These results were viewed as supportive of the hypothesis that hyperinsulinaemia may mediate the increased risks of certain types of cancer among type 2 diabetes patients.¹³⁶

Some authors have also disputed associations between type 2 diabetes and cancer incidence, suggesting that the effect observed may be mediated by screening effects, and by the

treatments used in type 2 diabetes rather than the condition itself.¹³⁷ This is due to the fact that exogenous insulin and insulin secretagogues such as sulfonylureas increase blood insulin levels in patients with type 2 diabetes.

3.1.3. Anti-diabetic treatments and colorectal cancer incidence and outcomes

To test the hypothesis that links between diabetes and cancer may be explained by the effects of anti-diabetic treatments, many studies have examined associations between individual diabetic treatments and cancer incidence and outcomes, including in colorectal cancer.

Insulin use has been the subject of many observational studies which have produced conflicting results; two recent meta-analyses suggested opposing effects of insulin treatment on colorectal cancer risk.^{138, 139} Meta-analyses of results regarding the effect of sulfonylurea drugs on cancer risk have also been conflicting.¹⁴⁰⁻¹⁴² Thiazolidinedione use has been linked to reduced incidence of colon cancer though evidence has differed for effects in other cancers.¹⁴³⁻¹⁴⁵ In the case of metformin, evidence from observational studies has broadly suggested a beneficial effect of the drug on cancer risk and outcomes for a variety of tumour types, including colorectal cancer.⁴⁷ However, some authors have argued that associations observed between anti-diabetic therapies and altered cancer incidence and outcomes are solely the result of methodological flaws in observational research.¹⁴⁶

The following sections will present and discuss the evidence for an association between metformin and cancer, particularly colorectal cancer. The history of this topic will be described, followed by presentation of the laboratory evidence and potential mechanisms of action for the role of metformin in cancer. The observational evidence will then be discussed followed by consideration of potential biases and methodological issues that are of concern in this type of research.

3.2. Metformin and Cancer

3.2.1. History

The literature regarding the role of metformin in cancer was identified by searching Medline® for the years 1990-2013 inclusive using the following MeSH terms: ("Biguanides"[MeSH] OR "Metformin") AND ("Neoplasms"[MeSH] OR "Cancer"). This search was updated on a weekly basis during this programme of research in order to identify new articles which might inform study design and interpretation. There was a significant increase in the volume of research on this topic in the past decade; the number of citations per year rose from 25 in the year 2000 to

286 in 2012. Between 2000 and 2012 inclusive, 1,694 citations were generated by the above search phrase.¹⁴⁷

Research into this topic in past decades was described previously.¹⁴⁸ The earliest Medline® citations relating to metformin as an anti-cancer agent comprise a series of preclinical research articles considering the effect of biguanides on tumour activity in the 1960s and examined effects relating to tumour respiration and gluconeogenesis.¹⁴⁹⁻¹⁵² Following this a number of articles explored the administration of biguanides to non-diabetic cancer patients in order to correct hormonal metabolic disturbances;^{153, 154} these studies involved using phenformin to achieve the 'metabolic rehabilitation' of breast and colon cancer patients.¹⁵⁵ In 2004 and 2005, emerging research outlining the mechanism of action of metformin in diabetes was published and suggested the importance of the known tumour suppressor LKB1 in metformin's anti-diabetic activity.^{106, 156} These revelations prompted a pilot observational study in 2005 with the hypothesis that metformin use in type 2 diabetes may reduce the risk of overall cancer.¹⁵⁷ Results showed that patients with any exposure to metformin (versus diabetics unexposed to metformin) were less likely to have cancer, and this association was greater in magnitude with longer duration of exposure.¹⁵⁷

Following this publication, many observational studies have been carried out to examine associations between metformin and cancer incidence and outcomes among type 2 diabetic patients.⁷⁶ These are described following discussion of the preclinical and mechanistic studies of metformin in cancer in the next sections.

3.2.2. Preclinical evidence for effects of metformin in colorectal cancer models

In response to initial observational research suggesting that metformin may exert a cancer-suppressing effect in diabetics, many preclinical studies have emerged examining the effects of metformin on cancer models in vitro and in vivo. Studies of metformin have been performed in a wide range of tumour models to examine effects on proliferation, spread and survival.⁴⁷ A number of studies have observed inhibition of proliferation of colon cancer cells in vitro following administration of metformin.¹⁵⁸⁻¹⁶⁰ Metformin has also been shown to induce or increase apoptosis (programmed cell death) in vitro¹⁶¹ and to inhibit cell migration of colon cancer cell lines.¹⁶²

Two in vivo studies have found that metformin is capable of suppressing polyp or tumour growth in non-diabetic mouse models. The first examined mice bred with a predisposition to

intestinal polyp development.¹⁶³ Tomimoto et al. administered 250mg dietary metformin per kg mouse weight and compared intestinal polyp numbers and size to untreated mice after 10 weeks. Insulin resistance, serum lipid levels and body weight were measured in the mice and were not found to be altered. Markers of apoptosis and proliferation were also not altered, but activation of AMPK and corresponding reduction of mTOR activity were both noted in protein assays;¹⁶³ the role of these proteins (AMPK and mTOR) as potential mediators of the effect of metformin is discussed in section 3.2.3. The same group later examined the effect of metformin in suppressing tumour development in a chemical carcinogen-induced colorectal cancer mouse model.¹⁶⁴ Metformin was again administered at a dose of 250mg/kg per day. Induction of aberrant crypt foci (ACF), a pre-cancerous marker associated with the development of colorectal tumours,¹⁶⁵ and colon polyps, were assessed by dissection. Metformin was found to significantly suppress the formation of ACF and the average size of ACF, while a modest inhibition of polyp formation was observed. Similar to the previous study, no significant changes in body weight or glucose concentration were found in the metformin-treated mice, though mTOR activity and cell proliferation were decreased.¹⁶⁴

Another group studied the effect of metformin on growth of colon cancer cell line xenografts in nude mice.¹⁶⁶ This group specifically examined whether a growth inhibitory effect of metformin could be modified by the presence or absence of the tumour suppressor p53.¹⁶⁶ Mice were injected with both p53-positive and p53-negative colon cancer cells (in different locations) and were treated with either metformin (250mg/kg) or saline control. After one month of metformin treatment, among the mice injected with p53-negative cancer cell lines, the average associated tumour volume was significantly smaller among mice treated with metformin (versus control). This significant difference was not observed among metformin and control-treated mice with p53-positive tumours. (The relevance of the factor p53 is discussed further in section 3.2.3)

Algire et al. have conducted in vivo experiments examining how the effect of metformin on colon cancer in vivo may be modified by diet.^{167, 168} Following the injection of colon cancer cells into mice on either high-energy or control diets, a subgroup from each diet group was given oral metformin at a relatively low dose of 50mg/kg per day. Mice in the high-energy diet group were found to have larger tumour volumes than mice on the control diet. However, within the high-energy diet group, metformin exposure was found to decrease tumour volume significantly as well as decreasing insulin levels, while having no effect within the control diet mouse group. Apoptosis was increased by metformin regardless of diet, though a greater level

of metformin-induced increase in apoptotic activity was observed among the high-energy diet mice.¹⁶⁷

3.2.3. Proposed mechanisms of action of metformin in cancer

Various mechanisms of action have been proposed to rationalise a potential anti-cancer effect of metformin, many of which are thought to relate to the mechanisms of action of metformin in type 2 diabetes (see section 2.2.3).⁴⁷ The similarities between type 2 diabetes and cancer, two complex diseases, were summarised in a recent review on the topic of metformin in cancer,¹⁶⁹ as well as sharing common risk factors (e.g. obesity) these diseases have biologic factors in common, including the insulin/insulin-like growth factor pathway, disordered cellular metabolism and common genetic components. As noted by the authors,

“Because metformin reduces insulin levels, suppresses growth factor signalling and alters metabolism, it possesses features that are desirable as an anti-cancer drug.”

The most established theories regarding the mechanism of action of metformin are illustrated in Figure 3.1 and are described in detail below.^{47, 169, 170} Common to these theories is the function of metformin in altering cellular energetics. As described previously in the context of its anti-diabetic mechanism of action (section 2.2.3), metformin’s activity in mitochondria inhibits the production of ATP, the carrier of energy within living cells.¹⁷¹ The energy sensor AMPK is activated in response and in turn activates a variety of pathways with the overall aim of promoting energy conservation and ATP production; biosynthetic pathways (which use up ATP) are switched off while other pathways which generate ATP are switched on.¹⁷¹ Effects in type 2 diabetes, such as reduced production of glucose by the liver and increased glucose uptake into muscle cells, thus represent energy-saving efforts, and result in the reduction of blood insulin levels. In the cancer setting, this reduction of hyperinsulinaemia may be a very important effect of metformin in tumours which are responsive to insulin. As mentioned in section 3.1, hyperinsulinaemia has been associated with colorectal cancer development and metastasis.^{172, 173} Reduction of hyperinsulinaemia with metformin may thus represent an indirect anti-cancer effect of the drug through its action in general (non-cancer) cells (Figure 3.1, pathway ‘A’).

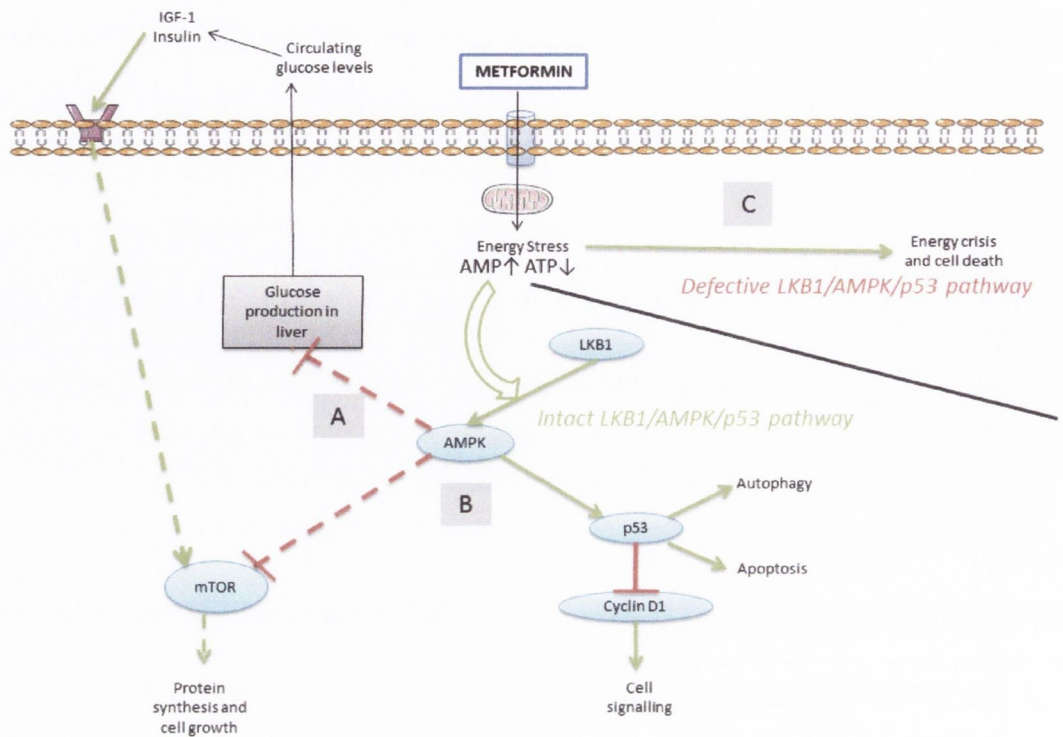


Figure 3.1: Potential mechanisms of action (A, B, C) of metformin in cancer cells ⁴

Following transport into the cell, metformin inhibits ATP production in the mitochondrion leading to energy stress. This activates the LKB1/AMPK pathway to preserve cellular energy reserves. AMPK activation leads to the inhibition of hepatic gluconeogenesis, which reduces circulating glucose levels and consequently reduces hyperinsulinaemia (pathway 'A'). Activation of mTOR by insulin signalling pathways is thereby minimised, representing an 'indirect' effect of metformin in preventing protein synthesis necessary for tumour growth. Inhibition of mTOR may also be achieved directly following AMPK activation by metformin (pathway 'B'). A third potential 'direct' effect of metformin involves generating an energy crisis and consequent cell death in tumour cells which have mutated such that they have lost function of LKB1, AMPK and/or p53 (pathway 'C').^{47, 169}

In the absence of hyperinsulinaemia, metformin may also have a number of anti-cancer actions through direct effects in cancer cells. Activation of AMPK has been viewed as a key component of mechanisms of direct anti-cancer activity attributed to metformin.¹⁰⁶ This link was made in light of the finding that LKB1 is the main upstream kinase responsible for AMPK activation; LKB1 is a known tumour suppressor, and this fact led to the suggestion of a role for AMPK in cancer.¹⁰⁶ Research into the effects of metformin in cancer cell lines and increased understanding of the role of AMPK in cell metabolism have furthered theories related to the action of metformin in cancer cells.¹¹⁰ For example, as activation of AMPK results in the

⁴ Components of this image produced using Servier Medical Art (www.servier.com)

switching off of biosynthetic pathways, the production of macromolecules required for tumour growth is prevented, thus arresting tumour development.⁴⁷ One of the main effects of AMPK in this manner is to inhibit the mammalian target of rapamycin (mTOR) (Figure 3.1, pathway 'B') and cancer cell inhibition through metformin effects on the mTOR pathway has been demonstrated in a number of studies,¹⁷⁴ including also in an AMPK-independent manner.¹⁷⁵ mTOR, activated via the insulin/IGF-1 pathway, serves to balance nutrient availability with cell growth and thus plays a key role in the proliferation of cancer cells. mTOR pathway inhibitors have undergone much investigation in recent years as potential treatments for colorectal cancer.¹⁷⁶ Several in vitro studies of colorectal cancer cell lines have found anti-cancer effects of metformin linked to AMPK activation and consequent mTOR inhibition and reduction of protein synthesis.^{158, 166, 167, 177}

In contrast to the above theories, some studies have found that successful treatment of pre-existing tumours in mice using biguanides occurred only in models lacking a functional LKB1-AMPK pathway.^{168, 178} The mice in these studies were not hyperinsulinaemic and a reduction in circulating insulin levels was not observed, ruling out an indirect effect of metformin. Furthermore, in vitro studies of inhibition of colon cancer cell lines in response to metformin revealed LKB1 and the glucose concentration of the growth environment to be influential variables.¹⁶⁸ Cancer cells lacking LKB1 were inhibited by metformin under growth conditions with low glucose concentrations similar to physiologic glucose levels, but not in conventional high glucose laboratory growth media.¹⁶⁸ The authors explained this effect as hypersensitivity to metformin; tumour cells lacking a functioning AMPK/LKB1 pathway are suspected to be incapable of compensating for the energy stress induced by metformin and therefore cannot survive (Figure 3.1, pathway 'C'). This group also conducted an in vivo experiment treating both diet and LKB1 expression as variables that may influence the effect of metformin.¹⁶⁸ Metformin was found to abolish the tumour-promoting effect of the high-energy mouse diet regardless of LKB1 expression in the tumour, and this anti-tumour effect was found to be associated with a reduction in circulating insulin levels. In the control diet mice (non high energy), metformin was found to inhibit the growth of tumours only in the LKB1-deficient group, and this effect was not related to a reduction in insulin levels. The authors concluded from these results that clinical anti-cancer activity of metformin is likely to vary according to the metabolic characteristics of the patients and the molecular pathology of their tumours.

Another study examined the influence of loss of function of the tumour suppressor p53 on the effect of metformin in colon cancer cells.¹⁶⁶ While the loss of p53 is conventionally associated with enhanced tumour growth, the activation of p53, which lies within the energy-saving

AMPK pathway, may enable energy-hungry tumour cells to survive the energy-deprived conditions produced by metformin. Indeed, colon cancer cells with functioning p53 were found to survive treatment with metformin as they underwent autophagy (breakdown of cell contents) in order to produce energy. However, metformin treatment was found to be toxic to p53-deficient cancer cells.¹⁶⁶

These revelations represent an interesting paradox. Loss of tumour suppressors such as LKB1 or p53 may accelerate proliferation of cancer cells in certain circumstances. However, under conditions of energy stress, as induced by metformin, the loss of these suppressors may in fact sensitise the cancer cells to metformin treatment.⁴⁷ As hypothesised in a recent review, while metformin may act through AMPK activation to suppress tumour growth during initial tumour development, metformin may be more effective in treating existing tumours when the function of the AMPK pathway has been lost.¹¹⁰ Also, as several tumour types possess mutations that result in hyperactivation of mTOR, or deficiency of p53 or LKB1, yet have very high glucose requirements, the production of an energy stressed environment by metformin may result in toxicity to these tumours, but not to the host (whose metabolic compensatory mechanisms are intact).¹⁷⁹

This concept has been referred to as 'metabolic synthetic lethality' and is an exciting theory regarding the potential of metformin as an anti-cancer treatment.¹⁷⁹ The 'synthetic lethality' induced by metformin has been described as *'taking advantage of oncogenetically driven addiction to nutrients functioning in the presence of deficient metabolic checkpoints'*.¹⁷⁹ Metformin has additionally recently been found to demonstrate this effect in cancer stem cells.¹⁷⁹ Cancer stem cells are self-renewing cells that give rise to the cells comprising the bulk of a tumour.²⁹ Colorectal cancer is thought to be highly reliant on cancer stem cells, with roles suggested for these cells in the continued growth, invasion and metastasis of a colorectal tumour, as well as resistance to standard cancer therapies.^{180, 181} A number of studies have shown that metformin is capable of specifically inhibiting cancer stem cells in various cancer cell lines,¹⁸²⁻¹⁸⁵ providing a promising hypothesis for the action of metformin in colorectal cancer.

In addition to the above-described possible effects of metformin, which represent the most established theories,^{47, 169, 186} further individual mechanisms of action have been proposed and include anti-oxidant effects,¹⁸⁷ alteration of microRNA expression,¹⁸⁸ inhibition of the unfolded protein response,¹⁸⁹ AMPK-independent mTOR inhibition¹⁷⁵ and many others, though further research will be required to determine the importance of such effects.

3.2.4. Metformin and cancer incidence: Observational studies

The majority of studies which have examined associations between metformin and cancer have investigated cancer incidence, as opposed to outcomes. These studies have mainly examined populations derived from individual hospital records, health administration databases or general practice research databases. A number of meta-analyses have collated such observational studies to examine the effect of metformin on the incidence of a range of tumour types; in this way metformin has been found to be associated with lower incidence of cancer for liver, pancreatic, breast, colorectal and other tumours.^{140, 142, 190-196} However, secondary analysis of data from clinical trials which studied the efficacy of anti-diabetic drugs did not find that metformin lowered cancer incidence.¹⁹⁷

3.2.5. Metformin and colorectal cancer incidence

Several meta-analyses of observational studies have been published which examined associations between metformin and colorectal cancer incidence.^{140, 190, 194-196} Studies included in these meta-analyses are listed in Table 3.1 and are summarised in Table 3.2. Additional evidence for the effect of metformin on colorectal cancer incidence has arisen from clinical trial data originally intended to examine the anti-diabetic efficacy of metformin versus other drugs, namely the manufacturer-sponsored 'ADOPT' study which compared metformin to the alternative anti-diabetic agents rosiglitazone and glyburide.¹⁹⁸ This data has been re-analysed to study the incidence of cancer in the trial, where malignancies were reported as an adverse event.¹⁹⁹ As listed in Table 3.1, each of the five meta-analyses conducted since 2010 have found that metformin exposure was associated with a significant decrease in incidence of colorectal cancer of between 30-40%.

While an apparent consistent beneficial association for metformin exposure and colorectal cancer incidence is observed across the meta-analyses listed in Table 3.1, caution is advised in interpreting these results, as discussed by Iannou et al.²⁰⁰ As these are meta-analyses of observational studies, they carry the limitations of the observational studies they comprise; such limitations include confounding by indication, whereby metformin may, for example, be preferentially prescribed to healthier (although likely obese) patients, or, confounding by additional factors over which no control may have been held in these retrospective studies.²⁰⁰ Additionally, many of the studies included a relatively short follow-up time from exposure to cancer incidence, which is unlikely to represent a biological effect due to the typical lengthy transition time from adenoma to carcinoma.²⁰⁰ Several of the observational studies included in these meta-analyses (for example, ²⁰¹⁻²⁰⁴) have come under additional criticism due to the

suggested presence of time-related biases in their methodology.¹⁴⁶ It has been suggested that immortal time bias, time-window bias or improper treatment of time-lagging issues in these studies have greatly inflated associations between metformin and cancer incidence, as follows.¹⁴⁶

Immortal time is a period of cohort follow-up during which, due to the exposure definition, the outcome under study cannot possibly occur.²⁰⁵ For example, if a patient is defined as exposed to metformin if they receive metformin at any stage during follow-up, the time between the start of follow-up and the receipt of metformin will be immortal; this follow-up time should instead be classified as unexposed.¹⁴⁶ Time-window bias may occur in case-control studies if the cases and controls are not matched on duration of exposure opportunity time (that is, duration of follow-up).¹⁴⁶ Time-lagging bias may occur where studies comparing diabetes drugs for effects on cancer incidence do not match patients by duration of diabetes.¹⁴⁶

Protopathic bias has also been suggested as a potential explanation for observed reduced incidence of colorectal cancer among metformin exposed patients. Van Staa et al. did not observe differences in long-term overall cancer risk between patients treated with metformin compared with patients treated with insulin and sulfonylureas and proposed that the discrepancy observed between this and other studies is most likely demonstrative of this bias.²⁰⁶ It was argued by the authors that changes in diabetes treatment are likely to occur in the few months prior to the diagnosis of cancer. As such, increased cancer rates may be observed shortly after commencing treatment, thereby potentially falsely implicating the treatments as the cause of the cancer.²⁰⁶

In the absence of specifically designed prospective studies of the association between metformin and cancer incidence, it has been suggested that the most appropriate source of information at present may be the secondary analysis of data from randomised controlled trials,²⁰⁰ as demonstrated by Home et al. in their analysis of the ADOPT study data.¹⁹⁹ However, a review by the Diabetes and Cancer Research Consortium¹²¹ has referred to the shortcomings of this approach, particularly the lack of power in trial data to assess adverse outcomes.^{207, 208}

The choice of the comparator to the exposure in studies of effects of metformin may be the most difficult source of potential bias to address. It is possible that any beneficial effect observed for metformin may simply represent a relative lack of harm, when compared to other anti-diabetic agents which may increase the risk of cancer or cancer mortality.²⁰⁰ This concern, and the manner in which it is addressed within the content of this thesis, is discussed in section 5.5.

Table 3.1: Meta-analyses of metformin exposure (versus no metformin exposure) and colorectal cancer incidence, and list of studies included

Studies Included in Meta Analyses ↓	Meta-analyses: First author and year of publication, Pooled risk estimate for colorectal cancer (95% Confidence Interval)					
	Franciosi M, 2013 ²⁰⁹	Zhang P, 2013 ¹⁹⁰	Noto H, 2013 ¹⁹⁴	Soranna D, 2012 ¹⁴⁰	Zhang ZJ, 2011 ¹⁹⁵	DeCensi A, 2010 ¹⁹⁶
	RR=0.83 (0.74-0.92)	RR=0.77 (0.64-0.91)	RR=0.68 (0.53-0.88)	RR=0.64 (0.54-0.76)	RR=0.63 (0.50-0.79)	RR=0.64 (0.38-1.08)
Observational studies						
Van Staa T, 2012 ²⁰⁶	✓					
Bodmer M, 2012 ²⁰⁴	✓	✓				
Ruiter R, 2012 ²¹⁰	✓	✓				
Tseng CH, 2012 ²¹¹		✓				
Ferrara A, 2011 ²¹²	✓					
Lee MS, 2011 ²⁰¹	✓	✓	✓	✓	✓	
Morden NE, 2011 ²¹³	✓	✓	✓			
Currie CJ, 2009 ²⁰³	✓	✓	✓	✓	✓	✓
Libby G, 2009 ²⁰²	✓	✓	✓	✓	✓	✓
Chung YW, 2008 ²¹⁴	✓	✓			✓	
Lewis JD, 2008 ²¹⁵	✓					
Oliveria S, 2008 ²¹⁶	✓			✓		
Yang YX, 2004 ²¹⁷		✓	✓	✓	✓	✓
Analysis of clinical trial data						
Home PD, 2010 ¹⁹⁹			✓			

RR: relative risk

Table 3.2: Characteristics of retrospective studies of associations between metformin exposure (versus no metformin exposure) and colorectal cancer incidence

First Author, Year, Country <i>Journal</i>	Study Objective	Study type, Data Source	Inclusion/Exclusion Criteria	Exposure/Comparator Outcome	Confounders in Model: Selection method, Confounders selected	Result for metformin exposure: Effect estimate (95% Confidence Interval)
Observational Studies						
Qiu H, 2013, UK <i>Diabetes Obes Metab</i> 218	Compare cancer incidence among T2DM patients newly exposed to metformin or sulfonylurea monotherapy.	Cohort study. UK CPRD	Patients who initiated OHA therapy during Jan 1 1995 – Dec 31 2008. Included only those who had at least 6 sequential Rxs for the same OHA. <u>Excluded:</u> <1year CPRD history prior to index, <1year follow up after index, Prior cancer, No cancer diagnosis date, Started with insulin only, Started with combination therapy, Diabetes<35yrs old or >80, No diabetes diagnosis.	Metformin versus sulfonylureas. Exposure for at least 6 months required for cohort entry. Cancer cases (Read codes). CRC identified as subgroup.	Change-in-estimate method, fixing age and gender in model. Age, gender, baseline HbA1c, BMI, smoking status, Chronic disease score, year of OHA start, no. of Rxs prior to OHA index date, hospitalisation, use of screening tests, duration of diabetes prior to OHA Index date – all considered. Only age and gender remained in the model.	HR for sulfonylureas (Reference= metformin): 1.17 (0.95-1.44)
Van Staa T, 2012, UK <i>Diabetologia</i> 206	Compare cancer risk among users of different classes of glucose-lowering agents.	Cohort study. UK CPRD	Patients >40 years with a Rx for insulin or OHAs at least one year after start of data collection. Included only new-users of drugs in analyses. <u>Excluded:</u> T1DM, history of cancer.	Newly prescribed metformin versus other treatments.	Age, sex, calendar year, socioeconomic status, smoking status, alcohol, BMI, comorbidities, ACE inhibitors, anti-platelets, beta blockers, calcium channel blockers, diuretics, nitrates, NSAIDs, aspirin, statins.	OR: 0.74 (0.53-1.03)
Bodmer M, 2012, UK <i>Cancer Epidemiol Biomarkers Prev</i> 204	Case-control analysis to explore whether metformin use is associated with an altered risk of colorectal cancer.	Case control. UK CPRD	Diabetes <age 90. Controls matched on index date, age, sex, GP, number of years of database history. <u>Excluded:</u> <3 years active history prior to cancer index date. Prior history of other cancer. Alcoholism, HIV prior to index date.	Comparator: No metformin Exposure: 1-9 metformin Rxs 10-29 metformin Rxs 30-49 metformin Rxs ≥50 metformin Rxs	Covariables that changed the univariate relative risk of cancer by >10% included in analyses. Diabetes duration, use of statins, NSAIDs, aspirin, smoking, BMI. Concomitant use of other anti-diabetic drugs.	1-9 metformin Rxs: OR 1.05 (0.83-1.33) 10-29 metformin Rxs: OR 1.05 (0.84-1.33) 30-49 metformin Rxs: OR 1.17 (0.88-1.55) ≥50 metformin Rxs: OR 1.43 (1.08-1.90)

Table 3.2 (continued)

First Author, Year, Country <i>Journal</i>	Study Objective	Study type, Data Source	Inclusion/Exclusion Criteria	Exposure/Comparator Outcome	Confounders in Model: Selection method, Confounders selected	Result for metformin exposure: Effect estimate (95% Confidence Interval)
Ruiter R, 2012, Netherlands, <i>Diabetes Care</i> ²¹⁰	Compare incidence of cancer between incident users of metformin and sulfonylurea derivatives.	Cohort study. Linked hospital discharge and pharmacy claims database.	Incident metformin/sulfonylurea users with >1 Rx 1998-2008. <u>Excluded:</u> Insulin only, <18years, Prior cancer.	Cumulative metformin (versus sulfonylureas) exposure in days until end of follow up. Diagnosis of any cancer. Subanalysis: CRC (ICD-9-CM 153-154).	Covariables that changed the HR of cancer risk by >10% or were considered clinically relevant. Age, sex, year of first Rx, no. unique drugs used in the year, no. hospitalizations in year prior to start of exposure.	HR: 0.91 (0.88-0.94)
Tseng CH, 2012, Taiwan <i>Experimental Diabetes Research</i> ²¹¹	Investigate whether the T2DM - CRC link is independent of detection bias. Examine whether metformin and duration of its use are associated with a protective effect against colon cancer.	Cohort study. Population-level health insurance database.	<u>Excluded:</u> T1DM, Region of residence unknown, CRC diagnosed pre 2003.	Metformin (<1 year, 1-3years, ≥3years) versus other OHAs. Colon cancer (ICD-9-CM 153).	No confounder selection methodology described. Age, sex, dyslipidaemia, obesity, hypertension, COPD (as smoking surrogate), asthma, stroke, nephropathy, ischemic heart disease, peripheral arterial disease, eye disease, statins, fibrates, ACEIs/ARBs, CCBs, aspirin, dipyridamole, clopidogrel, NSAID, sulfonylurea, insulin, acarbose, thiazolidinedione, region of residence, occupation, CRC detection examinations.	HR: 0.73 (0.58-0.92) Metformin ≥3years: HR: 0.64 (0.49-0.85)
Ferrara A, 2011, USA <i>Diabetes Care</i> ²¹²	Explore effect of pioglitazone on cancer risk.	Cohort study. Linked diabetes registry and cancer registry.	≥40 years, 1997-2005. <u>Excluded</u> Prior cancer diagnosis, gap in medication receipt for >4 months in the 4 months after cohort entry.	At least two Rxs after cohort entry. (Follow up started 6 months after cohort entry) Metformin comparator not detailed in report.	Use of individual diabetic medications, age, sex, year of cohort entry, race, income, smoking, glycaemic control, diabetes duration, ceratinine level, congestive heart failure.	OR: 0.90 (0.70-1.20) Source: ²⁰⁹

Table 3.2 (continued)

First Author, Year, Country <i>Journal</i>	Study Objective	Study type, Data Source	Inclusion/Exclusion Criteria	Exposure/Comparator Outcome	Confounders in Model: Selection method, Confounders selected	Result for metformin exposure: Effect estimate (95% Confidence Interval)
Lee MS, 2011, Taiwan <i>BMC Cancer</i> 201	Assess gastro-enterological cancer incidence in relation to metformin usage among T2DM.	Cohort study. Population-level health insurance database.	Incident T2DM patients. <u>Excluded:</u> Prevalent T2DM prior to 2000. Insulin use.	Metformin versus other OHAs. At least 2 Rx for metformin 2000-2007. CRC (ICD-9-CM 153-154) (and other GI cancers).	Age, gender, other OADs, comorbidity score, duration of metformin exposure.	HR: 0.36 (0.13-0.98)
Morden NE, 2011, USA <i>Diabetes Care</i> 213	Compare incident cancer in older patients with type 2 diabetes using various forms insulin.	Cohort study. US Medicare database	Elderly T2DM patients <u>Excluded:</u> Prevalent cancers, patients on antineoplastic prophylaxis.	Metformin (versus no metformin) entered as a covariate in Cox Hazards model. Various cancer sites including colon cancer; identified using ICD-9 codes and Clinical Classification Software.	Age, race, sex, income level, obesity, smoking, Charlson comorbidity score, diabetes complications, insulin use.	HR: 0.94 (0.72-1.22)
Currie CJ, 2009, UK <i>Diabetologia</i> 203	Compare incidence of solid tumours (including CRC) between metformin users and users of other OHAs.	Cohort study. UK general practice database (THIN).	Diabetic patients >40 years of age, ≥6 sequential Rx for OHAs. <u>Excluded:</u> <6 months case history prior to the index date. <6 months exposure to drug regimen. Prior cancer.	Metformin versus: sulfonylureas / metformin+sulfonylureas as/ insulin therapy. (≥6 months exposure) CRC (undefined).	Age, sex, smoking status.	Metformin versus sulfonylureas: HR: 1.8 (1.29-2.53) Metformin versus metformin+sulfonylureas: HR: 1.43 (1.05-1.94) Metformin versus insulin HR: 1.69 (1.23-2.33)

Table 3.2 (continued)

First Author, Year, Country <i>Journal</i>	Study Objective	Study type, Data Source	Inclusion/Exclusion Criteria	Exposure/Comparator Outcome	Confounders in Model: Selection method, Confounders selected	Result for metformin exposure: Effect estimate (95% Confidence Interval)
Libby G, 2009, UK <i>Diabetes Care</i> 202	Investigate if metformin use is associated with a reduced risk of cancer in people with T2DM.	Cohort study. Population-based diabetes register with linked clinical data.	Incident metformin users. <u>Excluded:</u> Prior cancer.	Incident metformin use (≥ 1 Rx) versus receipt of other OADs. Colorectal cancer (ICD-9-CM 153-154, ICD-10-CM C18-C20).	Age, sex, smoking status, deprivation, BMI, HbA1c, use of sulfonylureas and insulin.	HR: 0.60 (0.38-0.94)
Chung YW, 2008, South Korea <i>Disease of the Colon and Rectum</i> 214	Investigate if long-term insulin therapy was associated with colorectal cancer incidence in T2DM patients.	Case-control. Hospital-based study.	T2DM patients who underwent total colonoscopy 2003-2006. <u>Excluded:</u> <1 year follow-up, <1 year insulin therapy (among insulin users), CRC history, GI disorder history, lipid-lowering agent history.	One or more years of metformin use entered into model.	Age, sex, BMI, duration of diabetes, HbA1c, lipid levels, insulin use, metformin use, aspirin use.	OR: 0.7 (0.3-1.4)
Lewis JD, 2008, USA <i>Gastroenterology</i> 215	Examine the association between thiazolidinedione therapy and colorectal cancer in diabetics.	Nested case-control. Linked diabetes registry and cancer registry.	T2DM diagnosed 1994-1996. <u>Excluded:</u> Inflammatory bowel disease, FAP, HNPCC.	At least two Rx's after cohort entry. (Follow up started 6 months after cohort entry) Metformin comparator not detailed in report.	Age, sex, calendar year of index date, use of other diabetes medications	OR: 0.77 (0.67-0.88) Source: ²⁰⁹

Table 3.2 (continued)

First Author, Year, Country <i>Journal</i>	Study Objective	Study type, Data Source	Inclusion/Exclusion Criteria	Exposure/Comparator Outcome	Confounders in Model: Selection method, Confounders selected	Result for metformin exposure: Effect estimate (95% Confidence Interval)
Oliveria SA, 2008, USA <i>Diabetes and Metabolic Syndrome: Clinical Research and Reviews</i> 216	Quantify and compare the incidence of colorectal cancer (and other cancers) in users and non-users of anti-diabetic pharmacotherapies.	Cohort study. Health insurance claims database.	Diabetic patients with claims information 2000-2004 and at least 1 year claims history prior to start of follow-up. <u>Excluded:</u> Prior cancer	≥1 prescription for metformin versus none.	Age, gender, polyp history, ulcerative colitis, Crohn's disease.	Metformin monotherapy RR: 0.67 (0.52-0.8) Metformin/sulfonylurea dual therapy RR: 0.68 (0.51-0.91)
Yang YX, 2004, UK <i>Gastroenterology</i> 217	Investigate if long-term insulin therapy was associated with colorectal cancer incidence in T2DM patients.	Cohort study. UK CPRD	T2DM patients 1987-2002. <u>Excluded:</u> <3years follow-up post T2DM diagnosis, <1year insulin therapy among insulin users. CRC diagnosis within 3 years of follow-up/within 1 st year of insulin. therapy.	Insulin exposure versus non-insulin exposure. Metformin use ≥3years included in model. Incident colorectal cancer during follow-up.	A priori confounder selection. Sex, cholecystectomy history, smoking, duration of T2DM, BMI, metformin, sulfonylurea, NSAID, aspirin use.	OR: 1.0 (0.6-1.7)
Analysis of clinical trial results						
Home PD, 2010, (multi-centre) <i>Diabetologia</i> 199	Compare malignancy rates in participants randomised to metformin or to other oral glucose-lowering agents (rosiglitazone or glyburide).	ADOPT (Manufacture -sponsored oral glucose-lowering agent trial).	T2DM patients aged 30-75. <u>Excluded:</u> Numerous exclusions, including unstable/severe angina, uncontrolled hypertension, steroid-treated chronic disease, drug/alcohol abuse, body weight variations.	Metformin exposure versus rosiglitazone or glyburide.	n/a	(Only overall cancer effect reported in study report. Effect estimate for colorectal cancer not reported.)

CRC: colorectal cancer; HR: hazard ratio; OHA: oral hypoglycaemic agent; OR: odds ratio; RR: relative risk; Rx: prescription. T1DM: type 1 diabetes mellitus; T2DM: type 2 diabetes mellitus (Other abbreviations: See 'List of Abbreviations', page xviii)

3.2.6. Metformin and cancer outcomes: Observational studies

In comparison to studies of cancer incidence, fewer studies have examined associations between metformin and cancer outcomes. However, meta-analyses of associations between metformin and overall cancer mortality have recently been published (Table 3.3).^{190, 194, 197} Zhang et al. examined studies published by June 2012, tabulating the main characteristics and risk estimates provided by all included studies in their meta-analysis. The researchers included five cohort studies in their meta-analysis of effects on overall cancer mortality and reported that metformin exposure was associated with a risk reduction of 18% (Pooled Relative Risk=0.82, 95% CI 0.40-0.99).²¹⁹ Noto et al. included four cohort studies and the results of one randomised controlled trial published as of October 2011 and reported a pooled relative risk of mortality of 0.66 (95% CI 0.49-0.88).¹⁹⁴ However, Stevens et al. performed a meta-analysis of results of randomised clinical trials of metformin as a diabetes treatment and found no statistically significant benefit for metformin in reducing cancer mortality (Relative Risk=0.94, 95% CI 0.79-1.12).¹⁹⁷ The discrepancy between the results of this latter meta-analysis and those which analysed observational studies is discussed by Stevens et al.¹⁹⁷ The general limitations of observational studies, including confounding by indication, are suggested as potentially being responsible for over-estimating a beneficial effect of metformin. However, the authors also acknowledge the limitations of their study, including the short follow-up time of the included studies and low number of cases.

Several studies have examined the influence of metformin on outcomes in specific cancer types, including breast cancer,²²⁰⁻²²⁴ ovarian cancer,^{225, 226} lung cancer^{227, 228} and other tumour types.²²⁹⁻²³² Meta-analyses for some individual tumour types are presented as subgroup analyses within the meta-analyses by Zhang et al. and Noto et al.^{190, 194}

Table 3.3: Meta-analyses of metformin exposure (versus no metformin exposure) and overall cancer mortality or survival, and list of studies included.

Studies included in Meta-analyses ↓	Meta-analyses: First author and year of publication, Pooled relative risk for Overall Cancer Mortality (95% Confidence Interval)		
	Zhang P, 2013 ¹⁹⁰	Noto H, 2012 ¹⁹⁴	Stevens R, 2012 ¹⁹⁷
	RR=0.82 (0.40-0.99)	RR=0.66 (0.49-0.88)	RR=0.94 (0.79-1.12)
Observational studies			
Bo S, 2012 ²³³	✓		
Currie C, 2012 ²³⁴	✓		
Baur D, 2011 ²³⁵	✓		
Mellbin L, 2011 ²³⁶		✓	
Bowker S, 2010 ²³⁷	✓		
Landman G, 2010 ²³⁸	✓	✓	
Libby G, 2009 ²⁰²		✓	
Bowker S, 2006 ²³⁹		✓	
Analysis of clinical trial data			
UKPDS 34, 1998 ²⁴⁰		✓	✓
ADOPT, 2006 ²⁴¹			✓
COSMIC, 2005 ²⁴²			✓
Knowler W, 2005 ²⁴³			✓
EDIT, 2003 ²⁴⁴			✓
IDPP, 2006 ²⁴⁵			✓
Pfutzner A, 2011 ²⁴⁶			✓
QUARTET-M, 2004 ²⁴⁷			✓
QUARTET-C, 2004 ²⁴⁸			✓
RECORD, 2009 ²⁴⁹			✓

RR: Relative Risk

3.2.7. Metformin and colorectal cancer outcomes

Three published studies have specifically analysed associations between metformin exposure and survival among colorectal cancer patients.²⁵⁰⁻²⁵² A fourth study examined colorectal cancer as part of a stratified analysis of an overall study of solid tumours.²³⁴ Three of these four studies were published prior to the survival analysis described in Chapter Six.^{234, 250, 251} The characteristics of these studies are listed in Table 3.4 and are discussed as follows.

3.2.7.1. General study characteristics

All of the four studies listed in Table 3.4 are retrospective observational studies which compare patients exposed to metformin to patients not exposed to the drug. In all but the study by Currie et al., diabetic patients served as the reference group, though Cossor et al. used non-diabetic patients as the reference group in secondary analyses. All of the studies used a Cox proportional hazards modelling approach to examine associations with survival; age was an adjustment variable common to all of the studies, as well as gender (though the study by Cossor et al. was restricted to females). All of the studies except that by Currie et al., which used general practice data, acquired their subjects from a single institution source rather than a population-level database. None of the studies obtained the cancer-related data from a cancer registry source and only Lee et al. and Cossor et al. used cancer-specific survival as the primary outcome. However, staging data in some form was available to all of the studies for potential adjustment, except in the case of the study by Currie et al. BMI was also adjusted for in all of the studies except that by Currie et al. These authors reported that BMI had been considered as a confounder in their model and that while significantly associated with survival, inclusion of the variable did not have a meaningful impact on the hazard ratios in the study and was therefore excluded. Garret et al. also reported that excluding BMI from their model did not alter the point estimates for the effect of metformin on survival. HbA1c was available in the data of Garrett et al., Currie et al., and Lee et al., but was only adjusted for by Lee et al., who were also the only group to adjust for years of diabetes duration.

3.2.7.1. Exposure information

Information regarding the extent of exposure to metformin was missing in several of the studies. Lee et al. classified the metformin-exposed group as patients taking metformin for a minimum of six months but noted that '*consistent use before and after colorectal cancer diagnosis was included*'; it is unclear at what stage in the time period close to cancer diagnosis that this minimum of six months of exposure occurred. This lack of clarity has led to the suggestion of the possible presence of immortal time in the study in a review of the literature published recently.¹⁴⁶ As the exposure time window used by Lee et al. included metformin exposure both before and after cancer diagnosis, patients in the study may have been required to remain alive for a certain period in order to meet the 'minimum of six months' exposure criterion. Immortal time would therefore be introduced to the analysis and may have resulted in over-estimation of the effect of metformin in prolonging survival.¹⁴⁶

Table 3.4: Characteristics of retrospective studies of associations between metformin and colorectal cancer survival

First Author, Year, Country, Journal	Study Objective	Study design, Data Source	Inclusion/Exclusion Criteria	Exposure and Comparator*	Analysis details	Outcome	Confounders in Model: Selection method, Confounders selected	Result for metformin exposure: Hazard ratio (HR) (95% Confidence Interval)
Cossor F, 2013 USA Cancer Epidemiol 252	Examine associations between metformin use and CRC-specific and overall survival among postmenopausal women.	Cohort study. Women’s Health Initiative (WHI) trials cohort.	Postmenopausal women aged 50-79 years enrolled at WHI centres across USA 1993-1998, diagnosed with CRC after cohort entry. Excluded: Type 1 diabetes, prior CRC, deceased at CRC diagnosis.	Interviewer-administered questionnaires at baseline and at years 1,3,6 and 9. Metformin exposure (ever/never – timing not specified) compared to (i) non-metformin diabetic, (ii) non-diabetic.	Propensity score-adjusted Cox proportional hazards model.	CRC-specific survival	Stratification by quintiles of propensity score for metformin use. Propensity score generated using age, race, BMI, smoking status, alcohol use, dietary history, physical activity level, stage at diagnosis, insulin, total number of anti-diabetic drugs, aspirin, NSAID use.	Metformin versus no metformin: HR: 0.78 (0.38-1.55) Metformin versus non-diabetic: HR: 0.86 (0.49-1.52)
Garrett CR, 2012 USA Br J Cancer 251	Assess association between metformin use and overall mortality.	Cohort study. Hospital-based study.	Diagnosis of CRC 2004-2008. Excluded: Type 1 diabetes, resident outside of USA	Exposure determined from hospital records. Metformin exposure compared to non-metformin diabetic.	Multivariate Cox proportional hazards model.	Overall survival	Backward stepwise selection. Adjusted for age, sex, race, BMI, aspirin usage, stage at diagnosis.	HR: 0.6 (0.5-0.8)

Table 3.4 (continued)

First Author, Year, Country, Journal	Study Objective	Study design, Data Source	Inclusion/Exclusion Criteria	Exposure and Comparator*	Analysis details	Outcome	Confounders in Model: Selection method, Confounders selected	Result for metformin exposure: Hazard ratio (HR) (95% Confidence Interval)
Lee JH, 2012 Korea <i>Int J Cancer</i> 250	Assess the effect of metformin on CRC-specific mortality.	Cohort study. Hospital-based study.	New diagnosis of CRC 2000-2008. Excluded: diabetes diagnosed after CRC, incomplete records, metformin use <6months, any prior cancer.	Metformin exposure compared to non-metformin diabetic.	Multivariate Cox proportional hazards model.	CRC-specific survival	Age, sex, stage, BMI, diabetes duration, smoking status, HbA1c level, use of aspirin, insulin, sulfonylurea, thiazolidinedione.	HR: 0.66 (0.45-0.98)
Currie CJ, 2012 UK <i>Diabetes Care</i> 234	Determine if post-cancer survival is related to type of diabetes medication.	Cohort study. Primary care data.	Subjects with at least 2 years of data following cohort entry 1990-2009. Excluded: Type 1 diabetes, age <35 years at diagnosis, haematological cancer prior to solid tumour.	Metformin monotherapy in 90 days prior to cancer diagnosis. Also identified metformin monotherapy in 90 days post diagnosis. Compared to non-metformin diabetic.	Multivariate Cox proportional hazards model.	Overall survival	Age, sex, smoking status, deprivation status, Charlson comorbidity score, number of primary care contacts, year of diagnosis.	Metformin in 90 days pre diagnosis: HR: 0.89 (0.56-1.42)

CRC: colorectal cancer

HR: hazard ratio

*As discussed in section **Error! Reference source not found.**, it is not clear whether or not metformin exposure included exposure following cancer diagnosis, except for the study by Currie et al., which details the time windows used.

Currie et al. provided a more detailed description of the exposure timing in their study, in which they compared patients exposed to metformin monotherapy in the 90 days prior to cancer diagnosis to non-diabetic patients. In sensitivity analyses, the researchers also examined patients with metformin monotherapy in the 90 days post cancer diagnosis, but stated that *'these data need cautious interpretation'*. They did not specifically state that follow-up time for this sensitivity analysis was calculated following the 90 days post diagnosis, a measure which would avoid immortal time bias. However, the number of cases for this analysis was less than the number available in the main analysis, indicating that this measure was taken.

Garrett et al. and Cossor et al. provided little information on the timing or nature of the metformin exposure in their studies. Garrett et al. did not state at what time point in relation to the patient's diagnosis the exposure was examined but noted that data were not present regarding the duration of exposure of the patient to metformin. Cossor et al. defined exposure as 'use of metformin at any time' but acknowledged the fact that this definition did not incorporate timing, duration or dosage of exposure, an important limitation of the study.

None of the studies examined the effect of dosage or intensity/frequency of exposure to metformin on survival. Currie et al. specified the exposure as 'metformin monotherapy'. In the analysis of all tumours carried out by Currie et al., metformin combined with insulin or sulfonylurea therapy was also examined.

3.2.7.2. Main findings

Lee et al. and Garrett et al. examined colorectal cancer-specific survival and overall survival, respectively, and found significant associations between overall metformin exposure (versus no metformin exposure) and survival, within diabetic populations (Table 3.4). Garrett et al. reported the greater reduction in mortality, with a hazard ratio of HR=0.6. (95% 0.5-0.8), though this was close to the hazard ratio of 0.66 reported by Lee et al. A similar survival improvement was observed by Cossor et al., though this was not statistically significant (HR=0.78, 95% CI 0.38-1.55). No association was observed by Cossor et al. between metformin exposure and survival from colorectal cancer in analyses with non-diabetic patients as the reference.

Currie et al. compared patients receiving metformin monotherapy to non-diabetics. Reduced overall mortality was observed in patients receiving metformin monotherapy for 90 days prior to cancer diagnosis, versus non-diabetic patients, when all cancer types were combined (HR

0.90, 95% CI 0.81-0.99). A similar point estimate, but not statistically significant, was found among colorectal cancer patients when analyses were stratified by site (HR: 0.89, 95% CI 0.67-1.19).

3.2.7.3. Influence of stage at diagnosis and tumour site

Both Lee et al. and Garrett et al. examined the effect of stage by providing Kaplan-Meier curves stratified by AJCC stage at diagnosis and using the log-rank test to compare survival between metformin users and non-users. Lee et al. provided separate curves for stages I, II, III and IV colorectal cancer. The colorectal cancer-specific mortality rate was not found to be significantly different according to metformin use in stage I, II or IV colorectal cancer patients. However, in stage III patients, a significantly higher colorectal cancer-specific mortality rate was observed for the metformin group versus the non-metformin group ($p=0.05$), though the authors did not suggest a rationale for this potential effect modification. Garrett et al. provided separate Kaplan-Meier curves for stage I-II, stage III and stage IV cancer patients. Significant differences were found between metformin users and non-users for patients with TNM stage I-II ($p=0.03$) and TNM stage III ($p=0.002$) colorectal cancer, but no difference was observed in stage IV (metastatic) patients.

Lee et al. also provided Kaplan-Meier curves stratified by location of cancer (colon and rectal). By the log-rank test, survival was found to be significantly higher in rectal cancer patients taking metformin versus patients not taking metformin ($p=0.047$). No significant difference between the groups was observed among colon cancer patients.

As all of these Kaplan-Meier curves were unadjusted for potential confounders, it is difficult to draw conclusions from these analyses on effects of metformin. However, they suggest potential differential effects which are worthy of exploration in more rigorous analyses.

3.2.8. Metformin and response to neoadjuvant therapy:

Observational studies

As discussed in section 1.1.4, pathologic complete response is an important marker of rectal cancer patient outcomes following neoadjuvant therapy, including disease-free survival and overall survival. In a univariate analysis, Garrett et al. compared the pathologic response rate for complete and minor response (i.e., $\leq 10\%$ residual tumour) among metformin-exposed versus non-exposed diabetic patients receiving neoadjuvant chemoradiotherapy for rectal cancer. A trend towards a higher response rate was observed for the metformin patients (14/19, 74% versus 9/19, 47%, $P=0.09$).²⁵¹ This is the only study to date to examine an association between metformin exposure and pathologic complete response in patients with

rectal cancer. However, studies in other solid tumour types have formally examined associations between metformin and pathologic response rate using multivariate regression approaches, which may be considered as more reliable evidence.^{221, 253}

The first publication to examine associations between metformin and pathologic response rate was a study in breast cancer patients receiving neoadjuvant chemotherapy.²²¹ Diabetic patients taking metformin, diabetic patients not taking metformin, and non-diabetic patients were compared and factors predicting pathologic complete response were assessed using multivariate logistic regression. After adjustment for diabetes, BMI, age, stage, grade, hormone receptor status and neoadjuvant taxane use in the model, exposure to metformin (versus no exposure, among diabetic or non-diabetic patients) was found to independently predict pathologic complete response (OR=2.95, $P=0.04$).²²¹ Most recently, associations between metformin and pathologic complete response rates have been explored in oesophageal cancer. Researchers used a similar study design to that in the aforementioned breast cancer study and found that after adjustment for tumour stage, grade, and lymphovascular invasion, the pathologic complete response rate was significantly higher in patients taking metformin than in diabetic patients not taking metformin or in non-diabetic patients ($P=0.04$), and was related to metformin doses of greater than 1,500mg/day.²⁵³

3.2.9. Clinical trials examining metformin and colorectal cancer

In light of increasing observational evidence and preclinical studies (as described above) supporting a role for metformin in the treatment of colorectal cancer, a large number of clinical trials have been initiated in the past number of years (www.clinicaltrials.gov). These include a large-scale phase III randomised trial of the effect of adjuvant metformin versus placebo on recurrence and survival in early-stage breast cancer patients (Trial number: NCT01101438).²⁵⁴ This study, which began in June 2010, aims to recruit 3,582 non-diabetic node-positive or high-risk node-negative breast cancer patients receiving standard therapy. Patients are randomised to 850mg metformin (i.e. a dose schedule within the therapeutic range for type 2 diabetes) or placebo twice daily for 5 years. The primary outcome is invasive disease-free survival with secondary outcomes of overall and distant disease-free survival, breast cancer-specific survival and adverse events. This study is due for completion in June 2016. Meanwhile, several 'window of opportunity' trials of metformin in breast cancer have taken place. In these studies metformin is administered (at doses used in type 2 diabetes) to non-diabetic women in the time interval between tumour diagnosis and tumour surgery.²⁵⁵⁻²⁵⁷ Each of these trials have noted some reduction in tumour Ki-67 levels, a biomarker of cell proliferation, to varying extents following metformin administration.

To date, no large-scale phase III clinical trials have been commenced to study metformin in colorectal cancer patients, though a number of phase I and phase II studies have recently begun. Only one trial of the effects of metformin in relation to colorectal cancer has been published thus far. The group involved in this trial previously found in an animal study that metformin was capable of suppressing the chemical induction of aberrant crypt foci (ACF), a pre-cancerous marker associated with the development of colorectal tumours¹⁶⁵, in non-diabetic mice.¹⁶⁴ A pilot trial was subsequently begun, examining a small number of non-diabetic patients with ACF, and involving the randomisation of patients to treatment with metformin (250mg/day) or control (untreated) for one month. The number of ACF per patient was examined by magnifying colonoscopy at baseline and following the intervention. Among the metformin patients (n=9) available for end-point analyses, the mean number (\pm SD) of ACF per patient decreased significantly from 8.78 ± 6.45 (baseline) to 5.11 ± 4.99 ($p=0.007$). No significant change was observed in the control group (n=14) (7.23 ± 6.65 at baseline versus 7.56 ± 6.75 at 1 month, $p=0.609$). In order to also examine proliferative activity in the rectal epithelium, the proliferating cell nuclear antigen (PCNA) labeling index was investigated. This was found to decrease significantly in the metformin group between baseline and follow-up, although no significant change was found in the percentage of apoptotic cells. Importantly, this prospective study examined non-diabetics and used a low dose of metformin (250mg/day).²⁵⁸

3.2.10. Consideration regarding dosage of metformin in the cancer setting

As discussed by Quinn et al. in a recent review, various factors may contribute to responsiveness of cells to metformin in the prevention/treatment of cancer.¹⁶⁹ These may include the concentration of metformin present, the timing of exposure to metformin in relation to presence or absence of the tumour, growth conditions surrounding cancer cells, and the underlying genetic landscape.¹⁶⁹

Of these suggested conditions, the concentration of metformin has been the most contentious factor. The generalizability of in vitro studies to the in vivo setting has been a frequent cause for concern in the consideration of metformin as a possible anti-cancer agent. Some authors have argued that it has been inappropriate of investigators to use in vitro evidence in support of commencing clinical trials of metformin in cancer; this is due to the high concentrations of metformin used in such in vitro studies.²⁵⁹ The majority of laboratory studies have been found to use concentrations of metformin between 1 and 20 millimoles per litre in cell line

research.²⁵⁹ However, such high doses are unlikely to be attained in a clinical setting as plasma levels of therapeutic metformin in diabetic patients have been estimated to be in the range of 5-15 micromoles per litre.²⁶⁰ Therefore, while it is plausible that metformin may directly inhibit cancer cells in vitro, it has been suggested to be more likely that anti-cancer effects observed in vivo, if they are truly caused by metformin, are attributed to indirect effects involving insulin signalling pathways.

Researchers in the preclinical setting have challenged this viewpoint with emerging results showing effects of metformin at low concentrations. One group carrying out in vitro work using ovarian cancer cell lines observed that cell death occurred following treatment with micromolar concentrations of metformin combined with low-dose chemotherapy.²⁶¹ However, no such cytotoxicity was observed using either micromolar levels of metformin or low-dose chemotherapy alone.²⁶¹ This group has hypothesised that metformin behaves synergistically with standard anti-cancer chemotherapy and that this explains the survival benefit found in observational studies of diabetic patients.²⁶¹ Other research groups have suggested that low doses of metformin may be capable of inhibiting cancer growth by specifically targeting cancer stem cells; a recent study examined this hypothesis with in vitro and in vivo experiments in pancreatic cancer.¹⁸² Low dose metformin (20 micromoles per litre, or 20mg/kg, which equates to anti-diabetic levels of dosing) had an anti-cancer effect both in vitro and in vivo and was found to selectively inhibit CD133⁺ cells. These cells are known to be cancer stem cells and are thought to contribute to the recurrence, metastasis, and resistance to adjuvant therapy of pancreatic cancer.¹⁸²

Menendez et al., who described in detail the concept of synthetic lethality in relation to metformin (as discussed above), have firmly disputed the relevance of the argument that effects of metformin observed in vitro are impracticable due to the high concentrations used.¹⁷⁹ This group have argued instead that in vitro experiments which reported no effect of metformin at relatively low doses are in fact subject to 'glucose-related biases', as follows. The authors have pointed out that glucose concentrations commonly used in cell culture methods are up to 10-40 times higher than those of the physiologic microenvironment of an actual tumour. This is in part due to the fact that glucose deprivation is a characteristic feature of tumours, arising as a result of poor nutrient supply and extremely high glucose consumption. Menendez et al. tested the ability of metformin to bring about apoptotic cell death in HER2+ breast cancer cells under conditions of (i) plentiful glucose, and (ii) glucose deprivation. Metformin-induced apoptosis was found to increase more than 100-fold under the glucose-deprived conditions, which were designed to mimic the in vivo tumour environment. The

authors concluded that, if functional *in vivo*, this inhibitory effect of metformin in a low-glucose tumour environment could be achieved with doses even lower than those currently used for type 2 diabetes.¹⁷⁹

Considering the observational research evidence, some studies have observed dose-response effects of metformin. For example, a study of gastroenterological cancers has found that the effect of metformin in reducing cancer incidence was dose-dependent; a reduction in incidence was observed even at the lowest usual dose of metformin used in clinical practice ($\leq 500\text{mg/day}$).¹⁹⁴ The original hypothesis-generating study of metformin and cancer incidence by Evans et al. examined associations between metformin and cancer incidence stratified by duration of exposure; in this case, only patients with the longest exposure duration ($>1,806$ days) had a significantly reduced incidence of cancer.¹⁵⁷ Also, a study examining cancer survival found a dose-dependent association of metformin with cancer-related mortality, suggesting a 42% decrease in mortality for every 1g increase in metformin dose.²³⁸ However, this effect has not been replicated in other studies.²²⁰

An additional factor worthy of consideration in this topic is that the dose of metformin necessary for an anti-cancer effect may be dependent on the means of transport of the drug into the cell. Metformin is known to be actively transported into liver cells by the organic cation transporter OCT-1.²⁶² This transporter has been investigated recently and found to be relevant to the anti-cancer effect of biguanide drugs, particularly phenformin, in ovarian cell lines.²⁶³ Metformin has also recently been found to be transported into cells by another organic cation transporter, OCT-3²⁶⁴ and this transporter has been shown to be over-expressed in a number of cancer cell lines, particularly in colorectal cancer.^{265, 266} Indeed, this transporter has been found to be necessary for the action of metformin in activation of AMPK and inhibition of mTOR in head and neck cancer cells.²⁶⁵ The possibility of differential transportation of metformin in cancer tissue versus normal tissue, and in different tumour types, could potentially give rise to differential effects of metformin according to tumour site.⁴⁷

3.3. Summary

In the past few years there has been a significant increase in scientific interest regarding potential effects of metformin as an anti-cancer agent. Evidence for these effects has arisen from laboratory-based studies in cancer models, observational research, including studies of cancer incidence and mortality of diabetic patients and secondary analyses of clinical trial results. All such study types are represented in the evidence base supporting a beneficial effect of metformin in colorectal cancer. However, observational studies of outcomes in colorectal

cancer are few. Also, existing studies have not provided information on effects of frequency of exposure or impact of co-prescription with other anti-diabetic drugs. While clinical trials have recently been commenced to examine metformin in non-diabetic cancer patients, some have argued that these investigations are premature and that more extensive and more robust supporting evidence is now required in order to inform future investigations. Nonetheless, emerging results from laboratory studies present a strong case for the effectiveness of this drug in the cancer setting.

Chapter Four

4. Aspirin: Current uses and potential applications in colorectal cancer

This chapter examines the pharmacology of aspirin in its existing clinical applications, followed by discussion of current scientific evidence for its potential role in colorectal cancer. The background to the role of aspirin as an anti-cancer agent will be presented with a summary of current scientific evidence for its mechanism of action. Preclinical, observational and clinical research will be discussed followed by consideration of the potential of aspirin for repurposing as a preventative agent or treatment for colorectal cancer.

4.1. History of aspirin and related drugs

The history of aspirin has been reviewed in detail previously.^{267, 268} The development of aspirin originates in the use of willow tree bark by ancient civilisations to treat pain and inflammation; willow powder was later used by scientists of the 18th and 19th centuries in clinical studies aimed at treating fever and inflammation.²⁶⁷ Also during the 19th century, the active compound salicin was identified in willow extract and later purified as salicylic acid. Salicylic acid was found to have analgesic and antipyretic qualities but also resulted in gastric irritation and bleeding. The compound was therefore redeveloped and chemically converted to the less irritant acetylsalicylic acid (Figure 4.1). This chemical was eventually registered by Bayer with the trade name Aspirin in 1899 and sold as an analgesic.²⁶⁷ Following the production of aspirin, other compounds referred to as 'aspirin-like drugs' such as indomethacin, were developed.²⁶⁹ These, together with aspirin, became known as 'non-steroidal anti-inflammatory drugs' (NSAIDs) as their properties were dissimilar from steroid-type anti-inflammatory compounds.²⁶⁹ The mechanism of action of aspirin and other NSAIDs as anti-inflammatory drugs was not discovered until the 1970s;²⁶⁸ Vane first described in 1971 how these drugs exert their analgesic and anti-inflammatory via inhibition of prostaglandin synthesis.²⁷⁰ Current usage of aspirin is largely in the prevention or treatment of cardiovascular disease. The first study to suggest this beneficial effect was published in 1948 after a physician noticed that none of his patients who were regularly treated with aspirin experienced a myocardial infarction.²⁶⁷ The first large-scale randomised clinical trial examining aspirin as a treatment for stroke was performed in 1978.²⁶⁸

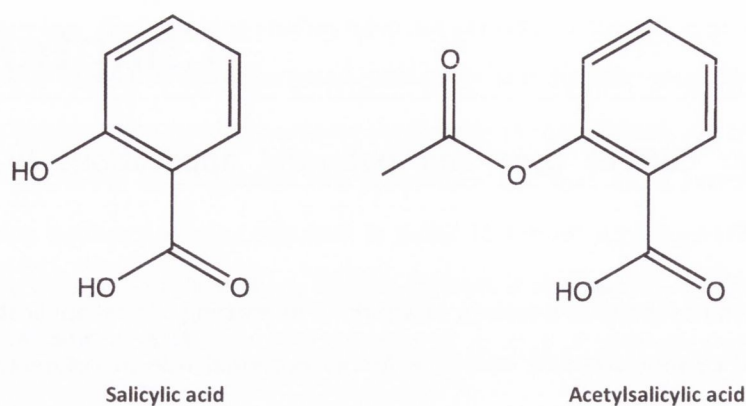


Figure 4.1: Chemical structures of salicylic acid (left) and acetylsalicylic acid (right)

4.2. Mechanism of action

Aspirin and other NSAIDs inhibit prostaglandin synthesis via inhibition of the enzyme prostaglandin G/H synthase (PGHS), known otherwise as cyclooxygenase, or COX.²⁷¹

Prostaglandins are signalling molecules with a variety of functions which include inducing pain, fever and inflammation. Some prostaglandins also have protective functions in preventing gastric ulceration and bleeding.²⁷² Other mediators derived from prostaglandins include thromboxane, which is involved in blood clotting by promoting the aggregation and activation of platelets.^{272, 273}

Prostaglandins are derived from arachidonic acid, a fatty acid released from the cell membrane under conditions of mechanical trauma or other triggers. Arachidonic acid is converted to the intermediate prostaglandins PGG₂ and PGH₂ by COX.²⁷² Following production of the prostaglandin PGH₂, this intermediate is further processed by other enzymes to generate various functional prostaglandins and thromboxane (Figure 4.2).

Cyclooxygenase exists in two forms referred to as COX-1 and COX-2.²⁷² The COX-1 form of the enzyme is mainly involved in basal, continuous prostaglandin synthesis, such as the synthesis of prostaglandins involved in protection of the gastric lining.²⁷² The COX-2 form of the enzyme, however, is largely found in inflammatory cells, is induced by the presence of bacterial cell components or tumour promoters, and is of particular importance in pain and inflammation.²⁷²

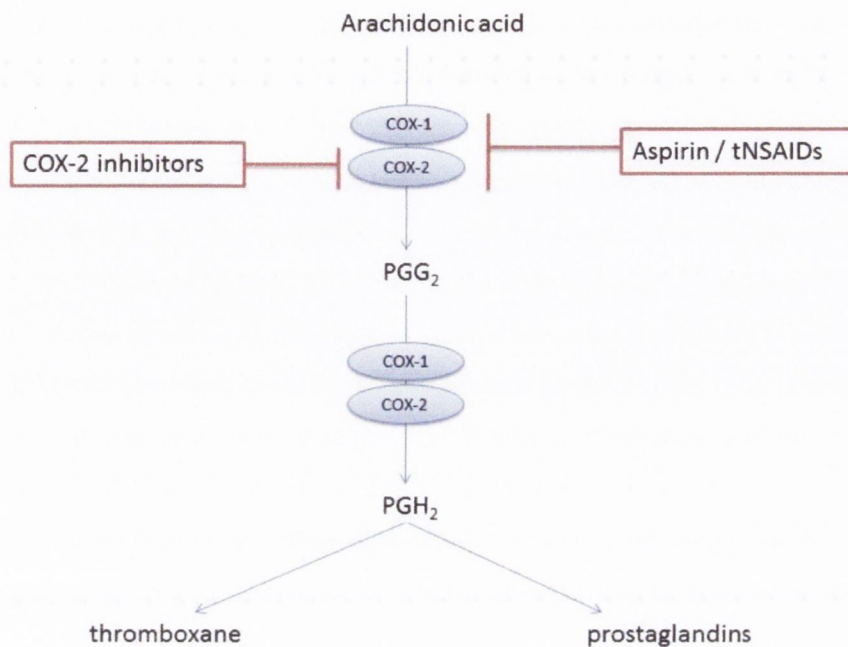


Figure 4.2: The role of COX in prostaglandin and thromboxane synthesis

The COX isoforms COX-1 and COX-2 convert arachidonic acid to the intermediates PGG₂ and PGH₂. PGH₂ is then converted to thromboxane or to a variety of prostaglandins by different enzymes. tNSAIDs: traditional NSAIDs

Aspirin and other NSAIDs have been found to inhibit COX-1 and COX-2 to different extents. For example, aspirin shows much greater selectivity for COX-1 than COX-2.²⁷⁴ Aspirin is unique in that it inhibits COX in an irreversible manner. This is accomplished due to the acetyl group in its chemical structure; aspirin covalently (thus, permanently) acetylates the domain of the COX enzyme which binds arachidonic acid, thereby preventing prostaglandin and thromboxane synthesis.²⁷⁵ Other NSAIDs reversibly and competitively inhibit COX; they do not permanently inactivate it.²⁷⁵ In recent years, anti-inflammatory drugs have been developed which are inhibitors that preferentially act on the COX-2 form of cyclooxygenase, e.g. celecoxib.²⁷¹ The rationale behind such drug development was to minimise inhibition of the COX-1 enzyme, thereby maintaining the synthesis of prostaglandins which serve to protect the gastric mucosa while inhibiting the synthesis of pro-inflammatory prostaglandins.²⁷¹ While the motivation behind development of drugs which specifically inhibit COX-2 was to avoid gastro-intestinal adverse effects of anti-inflammatory therapy, these drugs have been associated with cardiovascular adverse events leading to restriction of their use.²⁷¹

As aspirin inhibits COX-1 as well as COX-2, it is capable of inhibiting thromboxane production, thereby inhibiting platelet activation and aggregation. The discovery of this activity led to the consideration of aspirin as an anti-thrombotic agent.²⁶⁸ Aspirin is particularly important in this context due to its irreversible inhibition of the COX enzyme, which necessitates de novo synthesis of COX if enzyme activity is to be resumed. This re-generation of COX enzymes may be accomplished in normal cells within a few hours.²⁷⁴ However, mature platelets are cell fragments and do not contain a nucleus; re-generation of COX within platelets therefore cannot take place.²⁷⁴ Also, platelets are found in the portal circulation and are inhibited by aspirin before the drug, which has a half-life of approximately twenty minutes, undergoes first-pass metabolism.^{274, 276} For these reasons, regular doses of aspirin as low as 30mg per day (in contrast with the analgesic dose of 300-1,000mg every four to six hours) have been found to block platelet aggregation in clinical trials.²⁷¹ While a single dose of 30mg would be unlikely to inhibit platelet aggregation, the effect is cumulative with repeated doses of the drug as it takes approximately eight to twelve days for the functional platelet pool to be replaced following COX-1 inhibition.²⁷¹

4.3. Current use of aspirin

Aspirin, in its analgesic use, may be recommended for the relief of short-term mild to moderate pain.⁸⁰ Adult doses are between 300mg – 900mg every four to six hours, with a maximum daily dose of 4g; doses are advised to be taken with food in order to minimise gastric irritation.⁸⁰ Aspirin may also be prescribed for anti-inflammatory use, e.g. in rheumatoid arthritis, in divided doses up to a maximum of 4g daily. As aspirin is perceived to pose a relatively high gastrointestinal bleeding risk, particularly at high doses and in the elderly, this use has largely been superseded by other NSAIDs.^{80, 271, 277}

Aspirin administered for anti-thrombotic purposes (i.e. low-dose aspirin) is currently recommended in those who have experienced a cardiovascular event, or in patients at high cardiovascular risk.²⁷⁸ Aspirin has been confirmed as beneficial in the prevention of recurrent cardiovascular events also and may reduce the incidence of primary cardiovascular events.^{268, 278, 279} In the context of an acute cardiac event (unstable angina and myocardial infarction), aspirin is administered for immediate use (chewed or dispersed in water) at a dose of 300mg.⁸⁰ Immediately following coronary bypass surgery, aspirin may be given in a dose of 75-300mg. For long-term prevention against recurrent or primary cardiovascular events, aspirin is commonly used at a dose of 75mg daily.⁸⁰

4.4. Safety of aspirin

Aspirin poses serious bleeding risks as it acts to both inhibit platelet aggregation (and therefore prolongs bleeding time) and has the effect of inhibiting prostaglandins which serve to protect the gastric mucosa from damage; a single 300mg aspirin dose approximately doubles an average person's mean bleeding time for 5-7 days.²⁷¹ Even low-dose aspirin can increase the incidence of serious bleeds, including haemorrhagic stroke.²⁸⁰ The most recent results of the Women's Health Study clinical trial reaffirm the risks of non-fatal gastrointestinal bleeding and peptic ulcer with regular 100mg aspirin (HR=1.14, 95% CI 1.06-1.22, and HR=1.17, 95% CI 1.09-1.27, respectively).²⁸¹

Measures to reduce the risk of gastric injury and bleeding due to aspirin include contra-indication of the drug in patients with a history of active gastric ulceration or a bleeding disorder,²⁷¹ co-administration of drugs such as proton pump inhibitors (to reduce gastric acidity)⁸⁰ and the use of enteric-coated aspirin formulations, though there is little evidence to suggest a clinical benefit from this approach.^{282, 283} In addition to bleeding risks, some patients may experience severe allergic reactions to aspirin including the induction of asthma; the prevalence of aspirin intolerance has been estimated at under 0.3-0.9% of the population.²⁸⁴

Selective COX-2 inhibitors pose a lower risk of gastrointestinal bleeding than aspirin²⁸⁵ but have been shown in some cases to carry a significant higher risk of cardiovascular events compared to placebo.^{286, 287} It is also increasingly recognised that use of any NSAID can, to varying degrees, alter cardiovascular risk.⁸⁰ Due to these revelations, it is recommended that the lowest effective dose of NSAID be prescribed for the shortest period of time necessary to control symptoms and that the need for long-term treatment be regularly reviewed.⁸⁰

4.5. Aspirin prescribing trends in Ireland

In Ireland, aspirin is available for purchase in general retail outlets in packs containing a maximum of 24 oral dosage units; aspirin may also be purchased 'over-the-counter' in pharmacies in packs containing a maximum of 50 dosage units.²⁸⁸ However, formulations of aspirin available under these conditions contain aspirin doses in excess of 200mg per dosage unit; 'low-dose' aspirin intended for anti-thrombotic use, that is, aspirin in formulations that contain less than 200mg per dosage unit, is only available on prescription.

Aspirin is the most commonly prescribed product on the GMS scheme; in 2011, the drug was dispensed in its anti-thrombotic formulation 2,647,698 times, representing 4.6% of all GMS items dispensed.⁷⁸ Age-specific prevalence estimates for receipt of aspirin in the period 2002-

2009 are presented in Figure 4.3. Aspirin exposure was particularly prevalent in the 65-74 age-group and was found to increase steadily from 55% in 2002 to 74% in 2009. Over the span of the eight years, between 20% and 30% of patients over the age of 75 received aspirin. Between 14% and 21% of patients aged 45-64 received the drug.

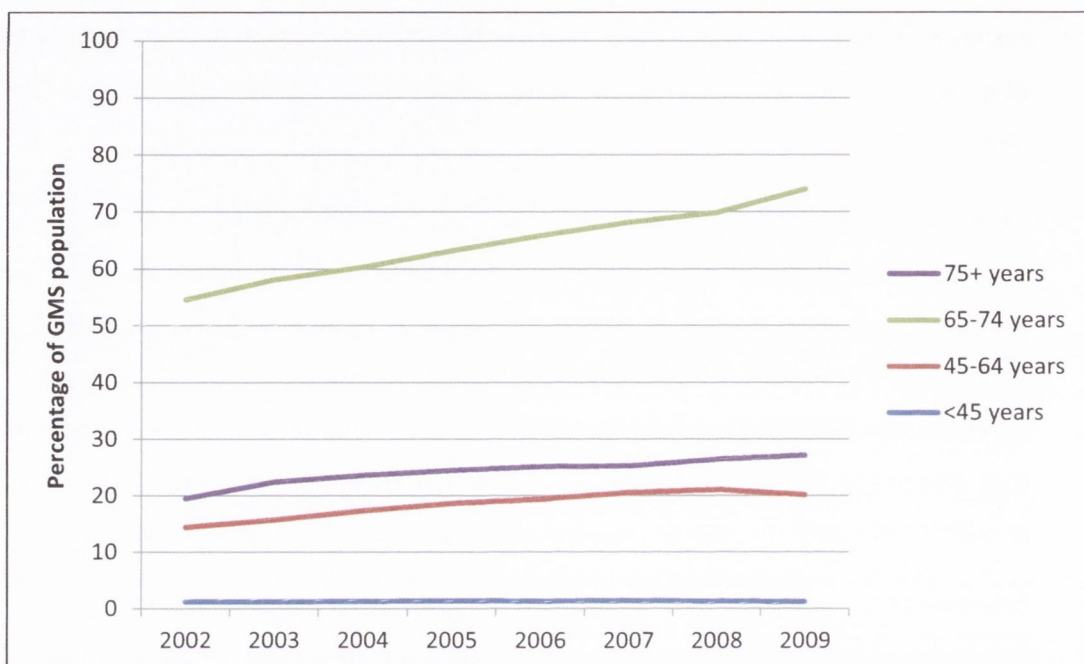


Figure 4.3: Age-specific prevalence of aspirin usage in the GMS population for the years 2002-2009.

GMS: General Medical Services scheme

4.6. Aspirin and Colorectal Cancer: History

Evidence reviewed within this chapter is focused on studies relating to effects of aspirin on colorectal cancer. Studies were retrieved by searching Medline® using the following MeSH terms: ("Aspirin"[MeSH] OR "Anti-Inflammatory Agents, Non-Steroidal"[MeSH]) AND "Colorectal Neoplasms"[MeSH].

The history of the investigation of aspirin as a potential chemopreventive agent or treatment for colorectal cancer has been discussed previously.^{289, 290} The initial evidence that aspirin might have an anti-cancer effect came from laboratory-based research performed in the 1970s, which suggested that aspirin might reduce tumour growth and metastasis due to anti-platelet effects (see section 4.8.1). Aspirin was studied shortly afterwards in a small

randomised clinical trial of sixty-six colorectal cancer patients; the drug was administered in 600mg doses twice daily as an adjuvant treatment but no effect was observed.²⁹¹ The first evidence of a possible association between aspirin and reduced incidence of colorectal cancer later came from a population-based case-control study in 1988, which found that patients with colorectal cancer were less likely to have used aspirin.²⁹² The authors stated the following in their discussion:

“This finding, whatever the mechanism may be, has potential significance in colorectal cancer chemoprevention and merits early confirmation.”

Observational studies investigating effects of aspirin in cancer prevention, together with analyses of randomised controlled trial data of aspirin in cardiovascular prevention, have formed the basis of systematic reviews and meta-analyses which have confirmed that aspirin reduces the long-term risk of colorectal cancer.²⁹³ Aspirin exposure has also been linked to reduced risk of metastasis in patients who develop colorectal cancer,²⁹⁴ and has been associated with improved colorectal cancer survival in some observational studies.²⁹⁵ These observational studies will be discussed in detail in sections 4.9-4.13.

4.7. Aspirin in colorectal cancer: preclinical evidence

Many studies have examined the ability of aspirin to prevent the development of colorectal cancer in cell culture and animal carcinogenesis models. In 1997, the WHO International Agency for Research on Cancer stated that there was sufficient evidence in experimental animal models to demonstrate the prevention of colon cancer by aspirin, though epidemiological studies were deemed as providing only limited evidence of an effect in humans at that time.²⁹⁶ However, in a review of some of the evidence of chemoprevention by aspirin in animal models, it was acknowledged that high doses were required for efficacy in some of the studies, which may affect the clinical relevance of these findings due to likely side effects at these doses.²⁹⁷

4.8. Proposed mechanisms of action of aspirin in cancer

The mode of action of aspirin in colorectal cancer is as yet not fully understood but is thought to involve the inhibition of platelets and/or inflammatory cytokines via blockade of the COX enzyme.²⁹⁵ Proposed mechanisms are informed by both laboratory studies and previous and on-going observational research, and are summarised below.

4.8.1. Effects mediated by COX-1: Platelet inhibition as anti-metastatic activity

The potential for an anti-cancer effect of aspirin acting through COX-1 has been investigated since the 1960s and 1970s, where laboratory studies were driven by the hypothesis that the formation of metastases is facilitated by platelet activity.²⁹⁸⁻³⁰⁰ At this time, aspirin was shown to reduce the development of experimentally-induced metastases in a mouse model.³⁰¹

The role of platelets in enabling metastasis is now increasingly recognised²⁹⁵ and many pro-metastatic actions of platelets have been identified.³⁰² Platelets are thought to aid in the cohesion of tumour cells being disseminated through the bloodstream, and may cloak tumour cells to prevent their elimination by the immune system.³⁰² They may also provide growth factors to tumour cells and aid in their attachment to epithelium.³⁰² In vitro studies of colon cancer cell lines have shown that thromboxane, a product of platelet COX-1 activation, stimulates tumour angiogenesis and growth in vivo, and the development of tumour metastasis,²⁷⁶ and platelet-derived thromboxane production has been enhanced within co-culture of platelets and colon cancer cells.²⁷⁶ Additional effects of platelets within tumours may include the stabilisation of tumour vasculature.³⁰²

As aspirin is capable of blocking the activation and aggregation of platelets through blockade of platelet COX-1, this may explain an anti-metastatic effect observed with aspirin exposure.²⁹⁴ In addition to actions occurring via platelet inhibition, inhibition of COX-1 by aspirin has been associated with reduced colon cancer endothelial tube formation (an in vitro model of angiogenesis).³⁰³

4.8.2. Effects mediated by COX-2: Inhibition of prostaglandin E2 and interruption of cell signalling pathways

The main mechanisms of action suggested to explain the effect of aspirin in preventing cancer relate to the action of the drug on the COX-2 enzyme.²⁷⁴ COX-2 is overexpressed in colorectal cancer; approximately 85% of colorectal tumours show upregulation of COX-2, in comparison to 20% of non-cancerous colorectal tissue samples.³⁰⁴ This over-expression is thought to be important in colorectal cancer development as it leads to an over-abundance of its product prostaglandin E2 (PGE2), the main prostaglandin produced by COX-2 in colorectal tissue.³⁰⁵ PGE2 has pleiotropic effects in colorectal cancer, being capable of enabling cell proliferation, survival, angiogenesis and invasion.³⁰⁴⁻³⁰⁶ The COX-2/PGE2 pathway was reviewed previously in relation to its effects on the six original 'hallmarks of cancer' defined by Hanahan and Weinberg in 2000.^{306, 307} In this review, the COX-2/PGE2 pathway was found to have a role in all

of these properties, that is, evasion of cell death, sustained proliferative signaling, insensitivity to growth suppressors, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis.³⁰⁶

Due to these biochemical effects of COX-2 expression in cancer, it may be expected that COX-2 over-expression in tumours affects clinical outcomes. This hypothesis was examined in a recent systematic review of observational study evidence regarding COX-2 and colorectal cancer prognosis.³⁰⁴ The authors of this review performed meta-analyses to examine associations between COX-2 expression and colorectal cancer prognosis. It was found that patients with tumours expressing COX-2 had a higher risk of tumour recurrence (HR=2.79, 95% CI 1.76-4.41) and poorer colorectal cancer-specific survival (HR=1.36, 95% CI 1.02-1.82) than patients with tumours not expressing COX-2.³⁰⁴ However, the authors suggested caution in interpreting these results due to the potential for publication bias.³⁰⁴ Also, as many of the studies included in the analyses did not adjust for stage or grade of tumour, it is not possible to conclude that COX-2 is an independent predictor of colorectal cancer-specific survival.³⁰⁴

The capacity to inhibit cancer development through COX-2 inhibition has mainly been studied using selective COX-2 inhibitors, but also with aspirin in some studies.³⁰⁵ COX-2 inhibitors have been found to decrease intestinal polyp formation and aspirin has been shown to suppress chemically-induced carcinogenesis in rat models with over-expression of COX-2.³⁰⁵ COX-2 selective inhibitors have also been shown to suppress the growth of human colorectal cancer cell xenografts in mouse models.³⁰⁵

4.8.3. COX-1 and COX-2-related mechanisms; impact of dose

Doses of aspirin required to inhibit COX-2 in cells are much larger than those necessary to inhibit COX-1 in platelets, and dosing must occur more frequently; a dosing schedule which will achieve sustained inhibition of COX-2 is likely to be in the range of 650mg of aspirin administered three to four times daily, in contrast to the 75mg once daily schedule which effectively inhibits platelet COX-1.²⁷⁴

Observational studies and randomised trials designed to study either cardiovascular outcomes or adenoma recurrence have found that low dose aspirin examined in this setting (75-100mg once daily) is associated with reduced colorectal cancer incidence with a maximal effect observed at these low doses.²⁷⁶ For reasons given above, it is not considered possible that such low doses could directly inhibit COX-2 in cancer cells.^{274, 276} Rather, these doses have been considered by some as indicative of a primary effect on platelet COX-1 activity and subsequent reduction of platelet activation by thromboxane.²⁷⁶ Recent hypotheses have, therefore,

suggested that this anti-platelet effect is primarily responsible for the anti-cancer action of aspirin.^{274, 276} Furthermore, these hypotheses propose that anti-platelet activity may result in a subsequent inhibition of COX-2 activity. Evidence for such an effect includes in vitro studies of colon cancer cells where platelets have been found to up-regulate COX-2 expression.^{308, 309} However, the most recent study on this topic did not observe inhibition of this effect by aspirin when platelets and colon cancer cells were co-cultured in vitro.³⁰⁸ Alternative evidence for a sequential effect of COX-1 and COX-2 activation includes findings that the deletion of COX-1 genes, as well as COX-2, is important in intestinal cancer development.^{310, 311}

In summary, it has been suggested that inhibition of platelet activation upstream, or COX-2 activity downstream, may interrupt the development of a colorectal tumour from normal tissue.²⁷⁴ Metastasis of a colorectal tumour via the bloodstream may also be interrupted by inhibition of platelet activation, as discussed in section 4.8.1.²⁷⁴

4.8.4. Other mechanisms of action

Effects of aspirin independent of actions on COX have also been demonstrated in colorectal cancer cell lines in vitro and in vivo. For example, aspirin has been found to inhibit signalling in the Wnt/ β -catenin pathway, which plays a crucial role in early colorectal carcinogenesis.³¹² Aspirin has also been found to alter the expression of DNA mismatch repair proteins, resulting in apoptosis.³¹³ Apoptosis may additionally be induced by aspirin through activation of the NF κ B signalling pathway, as seen with in vivo models of colorectal cancer.³¹⁴

Interestingly, aspirin has recently been found to inhibit mTOR signaling in colorectal cancer cells in vitro and in vivo.³¹⁵ This effect was demonstrated to occur via both AMPK-dependent and independent mechanisms.³¹⁵ Such properties have also been observed for metformin (section 3.2.3), and use of these two drugs together has shown a synergistic effect on mTOR signalling in vitro.³¹⁵ The effect of aspirin on mTOR has also been examined in a small, short-term clinical study of patients without cancer.³¹⁵ Three patients were administered 600mg aspirin once daily for seven days and S6 phosphorylation levels, a marker of mTOR inhibition, were measured in rectal tissue and found to be reduced.³¹⁵

While these proposed COX-independent mechanisms of action of aspirin suggest further effects that are worthy of investigation, some authors have suggested caution regarding the clinical relevance of these effects.^{274, 276} These authors suggest that the doses applied in the majority of these studies are too high to represent a meaningful effect in a clinical setting, and that more widely accepted mechanisms of action involving COX inhibition are more likely.^{274,}

4.9. Aspirin and colorectal cancer incidence: Observational studies

Observational evidence of associations between aspirin exposure and colorectal cancer incidence has largely arisen from two types of data sources:^{274, 316}

- Prospective cohorts such as the Nurses' Health Study and the Health Professionals Follow-up Study which were established to examine the long-term effects of various exposures on health outcomes.²⁹³
- Data from randomised clinical trials designed to examine the effect of aspirin exposure as a treatment or preventive agent in cardiovascular disease.³¹⁷

Many smaller cohort and case-control studies have also been carried out and have contributed to meta-analyses of the effects of aspirin on colorectal cancer.^{293, 318, 319}

Rothwell et al. have performed several secondary analyses of data from cardiovascular prevention trials.^{293, 317} Pooled data from twenty years of follow-up of five trials suggested that patients allocated to aspirin at any dose, versus no aspirin, had a 24% reduced risk of colon cancer over twenty years but no significant reduction in risk of rectal cancer.³¹⁷ Risk of colon cancer was also examined separately for the proximal (ascending) and distal colon and an effect was observed for reduced risk of proximal colon cancer (by 55%) but not distal cancer. These results are not expected to be biased by early detection of colorectal cancer among aspirin patients (potentially due to bleeding side effects) as there was no evidence of earlier cancer diagnosis in the aspirin group.³¹⁷ Of note, the decrease in colorectal cancer incidence associated with low-dose aspirin in this review was noticeable only after a latent period of greater than ten years of follow-up.³¹⁷ Recent results from Rothwell et al. found that allocation to low-dose aspirin was also associated with lower incidence of overall cancer from three years of follow-up onwards (OR=0.76, 95% CI 0.66-0.88).³²⁰

Systematic reviews of observational studies examining aspirin and colorectal cancer incidence and metastasis have also been performed by Rothwell et al.^{293, 318} Results of methodologically rigorous observational studies were found to be consistent with those obtained from secondary analysis of cardiovascular prevention trials. However, the authors stressed the importance of rigour in definition of drug exposure (e.g. dose and frequency of exposure) in future studies.³¹⁸ Other meta-analyses of observational studies have produced broadly consistent findings of reduced colorectal cancer incidence following long-term aspirin exposure.^{317, 319-322}

4.10. Aspirin and colorectal cancer incidence: Clinical trials of effects

The Women's Health Study, a randomised controlled trial commenced in 1993, was designed to examine the effects of 100mg alternate day aspirin exposure, versus placebo, on both cardiovascular disease and cancer incidence.²⁸¹ After ten years of data follow-up, no effect of low dose aspirin on colorectal cancer incidence was observed.³²³ However, after a median follow-up of eighteen years, aspirin exposure was recently found to be associated with lower incidence of colorectal cancer, particularly in proximal colon cancers (HR=0.73, 95% CI 0.55-0.95).²⁸¹ A potential explanation for this delayed effect is that it takes approximately ten years for an initial colorectal adenoma (the development of which aspirin is thought to prevent) to present clinically; a follow up time of 15 to 20 years after initial treatment may therefore be necessary to identify an effect of aspirin in patients.³²⁴

Randomised controlled trials have also been carried out to study the effects of aspirin on development of colorectal adenomas in patients with previous colorectal cancer.³²⁵⁻³²⁷ A meta-analysis of these trials found that aspirin at doses between 81 and 325mg per day was effective in reducing the risk of any colorectal adenoma by 17% over a median of 33 months.³²⁸ Other trials of aspirin have studied polyp development in patients with familial adenomatous polyposis (FAP) or hereditary non-polyposis colorectal cancer (Lynch syndrome) (section 1.1.4).^{16, 329, 330} Recent results from a trial of aspirin in patients with Lynch syndrome found that those patients who received aspirin for ≥ 2 years of treatment (versus placebo) had a significant 60% lower risk of colorectal cancer (HR=0.41, 95% CI 0.19-0.86).

4.11. Non-aspirin NSAIDs, selective COX-2 inhibitors and colorectal cancer incidence

Non-aspirin NSAIDs and selective COX-2 inhibitors have also been investigated for their effects on incidence and outcomes in colorectal cancer.^{328, 331} Selective COX-2 inhibitors have been studied in randomised controlled trials and found to reduce the risk of recurrent colorectal adenomas.^{332, 333} However, their use is associated with a significant increase in serious cardiovascular adverse effects which has led to the termination of such trials.³³³ The risks associated with general NSAIDs have also previously been considered too great to outweigh a potential benefit in routine use for primary prevention of colorectal cancer.^{334, 335} The US Preventive Services Task Force has recently published a draft research plan for an upcoming systematic review of the evidence regarding primary prevention of colorectal cancer with aspirin; this draft plan excludes the review of NSAIDs and selective COX-2 inhibitors due to safety concerns.³³⁶

4.12. Aspirin and colorectal cancer metastasis

Rothwell et al. have also examined the evidence regarding effects of aspirin on risk of cancer metastasis.^{294, 318} Analyses were performed on data from five large randomised controlled trials of aspirin versus placebo in cardiovascular prevention.²⁹⁴ Among patients who developed colorectal cancer, those who had been exposed to aspirin were found to be less likely to have metastatic disease (at presentation or follow-up) (OR=0.36, 95% CI 0.18-0.74).²⁹⁴ Also, patients with colorectal cancer that was not metastatic at diagnosis, and who were exposed to aspirin, were found to have a lower risk of later metastasis (HR=0.26, 95% CI 0.11-0.57).²⁹⁴

Rothwell and Algra also examined evidence from observational studies published before January 2011; this data on effects of aspirin on risk of cancer metastasis was compared with evidence from clinical trial data.³¹⁸ Two studies included in this review detailed the proportion of colorectal cancers with distant metastasis (at diagnosis or during follow-up) in regular aspirin users versus non-users. In both cases, non-significant reductions in the proportion of cancers with distant metastasis were observed for aspirin users versus non-users³¹⁸: OR=0.77 (95% CI 0.32-1.84);³³⁷ OR=0.79 (95% CI 0.43-1.48).³³⁸

One recent observational study specifically examined the effect of aspirin use prior to colorectal cancer diagnosis on metastatic status at diagnosis³³⁹ (as opposed to the studies reviewed by Rothwell et al., which included this data in stratified analyses).³¹⁸ This study found that aspirin use in the year prior to cancer diagnosis was associated with lower rates of tumour extent and metastatic disease for colorectal cancer. However, this analysis was not adjusted for important potential confounders such as access to screening, smoking status, or comorbidities; it is therefore difficult to draw conclusions from the results.³³⁹

4.13. Aspirin and colorectal cancer survival: Observational studies

As is the case with studies of aspirin and colorectal cancer incidence, a large component of the literature on associations between aspirin and colorectal cancer mortality comprises secondary analysis of clinical trials in cardiovascular prevention. Rothwell et al. performed meta-analyses on the results of twenty years of follow-up of randomised trials in order to determine the risk of mortality due to colorectal cancer.³¹⁷ Results for any dose of aspirin taken on a daily basis suggested a significant reduction in risk of death due to colorectal cancer (OR=0.64, 95% CI 0.49-0.83).³¹⁷ Data was pooled across four trials of aspirin versus placebo and analyses were performed stratified by duration of scheduled aspirin treatment. In these analyses, patients with a scheduled aspirin treatment duration of ≥ 2.5 years had a further reduced risk of mortality due to colorectal cancer (HR=0.54, 95% CI 0.36-0.80) and the greatest

reduction in mortality was observed in patients with ≥ 5 years of scheduled treatment (HR=0.48, 95% CI 0.30-0.77).³¹⁷ The Rothwell study examined the risk of fatal cancer among all patients enrolled in the clinical trials rather than solely among patients who were diagnosed with cancer. These results are therefore likely to reflect the effect of aspirin use on cancer mortality due to both reductions in colorectal cancer incidence and subsequent mortality reductions in patients who do develop colorectal cancer. The relative risk of case fatality in aspirin users versus non-users, i.e. the risk of mortality among patients who were diagnosed with colorectal cancer, was also reported by Rothwell et al. and suggested a borderline significant mortality reduction with aspirin use (RR=0.85, 95% CI 0.72-1.00).³¹⁷ When stratified by tumour site, no significant associations were observed, as may be expected due to lower statistical power. However, there was the suggestion of a potential benefit in proximal colon tumours (RR=0.76, 95% CI 0.49-1.17), and, to a diminished extent, in rectal tumours (RR=0.82, 95% CI 0.59-1.12).³¹⁷ No effect was observed in distal colon tumours.³¹⁷ Analyses of time to death after colorectal cancer diagnosis did not suggest an association with aspirin exposure.³¹⁷

A recent review²⁹⁵ cited a study by Fuchs et al., presented at the American Society of Clinical Oncology annual meeting 2005,³⁴⁰ as the first evidence of a potential role for aspirin in reducing mortality in established colorectal cancer. This study collected information on post-diagnostic aspirin use and was a nested analysis performed within a clinical trial designed to compare different chemotherapy regimens.³⁴⁰ Aspirin use was examined during adjuvant chemotherapy and six months after treatment but pre-diagnostic aspirin use was not detailed in the report. Consistent aspirin users (both midway and at six months post adjuvant treatment) were found to have a lower risk of the composite outcome of disease recurrence and/or death (HR=0.48, 85% CI 0.24-0.99).³⁴⁰ When these outcomes were examined separately, aspirin was significantly associated with lower colorectal cancer recurrence but a significantly lowered risk of death was not observed.

In the years since the study by Fuchs et al., numerous observational studies have been carried out to examine the influence of aspirin exposure on survival in patients with established colorectal cancer. These studies are listed along with their methodological details in Table 4.1. The main findings of the studies, including overall conclusions reached by the study authors, are detailed in Table 4.2. Results are listed according to (i) the influence of timing of aspirin exposure; (ii) the influence of aspirin dose or duration of use; (iii) the influence of tumour site. These findings are discussed further in the following section (4.13.1). As some studies additionally examined how survival following aspirin exposure differed in relation to the

presence or absence of certain biomarkers, these biomarker-specific findings are discussed in section 4.13.2.

4.13.1. Observational study findings: Effects of exposure timing, dosage and duration of treatment, and tumour site.

4.13.1.1. Timing of aspirin exposure

Several observational studies examined only the effect of pre-diagnostic aspirin exposure on colorectal cancer-specific survival (Table 4.2).³⁴¹⁻³⁴⁶ Zell et al. found a significant effect of regular pre-diagnostic aspirin use in a female cohort.³⁴² Coghill et al. performed a study using cancer registry data to investigate effects of both NSAID use and hormone therapy in women.³⁴⁴ This study had small numbers and did not observe an overall effect of pre-diagnostic aspirin on colorectal cancer death. Coghill, in a larger study of male and female patients, found improved survival in patients with pre-diagnostic aspirin use, including either current use at the time of diagnosis or former use.³⁴³ More recently, Coghill et al. examined data from the Women's Health Initiative cohort but did not observe an effect for pre-diagnostic aspirin use.³⁴⁵ Neither was an effect found by Din et al. for pre-diagnostic aspirin use among men and women.³⁴⁶

Among studies that examined the individual effects of pre and post-diagnostic aspirin exposure, varying results were observed. Bastiaannet et al. and Walker et al. both found a significant effect of aspirin in improving colorectal cancer survival for patients who had both pre and post-diagnostic aspirin exposure.^{347, 348} Bastiaannet et al. also observed a protective effect for only post-diagnostic use of aspirin, but this was not observed by Walker et al. Chan et al. additionally found a significant effect for aspirin in patients with only post-diagnostic aspirin use; no effect was observed in patients who also had pre-diagnostic aspirin use.³⁴⁹ A recent study examined a subset of elderly colon cancer patients from the data previously presented by Bastiaannet et al.³⁵⁰ Only post-diagnostic aspirin use was considered and a significant survival gain was observed.³⁵⁰ McCowan et al. entered both pre-diagnostic and post-diagnostic aspirin exposure terms into their model of colorectal cancer survival but only post-diagnostic aspirin was associated with a survival benefit.³⁵¹

It is difficult to explain observations where no significant survival benefit was found in patients with both pre-diagnostic and post-diagnostic aspirin exposure, e.g. the findings of Chan et al.³⁴⁹ Some authors have suggested that tumours involved in these cases may be resistant to the effects of aspirin; these authors suggest that tumours which develop under continuous aspirin exposure may show less susceptibility to the effects of the drug following clinical

presentation.^{295, 352} Caution is also advised in interpreting associations between post-diagnostic aspirin exposure and colorectal cancer survival as they may be subject to time-varying confounding, e.g. by disease progression, as discussed in section 5.4.2.³⁵³ Statistical methods which may minimise time-varying confounding, such as the use of marginal structural models, were not used in the studies of post-diagnostic exposure listed above.³⁵³

4.13.1.2. Dosage or duration of aspirin treatment

Few observational studies of aspirin and colorectal cancer survival have conducted analyses stratified by dose of aspirin. Bastiaannet et al. reported that the vast majority of patients (95%) received 80mg aspirin daily and observed a significant association between de novo post-diagnostic aspirin use and colorectal cancer survival.³⁴⁷ Din et al. examined only 75mg daily aspirin and did not observe an effect on cancer survival.³⁴⁶ Walker et al. stratified analyses according to aspirin dose; a significant effect was not observed with either high-dose or low-dose aspirin, for post-diagnostic use, but the hazard ratio for aspirin at doses ≤ 75 mg was closer to significance than that for high-dose aspirin (Table 4.2). Chan et al. also stratified analyses according to quantity of 325mg aspirin tablets consumed per week post-diagnosis.³⁴⁹ Patients who consumed 0.5-5 tablets per week had a significant reduction in mortality whereas no effect was seen in patients consuming greater than five aspirin tablets per week.³⁴⁹

Zell et al. stratified associations of regular aspirin use and colorectal cancer survival according to exposure for less than or greater than five years. A significant association between aspirin and survival was found only for exposure with a duration ≥ 5 years (Table 4.2).

4.13.1.3. Tumour Site

Seven of the listed observational studies performed analyses with stratification by tumour site (Table 4.2). Two studies did not identify a difference in results according to tumour site^{348, 349} while the remainder noted a greater benefit in proximal colon (or colon overall) versus the distal colon or rectal cancer^{342-344, 347, 351}.

A recent review discussed this pattern,²⁹⁵ noting the greater effect of aspirin in proximal colon tumours which was observed in two of these studies.^{343, 347} Several explanations for this effect were proposed, including potential confounding by availability of access to treatment advances for rectal cancer, and phenotypic differences in proximal versus distal tumours resulting in differential response to aspirin.²⁹⁵ Current opinion suggests that a gradient exists in the prevalence of different genetic phenotypes from the ascending colon to the rectum, which would provide a rationale for different effects of aspirin at different sites.³⁵⁴

Aspirin has previously been associated with differential effects on colorectal polyp or cancer incidence depending on location within the lower gastrointestinal tract.^{317, 355} In a clinical trial of polyp prevention with aspirin, greater reductions in polyp incidence were observed for effects of aspirin on proximal colon polyps than for the distal colon.³⁵⁵ Secondary analysis of randomised trial data has echoed these findings.³¹⁷

4.13.2. Observational study findings: Influence of biomarkers

Researchers who have published observational studies using the Nurses' Health Study and the Health Professionals Follow-Up Study cohort data have recently had the opportunity to stratify their studies according to the presence or absence of certain molecular characteristics in the tumours of patients.³⁵⁶ Some of the information to which these researchers have access include whether or not tumours recorded in the dataset have over-expressed COX-2, or whether they contain mutations of certain genes involved in tumour growth.

As discussed in section 4.9, Chan et al. examined the influence of aspirin on colorectal cancer incidence and observed that aspirin use is associated with a lower risk of COX-2 positive colorectal tumours, but not COX-2 negative tumours.³⁵⁷ As detailed in Table 4.1 and Table 4.2, the same research group later examined colorectal cancer-specific survival following aspirin exposure; tumour COX-2 expression levels had been identified using immunohistochemistry and were incorporated as a stratifying variable in the analysis.³⁴⁹ In the non-stratified analysis, a significant survival benefit was observed for post-diagnostic aspirin users versus non-users (HR=0.71, 95% CI 0.53-0.95). When stratified by COX-2 expression status, patients with COX-2 positive tumours had a greater reduction in mortality (HR=0.39, 95% 0.20-0.76) while no survival benefit was observed among COX-2 negative tumours.

PIK3CA (a subunit of the enzyme PI3K) and BRAF are components of signalling pathways which are activated upon stimulation of the cell by growth factors, and which are involved in the activation of mTOR, a protein which plays a crucial role in tumour cell proliferation.³⁵⁸ These signalling components commonly exist in mutated forms in colorectal cancer.³⁵⁹ The potential effects of the presence of these mutations on associations between aspirin exposure and survival have been investigated recently, as follows.^{359, 360}

Liao et al. examined colorectal cancer survival following post-diagnostic aspirin exposure in patients according to PIK3CA mutation status.³⁶¹ PIK3CA is one of the most commonly mutated genes in colorectal cancer and is found in 10-20% of colorectal tumours.³⁶⁰ Among patients with mutated PIK3CA, aspirin exposure (pre and post-diagnostic, versus no post-diagnosis use) was associated with an enhanced improvement in survival (HR=0.18, 95% CI 0.04-0.92). No

survival improvement was observed for patients who had a non-mutated PIK3CA tumour profile.

Nishihara et al. most recently examined aspirin use and risk of colorectal cancer according to BRAF mutation status, as reported in section 4.9.³⁶² BRAF is mutated in 15% of colorectal cancers.³⁵⁹ Patients exposed to aspirin had a 27% lower risk of being diagnosed with a tumour that did not contain mutated BRAF. However, patients exposed to aspirin did not have a lower risk of developing a tumour with mutated BRAF. In subsequent analyses of survival, use of aspirin did not alter survival outcomes for patients with either BRAF-mutated or non-mutated tumours.³⁶²

The authors of these studies have acknowledged that their results require replication.³⁶² However, if aspirin is proven to be more effective in patients with certain tumour molecular profiles, the authors suggest that their findings may influence how aspirin might be given as a future treatment in colorectal cancer.³⁶¹ There is evidence of cellular cross-talk between the signalling pathways which include PIK3CA and BRAF, and evidence also of connection of these pathways to COX-2 expression.^{361, 362} Therefore, if the findings of these groups stand up to testing, these results may provide valuable insights regarding the mechanism of action of aspirin and which patients may derive benefit from the drug.³⁶³

Table 4.1: Details of methodology of observational studies examining associations between aspirin exposure and colorectal cancer survival.

Study		Methodology				
First Author, Year, Country, Journal	Study type, Data Source	Inclusion/Exclusion Criteria	Exposure and Comparator	Analysis details	Outcome	Confounders in Model
Nishihara R 2013 USA <i>JAMA</i> 362	Cohort study Nurses' Health Study, Health Professionals Follow-up Study	Included CRC diagnosed pre July 2006 with available tumour tissue data, aspirin use information and survival data.	(Biennial patient-reported aspirin use) Exposure: Regular use of aspirin during most weeks post diagnosis. Comparator: Non-use of aspirin during most weeks.	Multivariate Cox proportional hazards model.	CRC-specific survival	Sex, age, year of diagnosis, time from diagnosis to first measurement of aspirin after diagnosis, regular use or nonuse of aspirin before diagnosis, tumour location, tumour differentiation, BMI, various tumour genetic status covariates (e.g. MSI, BRAF, KRAS).
McCowan C 2013 UK <i>Eur J Cancer</i> 351	Cohort study Linked cancer registry and prescription records.	Included CRC diagnosed 1997-2006 where patients had post-diagnostic aspirin use. Excluded previous CRC or diagnosed at death.	Aspirin use post-diagnosis, or pre-and-post diagnosis, versus no aspirin use.	Multivariate Cox proportional hazards model.	CRC-specific survival	Sex, age, stage at diagnosis, socioeconomic status, aspirin use pre diagnosis.
Reimers MS 2012 Netherlands <i>J Am Geriatr Soc</i> 350	Cohort study Eindhoven Cancer Registry linked to PHARMO prescription database	Included Colon cancer diagnosed 1998-2007 where patients were ≥70 years. Excluded pre-diagnostic aspirin users.	Exposure: At least one Rx for low-dose aspirin (80mg) for at least 14 days. Post-diagnostic use only. Comparator: 'never' aspirin user (Subgroup analysis of Bastiaannet et al. 2012)	Time-dependent multivariate Poisson regression survival analysis. Participants defined as users from first use to end of follow-up.	Overall survival	Sex, age, stage, adjuvant chemotherapy, comorbidity, surgery, grade, tumour location, year of diagnosis. Stratification by comorbidity, chemotherapy, grade, stage, surgery, tumour location.

Table 4.1 (continued)

Study		Methodology				
First Author, Year, Country, Journal	Study type, Data Source	Inclusion/Exclusion Criteria	Exposure and Comparator	Analysis details	Outcome	Confounders in Model
Bastiaannet E 2012 Netherlands <i>Br J Cancer</i> 347	Cohort study Eindhoven Cancer Registry linked to PHARMO prescription database	Included CRC diagnosed 1998-2007. Excluded if prior CRC or no follow-up information.	Exposure: Aspirin or NSAID use for ≥ 14 days. Frequent user: ≥ 3 Rx. Comparator: 'never' aspirin/NSAID user. <u>Aspirin groups</u> Post-diagnosis Frequent post-diagnosis Pre & post-diagnosis	Time-dependent multivariate Poisson regression survival analysis. (Non time-varying for pre-and-post diagnosis analysis)	Overall survival	Sex, age, stage, adjuvant chemotherapy, radiotherapy, comorbidity, surgery, grade, year of diagnosis. Stratification by tumour site.
Coghill AE 2012 USA <i>Cancer Epidemiol Biomarkers Prev</i> 345	Cohort study Women's Health Initiative cohort	Included postmenopausal women aged 50-79. Excluded if prior CRC or no follow-up information.	(Patient-reported aspirin use) Exposure: Current use at baseline. Continued use (baseline use and use ~ 3 years after baseline). Comparator: Non-use (no use at baseline or at ~ 3 years after baseline).	Multivariate Cox proportional hazards model.	CRC-specific survival	Age, BMI, smoking history, duration of smoking, history of diabetes/CV disease/ulcerative colitis, colonoscopy history, family history CRC.
Liao X 2012 USA <i>N Engl J Med</i> 361	Cohort study Nurses' Health Study, Health Professionals Follow-up Study	Included CRC diagnosed pre July 2006 where PIK3CA mutation data was available.	(Biennial patient-reported aspirin use) Exposure: Regular use of aspirin during most weeks post diagnosis. Comparator: Non-use of aspirin during most weeks.	Multivariate Cox proportional hazards model.	CRC-specific survival	Sex, age, year of diagnosis, time from diagnosis to first measurement of aspirin after diagnosis, regular use or nonuse of aspirin before diagnosis, tumour location, tumour differentiation, BMI, various tumour genetic status covariates (e.g. MSI, BRAF, KRAS).

Table 4.1 (continued)

Study		Methodology				
First Author, Year, Country, Journal	Study type, Data Source	Inclusion/Exclusion Criteria	Exposure and Comparator	Analysis details	Outcome	Confounders in Model
Walker AJ 2012 UK <i>Br J Cancer</i> 348	Cohort study UK CPRD (Clinical Practice Research Database)	Included CRC diagnosed at least 1 year after database entry (post 1987). Excluded if had previous CRC, <1 year of data history, or if deceased in year post diagnosis.	Exposure: ≥ 2 Rxs in year post diagnosis. Pre-diagnostic use examined as an effect modifier. Comparator: No aspirin use.	Multivariate Cox proportional hazards model.	Overall survival	A priori covariate selection: sex, age, comorbidity, smoking status.
Coghill AE 2011 USA <i>Gut</i> 343	Cohort study Interviews of SEER database participants (cancer registry data)	Included CRC cases aged 20-74 diagnosed 1997-2002. Excluded if deceased prior to interview, withdrawn from study or lost to follow-up prior to interview.	Exposure: See 'Aspirin groups' below. Comparator: Never use <u>Aspirin groups</u> 'Ever use': regular use (at least twice per week for ≥ 1 month) at any point prior to the 2 years pre CRC diagnosis. 'Current use': 'ever use' at the time of diagnosis. 'Former use': 'ever use' >2 years before diagnosis. 'Never user': no use/irregular use.	Multivariate Cox proportional hazards model.	CRC-specific survival.	Sex, age, BMI, smoking status, history of diabetes, prior inflammatory conditions, receipt of preventive colorectal screening, first-course treatment, stage of disease at diagnosis.

Table 4.1 (continued)

Study		Methodology				
First Author, Year, Country <i>Journal</i>	Study type, Data Source	Inclusion/Exclusion Criteria	Exposure and Comparator	Analysis details	Outcome	Confounders in Model
Coghill AE 2011 USA <i>Br J Cancer</i> 344	Cohort study Interviews of SEER database participants (cancer registry data)	Included female Caucasian CRC cases aged 20-74 diagnosed 1997-2002. Excluded if deceased prior to interview, withdrawn from study or lost to follow-up prior to interview.	<u>Aspirin groups</u> ‘Ever use’: regular use (at least twice per week for ≥1 month) at any point prior to the 2 years pre CRC diagnosis. ‘Current use’: ‘ever use’ at the time of diagnosis. ‘Former use’: ‘ever use’ >2 years before diagnosis. ‘Never user’: no use/irregular use.	Multivariate Cox proportional hazards model.	CRC-specific survival.	Sex, age, BMI, smoking status, family history, stage at diagnosis, history of preventive screening.
Din FVN 2010 UK <i>Gut</i> 346	Case-control Prospectively recruited population-based study, Scotland.	Included incident CRC cases aged 16-79 presenting to surgical units. Excluded due to death prior to ascertainment, or inability to complete questionnaires.	Patient-reported aspirin intake and number of months and days per week exposure. Pre-diagnostic exposure only.	Multivariate Cox proportional hazards model.	Overall and CRC-specific survival.	Sex, age, family history risk, stage at diagnosis.
Zell JA 2010 USA <i>Cancer Prev Res</i> 341	Cohort study California Teachers Study prospective cohort (female only)	Included incident invasive CRC. Excluded if other cancer present, lack of family history data, lack of NSAID use data, lack of meat dietary data.	Patient-reported NSAID (including aspirin) use including days per week and duration of use in years. Pre-diagnostic exposure only. Regular use: 1-3 days per week, 4-6 days per week, or daily.	Multivariate Cox proportional hazards model.	CRC-specific survival.	NSAID frequency/duration, age, stage at diagnosis, site, CRC family history, surgical treatment, meat consumption energy intake.

Table 4.1 (continued)

Study		Methodology				
First Author, Year, Country, Journal	Study type, Data Source	Inclusion/Exclusion Criteria	Exposure and Comparator	Analysis details	Outcome	Confounders in Model
Chan AT 2009 USA JAMA <small>349</small>	Cohort study Nurses' Health Study, Health Professionals Follow-up Study	Included confirmed stage I-III CRC diagnosed by end 2002 where aspirin use information was provided for the pre and post diagnostic periods. Excluded if stage IV CRC or prior cancer.	(Biennial patient-reported aspirin use) Aspirin use defined as regular use of aspirin during most weeks. Pre and post-diagnostic exposure assessed. Comparator: non-use of aspirin during most weeks.	Multivariate Cox proportional hazards model.	CRC-specific survival.	Sex, age, date of diagnosis of cancer, stage at diagnosis, site of cancer, histological grade, time from diagnosis to first measurement of post-diagnosis aspirin use, smoking, exercise level, BMI, family history.
Zell JA 2009 USA Cancer <small>342</small>	Cohort study California Teachers Study Prospective Cohort (female only)	Included incident invasive CRC. Excluded if other cancer present, lack of family history data, lack of NSAID use data.	Patient-reported NSAID (including aspirin) use including days per week and duration of use in years. Pre-diagnostic exposure only. Regular use: 1-3 days per week, 4-6 days per week, or daily.	Multivariate Cox proportional hazards model.	CRC-specific survival.	NSAID/aspirin-only use, age, stage at diagnosis, site, CRC family history, time from NSAID assessment to CRC diagnosis, treatment with surgery.

CRC: colorectal cancer

Table 4.2: Details of results of observational studies examining associations between aspirin exposure and colorectal cancer survival.

Study First Author, Year, Country Journal	Summary of results of aspirin exposure ^a			Authors' main conclusions
	Influence of exposure timing	Influence of dosage/duration of use	Influence of tumour location	
Nishihara R 2013 USA <i>JAMA</i> 362	(Results listed according to BRAF gene mutation status) Post-diagnostic exposure only assessed. Wild-type BRAF: HR: 0.77 (0.57-1.05) Mutant BRAF: HR: 0.72 (0.27-1.92)	n/a	n/a	Conclusions related primarily to effects on cancer incidence (data not shown in this table). No significant interaction was observed between post-diagnostic aspirin use and BRAF mutation status in cancer-specific survival.
McCowan C 2013 UK <i>Eur J Cancer</i> 351	Pre-diagnostic (versus no aspirin pre diagnosis) and post-diagnostic aspirin use (versus no post-diagnostic aspirin use) individually included in model. Pre-diagnostic: HR: 0.96 (0.84-1.11) Post-diagnosis: HR: 0.58 (0.45-0.75)	Dose not specified. Number of years of aspirin treatment entered into model; significant association observed suggests each additional year of therapy found to decrease risk of mortality.	When separate models were run, significant effects for <u>all-cause</u> mortality were observed for post-diagnostic aspirin in colon cancer but not in rectal or anal cancers. Colon: HR: 0.72 (0.57-0.91) Rectosigmoid: HR: 0.52 (0.36-0.74) Rectum: HR: 0.80 (0.58-1.11) Anal: HR: 0.66 (0.17-2.57)	Significant survival benefit observed in colon cancer patients receiving post-diagnostic aspirin exposure.
Reimers MS 2012 Netherlands <i>J Am Geriatr Soc</i> 350	Examined patients with only post-diagnostic exposure. Significant association observed. RR: 0.59 (0.44-0.81)	n/a	Examined only colon cancer. Significant association observed. RR: 0.59 (0.44-0.81)	Significant survival benefit observed in older colon cancer patients receiving post-diagnostic aspirin exposure.

Table 4.2 (continued)

Study		Summary of results of aspirin exposure ^a		
First Author, Year, Country Journal	Influence of exposure timing	Influence of dosage/duration of use	Influence of tumour location	Authors' main conclusions
Bastiaannet E 2012 Netherlands <i>Br J Cancer</i> 347	Pre and post-diagnosis: significant association observed. RR: 0.88 (0.83-0.94) Post-diagnosis only: significant association observed, higher in magnitude. RR: 0.77 (0.63-0.95)	95% patients used 80mg aspirin per day. Point estimate for 'frequent users' (≥3 Rxs post-diagnostic aspirin) suggests higher magnitude of survival benefit: RR: 0.70 (0.57-0.88)	Colorectal cancer: Significant association for post-diagnostic exposure. When stratified by site, effect only observed in colon cancer: Pre and post-diagnosis: significant association observed. RR: 0.89 (0.82-0.96) Post-diagnosis only: significant association observed, higher in magnitude. RR: 0.65 (0.50-0.84)	Significant survival benefit observed in colon cancer patients receiving post-diagnostic or pre-and-post diagnostic aspirin exposure.
Coghill AE 2012 USA <i>Cancer Epidemiol Biomarkers Prev</i> 345	Pre-diagnostic exposure only assessed. HR: 1.04 (0.80-1.35)	Amount of use of aspirin did not affect CRC case fatality estimates. Results for time to CRC mortality (measured from baseline questionnaire to end of follow-up post CRC diagnosis) found a marginal survival benefit following 6+ years of aspirin exposure.	n/a	Potential benefit observed with prolonged NSAID use in postmenopausal women.

Table 4.2 (continued)

Study	Summary of results of aspirin exposure ^a			Authors' main conclusions
First Author, Year, Country Journal	Influence of exposure timing	Influence of dosage/duration of use	Influence of tumour location	Authors' main conclusions
<p>Liao X 2012 USA <i>N Engl J Med</i> 361</p>	<p>(Results listed according to PIK3CA gene mutation status)</p> <p>Wild-type PIK3CA: Pre and post-diagnosis use versus no post-diagnosis use: HR: 0.92 (0.56-1.51)</p> <p>Only post-diagnosis use versus no post-diagnosis use: HR: 0.90 (0.53-1.54)</p> <p>Mutant PIK3CA: Pre and post-diagnosis use versus no post-diagnosis use: HR: 0.18 (0.04-0.92)</p> <p>Only post-diagnosis use versus no post-diagnosis use: HR: 0.28 (0.04-2.10)</p>	<p>Only examined 'regular' post-diagnostic aspirin use ('most weeks').</p> <p>n/a</p>	<p>n/a</p>	<p>Significant survival benefit observed in mutated-PIK3CA colorectal cancer but not wild-type PIK3CA cancer.</p>
<p>Walker AJ 2012 UK <i>Br J Cancer</i> 348</p>	<p>Pre-diagnostic only: HR: 1.04 (0.97-1.12)</p> <p>Pre and post-diagnosis: HR: 0.86 (0.76-0.98)</p> <p>Post-diagnosis only: HR: 0.99 (0.84-1.16)</p> <p>Survival improvement was observed for up to 5 years of exposure post diagnosis, but no later.</p>	<p>(Post-diagnostic use)</p> <p>≤75mg: HR: 0.94 (0.86-1.02)</p> <p>>75mg: HR: 1.13 (0.97-1.32)</p>	<p>Effects of post-diagnostic aspirin exposure did not differ substantially between colon and rectal cancer.</p> <p>Colon: HR: 0.89 (0.49-1.01)</p> <p>Rectal: 0.92 (0.77-1.09)</p>	<p>Significant survival benefit with aspirin exposure in first 5 years post diagnosis.</p>

Table 4.2 (continued)

Study		Summary of results of aspirin exposure ^a			Authors' main conclusions
First Author, Year, Country, Journal	Influence of exposure timing	Influence of dosage/duration of use	Influence of tumour location		
Coghill AE 2011 USA <i>Gut</i> 343	Pre-diagnostic exposure only assessed. Significant association observed. Aspirin Ever use: HR: 0.74 (0.60-0.92) Current use: HR: 0.77 (0.59-1.00) Former use: HR: 0.75 (0.56-1.00)	Average daily dose was not associated with colorectal cancer survival. Duration of regular use (months-years expressed as quartiles). (Below estimates for NSAIDs overall, not aspirin alone) <6months: HR: 0.82 (0.58-1.15) 6months-2.5years: HR: 0.72 (0.53-0.97) 2.5-7 years: HR: 0.70 (0.49-0.99) >7 years: HR: 0.83 (0.59-1.16)	<u>Proximal colon:</u> Current use: HR: 0.58 (0.38-0.96) Former use: HR: 0.46 (0.25-0.86) <u>Distal/rectal disease:</u> Current use: HR: 0.97 (0.63-1.50) Former use: HR: 1.11 (0.70-1.75)	Significant survival benefit with regular use of NSAIDs prior to diagnosis, particularly among cases diagnosed with proximal disease and in longer term NSAID users.	
Coghill AE 2011 USA <i>Br J Cancer</i> 344	Pre-diagnostic exposure only assessed. HR: 1.14 (0.77-1.69)	n/a	<u>Proximal colon:</u> HR: 0.75 (0.41-1.37) <u>Distal/rectal disease:</u> HR: 1.64 (0.96-2.78)	Overall NSAID use (aspirin & ibuprofen) was associated with a significant survival benefit among women with proximal colon tumours.	
Din FVN 2010 UK <i>Gut</i> 346	Pre-diagnostic exposure only assessed. (CRC-specific survival) HR: 1.03 (0.80-1.31)	75mg dose only assessed.	n/a	Aspirin use prior to CRC diagnosis was not found to influence survival.	

Table 4.2 (continued)

Study First Author, Year, Country Journal	Summary of results of aspirin exposure ^a			Authors' main conclusions
	Influence of exposure timing	Influence of dosage/duration of use	Influence of tumour location	
Zell JA 2010 USA <i>Cancer Prev Res</i> 341	Pre-diagnostic exposure only assessed. Point estimates for overall NSAID-use (not aspirin-specific) according to meat-consumption category: Within low meat consumption tertile: HR: 0.22 (0.06-0.82) Within medium meat consumption tertile: HR: 0.81 (0.22-3.00) Within high meat consumption tertile: HR: 1.08 (0.36-3.24)	n/a	n/a	Regular NSAID use (including aspirin use) was significantly associated with decreased CRC-specific mortality among patients in the lowest meat consumption tertile but not among patients in higher meat intake tertiles.
Chan AT 2009 USA <i>JAMA</i> 349	Adjustment for pre-diagnostic use: HR: 0.71 (0.53-0.95) Post-diagnostic use only: HR: 0.53 (0.33-0.86) Pre and post-diagnosis: HR: 0.89 (0.59-1.35)	Dose of aspirin used after CRC diagnosis: 0.5-5 aspirin 325 mg tablets per week: HR: 0.57 (0.32-0.99) ≥ 6 aspirin 325 mg tablets per week: HR: 0.49 (0.18-1.35)	Results not found to be affected when stratified by site (<i>P</i> for interaction = 0.56). (Individual hazard ratios not reported)	Regular aspirin use after the diagnosis of CRC is associated with lower risk of CRC-specific and overall mortality.
Zell JA 2009 USA <i>Cancer</i> 342	Pre-diagnostic exposure only assessed. Regular aspirin use (daily, 4-6 times per week, or 1-3 times/week): HR: 0.62 (0.41-0.94)	Regular aspirin use (daily, 4-6 times per week, or 1-3 times/week): < 5 years exposure: HR: 1.03 (0.62-1.72) ≥ 5 years exposure: HR: 0.33 (0.18-0.63)	Analyses restricted to (i) colon and (ii) rectal site tumours were performed for overall NSAID use. Significant results were observed for colon but not rectal tumours.	Regular NSAID use over a prolonged duration prior to CRC diagnosis was associated with decreased mortality among female CRC cases.

CRC: colorectal cancer. (a): Results are for aspirin exposure relative to no aspirin, unless otherwise specified.

4.14. Summary: Current opinion regarding the potential utility of aspirin in cancer

As outlined in section 4.10, clinical trials of patients at high risk of colorectal cancer have shown that aspirin reduces colorectal adenoma development ²⁹⁵ and it has recently been claimed that the evidence is now sufficient to recommend aspirin in those at high risk of colorectal cancer, particularly patients with Lynch Syndrome.³⁶⁴ Regarding patients who are not predisposed to colorectal cancer, observational studies and secondary analyses of randomised clinical trial data have consistently demonstrated that regular aspirin use is associated with lower colorectal cancer incidence. However, aspirin has not yet been recommended for use in the primary prevention of colorectal cancer among patients at low or moderate risk of the disease.^{317, 320}

The concept of using aspirin as an adjuvant treatment in established colorectal cancer is relatively novel; while the long-standing debate surrounding aspirin's utility has focused primarily on chemoprevention, the emerging observational studies, coinciding with studies by Rothwell et al., have prompted the consideration of aspirin as an adjunct cancer treatment.²⁹⁵ Analyses of cardiovascular prevention randomised trial results and observational studies have suggested that aspirin may improve survival among patients with colorectal cancer. However, evidence has been conflicting in some cases and there is little information on the dosage and/or intensity of aspirin required to demonstrate a possible survival benefit.

5. Patients and methods

This chapter will first describe the data which was used in the studies presented in this thesis. The processing of this data to prepare it for analysis and the methods used to analyse it, including approaches to minimise bias and confounding, will then be discussed.

5.1. Data Collection: Setting & Data Sources

All of the data analysed within this thesis was provided by the National Cancer Registry Ireland (NCRI) and comprised individual cancer patient records which have been linked to prescription dispensing data from Ireland's Health Services Executive (HSE) – Primary Care Reimbursement Services (PCRS) pharmacy claims database.

5.1.1. Patient and Cancer Data: NCRI

The NCRI was established as a statutory body in 1991 and commenced registering all incident cancers for the whole population usually resident in the Republic of Ireland in January 1994. Cases are actively ascertained and followed up by qualified tumour registration officers employed by the registry and based in hospitals throughout the country. Notification of cases is primarily by pathology report but also occurs via listing of cancer patients through the 'Hospital Inpatient Enquiry (HIPE) system', a computer-based system which details demographic, clinical and administrative data on discharges and deaths from acute hospitals; ³⁶⁵ notification may also occur following receipt of death certificates.³⁶⁶ Comprehensive details on all incident cancers are collected through multiple sources of information including pathology and radiology reports, and treatment records. Death certificates are supplied to the NCRI by Ireland's Central Statistics Office (CSO) following publication of the CSO's quarterly report. Cancer death certificates are matched against NCRI records and unmatched cases are followed up. For NCRI cases, deaths due to cancer have been recorded in the registry since the beginning of 1994; deaths due to any cause (cancer or otherwise) have been recorded for all cases registered as having cancer since this time.

Data quality at the NCRI has recently been reviewed by examining the comparability, completeness and validity of the data.³⁶⁶ Comparability has been achieved through the use of international data classification systems including ICD-10 codes and AJCC TNM tumour classification,¹⁹ and adherence to data assignment recommendations of the European Network of Cancer Registries. For all cancers combined (excluding non-melanoma skin cancer), data

completeness was 100% for name, gender and date of incidence of cancer cases; completeness for date of birth and date of death were 98.9% and 99.8%, respectively. Validity of the data was estimated by examining the percentage of cases for which tumour morphology had been microscopically verified; for colorectal cancer cases this figure was 91.5% for the years 2003-2007. The percentage of colorectal cancer cases with staged tumours was 90%³⁶⁶ In summary, the NCRI data has been found to meet high standards of completeness and validity. Analyses of the NCRI data have previously contributed to colorectal cancer epidemiology studies published in a variety of international journals.^{6, 367, 368}

5.1.2. Exposure Data: HSE-PCRS

The HSE-PCRS General Medical Services (GMS) scheme currently provides taxpayer-funded universal healthcare, including medicines, to approximately 37% (1.6 million) of the Irish population.⁷⁸ The scheme was originally administered through the Irish General Medical Services (Payments) Board, which was set up in 1970 by order of the Minister for Health under section 11 of the Health Act, 1970.³⁶⁹ This board provided general practitioner, pharmacy and dentistry services free of charge to eligible persons in the eight regional health boards of the time. These health boards, now defunct, served as the health administration divisions in the Republic of Ireland until 2005. The organisation of HSE-PCRS pharmacy claims data by health board has persisted.

Currently, eligibility for the GMS scheme is determined by means testing in order to accommodate persons '*...who are unable without undue hardship to arrange general practitioner, medical and surgical services for themselves...*'⁷⁸ Previously, eligibility was also extended to all persons over the age of 70 years between 1st July 2001 and 1st January 2009.^{369, 370} Persons eligible for the GMS scheme are registered with a doctor of their choice from a list of named doctors who have entered into GMS contracts. Prescribed drugs may then be obtained free of charge when dispensed off a GMS prescription form in any pharmacy which has entered into a GMS contract.³⁶⁹

Pharmacists operating in GMS-contracted pharmacies claim for re-imburement of dispensed medications from the Primary Care Reimbursement Services via an electronic data entry system. Where pharmacists submit paper-based claims, these are converted into an electronic format by the Primary Care Reimbursement Services. As reimbursement does not occur without submission of dispensing records, almost full data capture is anticipated of drugs dispensed under the scheme. Drugs dispensed are coded using the WHO Anatomical Therapeutic Chemical (ATC) classification system³⁷⁰ and individual product codes from which dosage strength and form may be ascertained. Demographic data, including age and sex, are

also recorded in this system and patient records are identified by an eight-character alphanumeric key. The identification number of the prescriber is also recorded. This data comprises the HSE-PCRS GMS database, resulting in detailed prescription claims information for all patients who have been dispensed drugs under the GMS scheme. The HSE-PCRS data is available to a limited number of researchers via defined user agreements and has been used extensively in the past decade for pharmacoepidemiological research, including numerous studies focusing on the use of anti-diabetic medications.^{119, 371, 372} Recent research on sources of medication data compared self-reported medication use with records from the HSE-PCRS data.³⁷³ Reported advantages of the pharmacy claims data included capture of topically applied medicines and drugs prescribed as needed, which may be underreported in patient interviews. Disadvantages of the pharmacy claims data included the inability to capture non-prescription drugs and supplement use.

5.1.3. Linkage of NCRI and HSE-PCRS databases

The NCRI and HSE-PCRS databases as described above have been linked by the NCRI. This process, outlined as follows, has been detailed in internal communication between the NCRI and the Department of Pharmacology and Therapeutics, Trinity College Dublin (2014).

In the case of all patients diagnosed with colorectal cancer between 1st January 2000 and 31st December 2006, linked prescription records are available for all prescriptions dispensed between 1st January 2000 and 31st December 2007. Identifiers used for the linkage process included first names, surnames, sex, date of birth, address, date of death, and Personal Public Service Number (PPS Number). Data processing and record linkage were accomplished using two software applications.

DataPipe, the first application, was developed at the NCRI. This programme was used to standardise the data prior to matching and to mine useful information from fields which contain poorly coded values. Simple mistakes in the data were corrected using the programme and the data was re-formatted to enable easier examination of the data using matching. This re-formatting can take the form of splitting complex fields into a series of smaller, simpler fields or creating codes that enable the matching programme to detect similarities even when words are spelled differently. The second application, Automatch (Matchware Technologies, Inc., Silver Spring, MD USA), was used to perform probabilistic record-linkage with 'fuzzy' comparison techniques, that is, returning the degree of similarity between records rather than declaring values simply as identical or non-identical. E.g. "Robert" and "Robert" are reported as 100% similar while "Robert" and "Rob" are reported as 40% similar. Having determined the degree of similarity for every pair of the fields, Automatch creates a similarity score which

indicates how similar the records are to one another. This score determines whether records: (i) are definite matches, (ii) are definite non-matches, or (iii) require further examination by the user. As such, some manual reviewing of the potential matches occurred during the linkage process.

A match rate of approximately 90% was achieved for patients over the age of 70 (all of whom were entitled to be registered with the GMS scheme in the time period covered by the data). Unmatched records may reflect patients who were not registered with the GMS scheme due to private insurance cover and/or high income. Due to the lack of a 'gold standard' for comparison purposes, it is not possible to accurately ascertain how 'complete' the data linkage was. A recent study examined factors affecting receipt of a GMS scheme membership card ('medical card') among colorectal cancer patients; greater age and deprivation prior to diagnosis were found to predict receipt of a medical card prior to cancer diagnosis.³⁷⁴

The linked NCRI- PCRS database has been used in a number of cancer pharmacoepidemiological studies since the completion of the record linkage process.^{63, 64}

5.1.4. Ethical Approval

Anonymised data with unique patient identifiers were provided by the NCRI under established usage agreements with the NCRI and HSE-PCRS. A formal ethical approval process was not required as all potentially traceable patient identifiers were removed from the data prior to receipt for analysis. The use for research of anonymised data held by the NCRI is covered by the Health (Provision of Information) Act 1997.

5.2. Data Collection: Data cleaning and preparation of the study cohort

All processing of the data obtained from the above sources was carried out using SAS[®], version 9.2 (SAS[®] Institute Inc, Cary, North Carolina).

5.2.1. Inclusion and exclusion criteria

Colorectal cancer patients were selected from the overall cancer registry using the three ICD-10 codes C18 (malignant neoplasm of colon), C19 (malignant neoplasm of rectosigmoid junction) and C20 (malignant neoplasm of rectum).³⁷⁵ Patients with C21 tumours (malignant neoplasms of anus and anal canal) were not included due to the marked differences in the nature of such tumours in terms of physiology, staging and treatment.¹⁹ Only patients who did not have prior invasive tumours were carried forward for analyses. In order to identify prior invasive tumours, tumour records in the cancer registry were classified as invasive or non-invasive using the ICD-10 codes C00–C97 (malignant neoplasms) and D37–D48 (neoplasms of uncertain or unknown behaviour) inclusive. 'Neoplasms of uncertain behaviour' were

considered as possible prior invasive tumours as a conservative measure. Patients diagnosed at autopsy, or whose date of diagnosis occurred after their date of death, were excluded from survival analyses.

Patients were also required to have a complete year of eligibility for the HSE-PCRS scheme in the year prior to diagnosis. Eligibility for the scheme was determined using a combination of GMS scheme membership card start and stop dates and dates of prescription dispensing. Gaps of 90 days or less between two periods of scheme coverage were considered as continuous coverage. In consideration of the generalisability of the data, survival was compared between patients who met the criterion of a complete year of eligibility for the HSE-PCRS scheme in the year prior to diagnosis (n=5,555, Figure 5.2), and patients who did not have eligibility for the scheme during this time (and were therefore not included in the studies within this thesis)(n=4,918). Using a Cox Proportional Hazards model (SAS® PROC PHREG) adjusted for age at diagnosis, no significant difference in colorectal cancer-specific survival was observed between the two groups (hazard ratio for scheme eligibility versus non-eligibility: HR=1.01, 95% CI 0.95-1.09).

The time range of data available for the study cohort is illustrated in Figure 5.1. In summary, patients diagnosed between 1st January 2001 and 31st December 2006 were eligible for inclusion in the study; patients diagnosed in 2000 could not be included as GMS information was not available for the full year prior to diagnosis of these patients. Prescription dispensing data was available for the years 2000-2007 inclusive. A flow chart illustrating the patient selection process for the studies is presented in Figure 5.2.

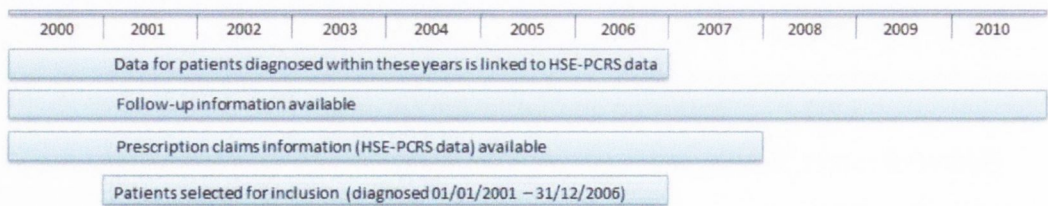


Figure 5.1: Timeline of range of data available for studies within this thesis

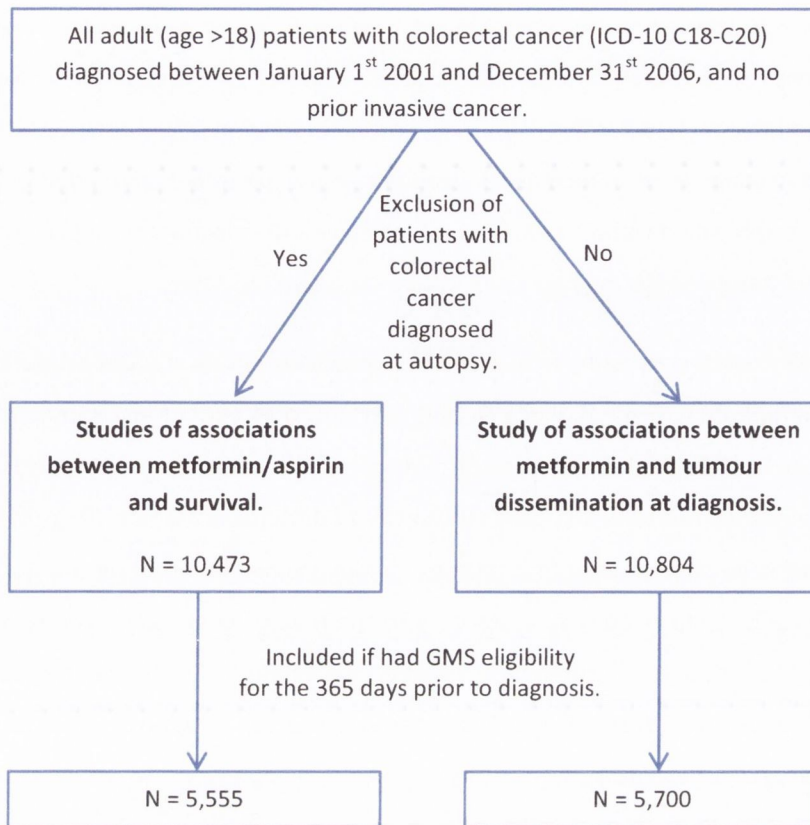


Figure 5.2: Flow chart of overall patient selection

5.2.2. Mortality Data

Patient mortality data was available for all deaths occurring until 31st December 2010. Survival time was calculated from the date of cancer diagnosis to end of follow up, which was the date of death or 31st December 2010, whichever came first. Cause of death information was recorded by the NCRI using death certificate data from the Central Statistics Office. The primary definition of colorectal cancer-specific mortality in the survival analyses in this thesis was any death with an ICD-10 code of C18, C19, C20 or C21 or an ICD-9 code of 153 or 154 (where ICD-10 coding was not used) listed as the main cause of death.

5.2.3. Exposure Information

Exposure to medications of interest was determined from HSE-PCRS data. The data for all of the eight regional health board areas in Ireland was compiled and prescription dispensing records for all patients in the study cohort were retained for processing. A separate reference file known as the 'DMA' (Drugs and Medical Appliances) file was merged, by product code, to

the records relating to the study cohort. The DMA file contains information on the strength (measured in milligrams (mg)), formulation, number of Defined Daily Doses (DDD), trade name and pack size for each product corresponding to a product code in the GMS data. Records referring to combination products were identified and additional variables were introduced to the dataset to enable separation of the individual chemical entities and listing of the associated dosage strengths in such combination products.

Two SAS® macro code programs developed internally within the Department of Pharmacology and Therapeutics, Trinity College Dublin, were used to extract and summarise information on exposure to individual drugs for each member of the study cohort (Appendices One and Two). The 'DailyDrug' macro is first used to identify all dispensings for the drugs of interest. Exposure to a particular class of drug (e.g. 'statins') is determined by entering the ATC codes of interest (i.e. any ATC code relating to a statin) into the macro parameters. The output file contains a row per patient and individual daily variables indicating each day of exposure (1st January 2000 to 31st December 2007 inclusive). The second macro, 'DrugExposure', outputs an exposure summary file for the drug of interest for a specified exposure time window. The macro extracts tumour date of incidence (diagnosis) data from the file containing the study cohort and identifies drug exposures which occurred within the time range of interest (e.g. within the 365 days prior to tumour date of diagnosis). The output file contains one record per patient and contains variables describing the number of days within the time window in which the patient was exposed to the drug, and the total dose of the drug that was received during that time. This information is also expressed as the proportion of the time window for which exposure occurred, and the average daily dose for the patient.

The intended daily dosage strength (or 'dose') of a particular drug is computed for each patient using the above-mentioned macros. Dose is calculated by multiplying the strength, in mg, of a dosage unit of the product dispensed, by the number of dosage units supplied per day. The number of dosage units supplied per day is in turn estimated in accordance with the knowledge that each dispensing of a HSE-PCRS prescription is restricted to providing a maximum of one month's medication supply (except for hospital-only prescriptions, which provide a maximum seven-day supply). For example, where a patient is dispensed sixty tablets of 500mg metformin, this is deduced to represent two dosage units (tablets) per day, resulting in a calculated intended dose of 1,000mg of metformin per day. In order to also quantify the average daily dose received by each patient in the year prior to colorectal cancer diagnosis (i.e. the average number of milligrams taken over this period), each intended daily dose that was dispensed during the period was summed and divided by 365.

Dosing intensity was defined as the proportion of days within the exposure time period of interest for which the patient had a supply available of the drug of interest.³⁷⁶ To calculate this parameter, the number of days of supply for a particular drug was summed, and this total was divided by 365. This was presented within the studies as a percentage.

5.2.4. Covariates

Covariates available in the NCRI-PCRS linked data relating to patient characteristics and properties of the tumour under investigation are described as follows.

5.2.4.1. Patient characteristics

Socio-demographic variables available in the NCRI-PCRS linked data include gender (male/female), age of the patient at diagnosis (years), and smoking status at diagnosis (current/former/never/unspecified). Socioeconomic status of the patient was derived from a census-based indicator of deprivation level, which has been recommended for application in national health research studies.³⁷⁷ This categorical indicator comprises five levels, from least deprived to most deprived, and a separate category for unspecified status.

As diagnostic codes for conditions other than cancer were not present in the dataset, an indication of comorbidity was derived for each patient based on the range of medications to which the patient was exposed. This medication-based comorbidity score was calculated by the number of distinct drug classes to which the patient was exposed in the year prior to diagnosis.³⁷⁸ Distinct drug classes were determined using the 4th level of the ATC classification system, which comprises five-character codes, e.g. biguanide drugs: ATC code = A10BA.³⁷⁰ Comorbidity scores based on number of prescribed medications have performed well in previous studies of comorbidity score validity.³⁷⁸ Methods of comorbidity scoring in the study of colorectal disease and cancer have recently been reviewed and no gold standard method has so far been identified.^{379, 380}

Marital status, occupational status, and patient location were additionally available covariates but were not considered as relevant to the pharmacoepidemiological studies in question and are therefore not described further.

5.2.4.2. Tumour-related covariates

Highly detailed covariates relating to tumour characteristics are contained within the NCRI database. Tumour staging covariates included AJCC TNM stage (I/II/III/IV/unspecified), T-stage (1/2/3/4/unspecified), N-stage (0/1/2) and M-stage (0/1/2)¹⁹; tumour grade was classified as well/moderately differentiated (1/2), poorly differentiated (3/4) or unspecified.¹⁹ Tumour

morphology, coded using the International Classification of Diseases for Oncology, 3rd edition (ICD-O3),³⁸¹ was classified as 'adenocarcinoma' or 'other' for analyses. Method of presentation was classified as 'symptoms', 'other', or 'unspecified'. Method of diagnosis was classified as 'histological', 'other', or 'unspecified'. Other tumour details included year of incidence (2001/2002/2003/2004/2005/2006) and site of the tumour, which was divided into 'colon' (ICD-10 C18) or 'rectal' (ICD-10 C19, C20).

Receipt of tumour-directed surgery, radiotherapy and/or chemotherapy, were determined from treatment procedure codes within the NCRI data. For the purpose of these studies, treatment was recorded as having occurred if the date of treatment fell within the 365 days following cancer diagnosis. This is because the year following diagnosis is the most likely timeframe for receipt of adjuvant therapy. Also, active follow-up of cancer treatment of cases by the NCRI occurs only in the first year following diagnosis and subsequent records of treatment are therefore not likely to be representative of the entire sample.

5.2.4.3. Missing covariate data

Where covariate values were missing, the variable value was coded as 'unspecified' for that patient and retained in the analysis as a separate category. Other methodological approaches for handling missing covariate data were reviewed, including the undertaking of a complete case analysis. Such an analysis would include only those individuals who have no missing data for any of the variables considered within the analysis. This approach was not found to be recommended in the literature due to: (i) the risk of introduction of bias, and (ii) the loss of precision and power associated with exclusion of a substantial proportion of the population sample.³⁸² In light of these factors and considering that studies within the diabetic population would feature low patient numbers, it was viewed that a complete case analysis approach would be inappropriate in this setting.

5.3. Optimising analyses conducted using the data sources

Careful design considerations for the analysis of the above-described data are necessary in order to minimise bias and confounding (section 1.4.1).

With respect to bias in selection of participants or measurement of covariates, such bias is minimised in these studies by the use of pre-collected registry and administrative data; reliance on population-based registries for case and prescription drug data is viewed as an excellent method of reducing the occurrence of selection bias.⁵⁴ Also, as one of the inclusion criteria for these studies was that all patients were required to have a year's worth of eligibility for the GMS scheme prior to diagnosis, measurement of drug utilisation, for example, should

be equal between exposure and comparator groups. The use of prescription claims data avoids recall bias and has been reported as the most accurate source of drug exposure data in observational studies using secondary data sources, as well as the most commonly used source.³⁸³ Also, linkage of vital statistics data from the Central Statistics Office to the cancer registry data provides reliable follow-up data. For some covariates, data was missing for a proportion of the population, as detailed in the previous section.

5.3.1. Confounder selection methodologies

Confounding was minimised in these studies by using multivariate regression analysis to adjust for potential confounders.⁵⁴ The approach adopted in selecting confounders for the multivariate models is described as follows.

A covariate is a true confounder if it is associated with both the drug exposure and the outcome of interest without being in the causal pathway between exposure and outcome (i.e. the confounder is not brought about by the exposure).⁵⁴ Identification of potential confounders for inclusion in multivariate models must take into account this definition or otherwise the introduction of further statistical bias is possible where covariates are inappropriately classified as confounders.³⁸⁴ The initial consideration in selecting covariates for adjustment is based on prior knowledge and expert opinion. Directed acyclic graphs (DAGs), otherwise known as causal diagrams, may then be used to clarify potential confounding relationships in a visual manner.³⁸⁵ DAGs also serve to aid in identifying minimally sufficient adjustment sets for analyses so that over-adjustment of models may be avoided; software programmes have been developed to aid in the production of DAGs and the determination of such minimal sufficient adjustment sets.³⁸⁶ While DAGs provide a rational basis for inclusion of covariates in a statistical model, they do not take into account the effect of sampling variation within the data.³⁸⁷ Selection of confounders for inclusion in a model has therefore historically been accomplished using statistics-based approaches such as stepwise selection of confounders based on changes in p-values upon model inclusion. However, risks associated with such approaches relate to the fact that these methods are data-driven and not combined with a-priori approaches.³⁸⁸ Recent studies have begun to combine the approaches of DAGs and statistical methods such as the change-in-estimate procedure to provide a more reliable process of confounder selection.^{387, 388}

For the current studies a similar and conservative approach to confounder selection was adopted. Prior knowledge and literature review were used to construct causal diagrams of associations between drug exposure and colorectal cancer outcomes using appropriate

software to generate a minimal adjustment set, which was then held fixed in the model ³⁸⁶. Additional variables judged a priori to have a likely confounding effect were then tested using backwards elimination based on a maximum cumulative change in the risk estimates of 10%, resulting in the final multivariate model selection. ³⁸⁹

5.3.2. Additional statistical considerations

This research comprised retrospective observational studies where data on additional subjects could not be collected. Power calculations were therefore not carried out as sample size was not a relevant concern, as outlined in the guide on methodological standards in pharmacoepidemiology produced by the European Network of Centres for Pharmacoepidemiology and Pharmacovigilance. ³⁹⁰

5.4. Methodological challenges common to studies of both metformin and aspirin

5.4.1. Detection bias

Detection bias occurs in cohort studies where the follow-up procedures for detecting adverse events are different for patients with and without the drug exposure. ⁵⁴ This form of bias is of concern in the case of both aspirin and metformin because the recognised adverse effects of these drugs may result in investigations such as colonoscopy. Such investigations may lead to earlier detection, and therefore better prognosis, of colorectal cancer. However, the importance of this bias is unclear. In the case of aspirin, no evidence of earlier diagnosis of colorectal cancer was found in secondary analysis of clinical trials, where patients assigned to aspirin may possibly have had more investigations due to effects such as gastrointestinal bleeding. ³¹⁷ Among diabetic patients, those using metformin, as compared to patients exposed to sulfonylureas, have been shown to have a slightly increased risk of colonoscopy, (HR=1.12, 95% CI 1.06-1.18) potentially due to patients experiencing gastrointestinal side effects following metformin treatment. ³⁹¹

An additional concern relating to detection bias is the 'healthy user/adherer' effect. This form of bias is highly prevalent in pharmacoepidemiology and is relevant to the studies within this thesis due to the consideration of the influence of dosing intensity on associations between users versus non-users and outcomes. ³⁹² The healthy adherer/user effect occurs as a result of patients who use a drug, or have high adherence to a drug therapy, being more likely to partake in healthy behaviours; for example, patients who adhere consistently to their drug therapy have been shown to be more likely to engage in cancer screening activities. ³⁹² In the

present studies, it is expected that adjustment for factors related to healthy choices (e.g. smoking status), or early detection of cancer (as represented by tumour stage), should reduce any potential healthy user bias.

5.4.2. Study of post-diagnostic drug exposures

In examining the effects of drug exposure on survival after cancer diagnosis, post-diagnostic exposure to the drug may be examined separately to pre-diagnostic exposure. However, as discussed in a recent review, a number of potential biases, including reverse causation and immortal time bias, may affect the validity of studies of post-diagnostic exposure in cancer survival research.³⁵³ Approaches, such as the use of time-dependent covariates and ‘lagging’ of exposure classification, may help to minimise some of these biases.³⁵³ Lagging of exposure is where patients are not considered exposed to the drug under investigation until a certain window of time following initiation of the drug has passed.³⁵³ This is intended to avoid reverse causation bias, which is where patients who die soon after treatment initiation may have been prescribed the drug under investigation in the lead up to their death due to this worsening prognosis. However, use of lagging may result in diminished statistical power to detect effects as patients who die within the lag time window do not contribute survival time to the exposure group in the analysis. Time-varying confounding may also be controlled for by the use of marginal structural models to account for the effects of changes in prognosis on prescribing, though complete ascertainment of tumour recurrence is required for this approach.³⁵³ It has been suggested that residual time-varying confounding may remain in analyses of post-diagnostic exposures and mortality even despite these measures.³⁵³

Accurate recurrence data was unavailable for the present studies and the quantity of post-diagnostic exposure data for both aspirin and metformin did not allow for the use of marginal structural models. Therefore, only pre-diagnostic exposures were considered. The method adopted in the studies within this thesis was an ‘intention-to-treat’ analysis style, whereby if a patient is classified as ‘exposed’ prior to diagnosis of cancer, this patient remains classified as exposed for the duration of follow-up, regardless of potential treatment switching. This mimics the approach commonly adopted by randomised clinical trials.³⁹³

5.5. Challenges of bias and confounding in the study of anti-diabetic drugs and cancer

The study of associations between anti-diabetic drugs and cancer incidence/outcomes is complex.¹³¹ Certain biases affecting associations between anti-diabetic drugs and cancer are particularly important to recognise and have increasingly been discussed in line with emerging

research on the topic.^{146, 200, 394, 395} 'Evaluating Cancer Risk with Diabetes Treatment' was the title of a plenary session on 'Hot Topics' at the 2012 International Conference on Pharmacoepidemiology and Therapeutic Risk Management which examined ways to avoid bias in such studies, including bias caused by choice of comparator, adherence bias, detection bias and time-related biases, amongst other considerations.³⁹⁶ While such discussions related specifically to studying cancer risk as an outcome, several of the concerns expressed apply also to survival analyses and study of the association of metformin exposure with tumour extent. Individual challenges in avoiding bias and approaches adopted within the present studies are discussed below.

5.5.1. Diabetic patients as study comparator

The most fundamental concern in studies of associations between metformin and cancer is perhaps the choice of comparator for these analyses. In order to avoid confounding by indication, conventional pharmacoepidemiological wisdom suggests choosing an active comparator (that is, a patient treated with a drug for the same indication, rather than an untreated patient) as the analysis reference.³⁹⁷ However, metformin is currently the first-line treatment in type 2 diabetes and choosing a suitable comparator drug is therefore challenging;^{69, 70} comparison of a patient taking metformin to another treated type 2 diabetes patient may involve the comparison of patients with early and late stage diabetes.²⁰⁰ Because of this there may be differences in the baseline characteristics of patients exposed to metformin versus patients exposed to non-metformin anti-diabetic drugs, including age, weight, and comorbidities.²⁰⁰ While information on factors such as age and comorbidity are available, measures of patient weight, e.g. BMI, are not available in the NCRI-PCRS data, representing a potential unmeasured confounder, as discussed below in section 5.5.5.

While the above concerns regarding metformin as a first-line therapy are noteworthy, it is also important to consider the prescribing patterns for metformin in the years of relevance to the studies of this thesis, that is, 2001-2006, and how this may affect the presence of such bias. As described in section 2.4, prescribing of metformin as first-line therapy was substantially lower in the early 2000s than in the latter part of the past decade.¹¹⁷ Indeed, consensus regarding the appropriateness of metformin as a first-line agent has only recently been reached; even in 2008 it was unclear in clinical practice as to which anti-diabetic agent should be preferentially prescribed.³⁹⁸ As likelihood of exposure to metformin in the period prior to cancer diagnosis is influenced by year of diagnosis in this way (due to changes in prescribing), and as year of diagnosis may affect cancer survival due to on-going improvements in colorectal cancer

survival, year of diagnosis was considered as a potential confounder covariate in the analysis described in chapter 6.

5.5.2. Non-diabetic patients as study comparator

As an alternative to an active diabetic comparator, a non-diabetic comparator could be considered. In a research guidance paper published on the topic of associations between type 2 diabetes and cancer mortality, several potential confounders of this relationship were identified; if comparing patients receiving diabetes medication to non-diabetic patients, poorer outcomes must be expected among the diabetic group unless appropriate adjustment is made for potential bias.¹³¹ Notwithstanding the arguments put forward by this guidance paper, the utility and application of a non-diabetic comparator has been defended by the authors of a recent paper examining metformin and breast cancer outcomes,³⁹⁹ although their use of non-diabetics as a comparator has generated some criticism.^{137, 400}

5.5.3. Final choice of comparator and exposure classification

In light of the above-described biases regarding comparator choice, in these analyses we opted to perform primary and secondary analyses of the effect of overall metformin exposure. In primary analyses, metformin exposure was compared to exposure to all other anti-diabetic drugs. Secondary analyses were then carried out where metformin exposed patients were compared to non-diabetic patients. Also, when examining metformin exposure, we opted to perform subgroup analyses where we explored the nature of the exposure in depth, taking into account both dosing intensity prior to diagnosis and the presence or absence of co-prescribed anti-diabetic medications. The following classifications for exposure in the year prior to diagnosis were used in this case:

- Exposure to anti-diabetic drugs not including metformin
- Exposure to both metformin and non-metformin anti-diabetic drugs, involving:
 - i. Low intensity exposure to metformin
 - ii. High intensity exposure to metformin
- Exposure to metformin but no other anti-diabetic drugs, involving:
 - i. Low intensity exposure to metformin
 - ii. High intensity exposure to metformin

It was concluded that the use of these exposure classifications would provide clarity on the effects of various modes of exposure to metformin relative to diabetic and non-diabetic comparators. A similar approach was recently adopted in a study of associations between metformin exposure and breast cancer risk and outcomes; mutually exclusive exposure groups

were used to provide a comprehensive examination of the effects of different diabetes treatments.²²⁴

5.5.4. Type 1 diabetes patients and non-drug treated type 2 diabetes

In these studies, due to the lack of diagnostic coding, patients with type 1 diabetes or patients with type 2 diabetes not treated with medication could not be specifically identified. Type 1 diabetes patients are treated solely with insulin (as opposed to use also of oral hypoglycaemic agents) and therefore may have been included in the comparator group for these studies. However, as type 1 diabetes patients represent approximately 5% of diabetes patients, it is not expected that significant bias would result from their inclusion.

Some patients with type 2 diabetes are at the early stages of disease and are not yet receiving drug treatment. Such patients may be referred to as 'diet control only' diabetics; they typically have less severe disease and may have lower levels of interaction with the health system than drug-treated diabetics. For these reasons it was not considered appropriate that such patients be included among drug-treated diabetes patients.

5.5.4.1. Adjustment for diabetes control

HbA_{1c} was not captured in the linked data available for these studies so could not be included in analyses. A number of previous studies assessing associations between metformin exposure and cancer mortality have adjusted for HbA_{1c}. However, if metformin is thought to exert an anti-cancer effect through an insulin-lowering mechanism, as discussed in section 3.2.3, adjustment for HbA_{1c} could potentially adjust out the associations between anti-diabetic drugs and cancer outcomes. Expressed otherwise, HbA_{1c} may be on the causal pathway between metformin and cancer survival and would not qualify as a confounder in this case.

5.5.5. Adjustment for obesity

As described in section 5.5.1, differential distribution of obesity (as measured by BMI) between metformin-exposed and unexposed patients is an important issue for consideration. Obesity has been widely accepted as a risk factor for increased incidence of a number of cancers, including colorectal cancer.^{401, 402} Effects of obesity on colorectal tumour characteristics and outcomes have also been explored; one recent study examined the effect of obesity on pathologic stage, positive lymph node status and degree of nodal involvement.⁴⁰³ Overall, no association was observed between obesity and these tumour characteristics. However, a significant association was observed for males in the cohort and for colon cancer cases.⁴⁰³

There is as yet no clear consensus in the literature regarding the effect of obesity on colorectal cancer survival.⁴⁰⁴ A number of studies have found associations between BMI and colorectal cancer survival^{405, 406} while others have found that BMI and different classifications of obesity did not influence survival of colorectal cancer patients.^{12, 403} One recent study found that pre-diagnostic raised BMI, but not post-diagnostic raised BMI, was an important predictor of survival in non-metastatic colorectal cancer.¹⁴ Reasons put forward for this apparent contradiction included the suggestion that obesity prior to diagnosis may lead to a more aggressive cancer phenotype.⁴⁰⁴

It is likely that conventional measurements of obesity used in these studies, such as BMI, are not entirely satisfactory for determining the influence of obesity on cancer outcomes; a recent study observed that visceral obesity, but not BMI, was associated with disease-free survival.⁴⁰⁷ Also, the timing of obesity measurements and site of cancer may modify the effect of obesity on cancer outcomes.⁴⁰⁴

In spite of the above limitations of the BMI variable, efforts were made to determine if it would be possible to perform external adjustment of the NCRI-PCRS data by collecting BMI data for a subset of patients. Ethical approval was granted to explore the St James's Hospital, Dublin, colorectal cancer database, which represents approximately 8% of the patients within the NCRI data, to identify potential unmeasured confounders, including BMI, within the NCRI data.⁴⁰⁸ While BMI data was found to be available for many patients within the St James's Hospital database, the number of patients of interest (i.e., exposed to metformin) for whom BMI data was available (n=9) was too low to permit the adjustment.⁴⁰⁹

5.6. Challenges of bias or confounding in the study of aspirin and cancer

While there are fewer and less complex sources of bias in the study of associations between aspirin and colorectal cancer, as compared to the study of anti-diabetic treatments and cancer, several issues do require consideration. The potential introduction of detection bias, and the limitations of time-varying confounding were discussed in section 5.4. An additional important concern is the risk of misclassifying patients who are exposed to aspirin as unexposed; this issue was highlighted in a recent review of studies examining associations between aspirin and improved colorectal cancer survival.²⁹⁵

The exposure data in the present study can be considered as highly reliable due firstly to the fact that low-dose aspirin is not available over-the-counter in Ireland; therefore, all exposure to aspirin as a cardio-protective treatment is likely to be captured in the linked NCRI-PCRS

data. Also, while analgesic doses of aspirin may be purchased over the counter in Ireland, patients eligible for the GMS scheme would be unlikely to pay 'out-of-pocket' for aspirin in this way, at least over a long-term basis, when analgesic aspirin is available to them free of charge under the GMS scheme. Therefore, while some patients with infrequent exposure to analgesic doses of aspirin purchased over the counter may have been misclassified as 'unexposed', the vast majority of aspirin exposure is expected to be correctly classified within this study. The specific definition of aspirin exposure used in this study will be described in chapter eight.

6. Metformin exposure and survival in patients with non-metastatic colorectal cancer ⁷

6.1. Introduction

As discussed in section 3.2.7, three observational studies have examined associations between metformin exposure and colorectal cancer survival among diabetic patients.²⁵⁰⁻²⁵² Two of these studies reported significant associations between metformin exposure (versus no metformin exposure) and improved survival,^{250, 251} while the most recent study, which was restricted to post-menopausal women, did not find a significant association.²⁵² This study of post-menopausal women also compared metformin exposed patients to non-diabetics, as did a fourth study which examined colorectal cancer patients in a sub-analysis of a larger study on diabetes and cancer mortality.²³⁴ Neither of these observed a survival improvement with metformin exposure as compared to non-diabetics.^{234, 252}

The studies which observed associations between metformin and survival in diabetic patients were conducted in individual institutions and only one of these examined cancer-specific survival (as opposed to overall survival). Also, as discussed in section 3.2.7, concerns regarding the presence of immortal time bias have been raised in the case of one of these studies, and further research using appropriate methodologies has been called for to address these concerns.¹⁴⁶

The cohort study described within this chapter uses national-level data and an intention-to-treat design, including consideration of different exposure categories, to add to the knowledge base on the associations between metformin exposure and cancer-specific survival. Previous studies did not find that metformin was associated with a benefit in stage IV (metastatic) colorectal cancer patients;^{250, 251} therefore this present study focused on stage I-III patients. Stage IV and unspecified-stage patients were included as part of sensitivity analyses (see below).

The study had the following specific aims: (i) to investigate associations between metformin exposure and colorectal cancer-specific survival in a diabetic population; (ii) to examine the

⁷ A version of this chapter has been published in *Cancer Epidemiology, Biomarkers and Prevention*. 2013 Aug;22(8): 1364-74.

influence of exposure intensity, and co-prescription with other anti-diabetic medications, on such associations; (iii) to investigate associations between metformin exposure and colorectal cancer-specific survival in comparison to non-diabetic patients.

6.2. Methods

6.2.1. Study design

Patients eligible for this study were identified from the basic study cohort as described in section 5.2.1, that is, patients over the age of eighteen diagnosed with colorectal cancer (ICD-10 C18-C20) between January 1st 2001 and December 31st 2006 inclusive. Patients were excluded from the cohort if their colorectal cancer was diagnosed at autopsy, if they had a prior history of an invasive cancer other than non-melanoma skin cancer, or if they did not have GMS eligibility for the full year prior to colorectal cancer diagnosis. Additionally, the main study was restricted to patients with a diagnosis of TNM stage I-III (pathologic or clinical staging,¹⁹) cancer, as outlined above.

The decision process for choice of comparator in studies of metformin exposure and colorectal cancer outcomes is described in section 5.3.1. Cohort members were classified into two groups; “diabetic” and “non-diabetic”. Individuals were classified as diabetic if they were identified, through the GMS pharmacy claims data, to have received a supply of at least one anti-diabetic drug (ADD: WHO ATC therapeutic subgroup A10)³⁷⁰ in the year prior to colorectal cancer diagnosis. All other patients were classified as non-diabetic. The main analyses were nested within the diabetic subgroup and considered diabetics receiving versus not receiving metformin. Analyses were subsequently repeated in the full cohort where the reference group was non-diabetics. These analyses were carried out to address the possibility that studies nested within a diabetic population may be biased due to differences in the severity of diabetes or the effectiveness of diabetes control between patients receiving metformin versus non-metformin anti-diabetic drugs.^{394, 410}

6.2.2. Exposure Definition

Metformin exposure was identified from linked prescription refill data using WHO ATC drug codes (Appendix Three). Exposure (yes/no) was defined according to whether or not the individual had a supply of metformin available at any point in the year prior to colorectal cancer diagnosis. Metformin dosing intensity was calculated as per section 5.2.3.³⁷⁶ This was presented as a percentage and stratified as ‘low’ or ‘high’ at the median.

6.2.3. Outcomes

The primary outcome was colorectal cancer-specific survival; overall survival was also examined in secondary analyses.⁴¹¹ The date and cause of death for each patient was identified using linked death certificate information from the NCRI database. Colorectal cancer-specific deaths were identified using the ICD-10 cause of death codes C18-C21 and ICD-9 codes 153 and 154 in earlier years, as described in section 5.2.2. Survival time was calculated from the date of colorectal cancer diagnosis to the first of death or end of follow-up (31st December 2010). Patients were censored if they were alive on the latter date.

6.2.4. Covariates

Patient covariates included in this study are described in detail in section 5.2.4. Patients' socio-demographic information and tumour and treatment details were abstracted from the NCRI database. Patient information included age at diagnosis (years), gender, smoking status at diagnosis (current, former, never, unspecified) and a census-based indicator of socioeconomic status.³⁷⁷ Tumour details included AJCC summary stage (I, II, III),¹⁹ tumour grade (well/moderately differentiated, poorly differentiated, unspecified), site (colon, rectum; Appendix Three), morphology (adenocarcinoma, other; Appendix Three), and year of diagnosis (categorical). Receipt of tumour-directed surgery, chemotherapy, and/or radiation in the year following diagnosis, and corresponding treatment commencement dates, were also abstracted. Linked prescription refill data was used to identify exposure (yes/no; Appendix Three) to non-metformin anti-diabetic drugs (sulfonylureas, insulin, other anti-diabetic drugs (thiazolidinediones, DPP4 inhibitors, meglitinides and alpha glucosidase inhibitors)) in the year prior to diagnosis. Exposure to aspirin was also identified due to increasing evidence of an effect for the drug in colorectal cancer.²⁹⁵ Consideration was given to other medications commonly used in diabetes, such as statins, beta-blockers and ACE-inhibitors, which have been examined in relation to colorectal cancer.⁴¹²⁻⁴¹⁴ However, in contrast to aspirin, compelling evidence was not found to suggest that these drugs significantly affect colorectal cancer survival and they were therefore not selected for potential inclusion in multivariate analyses. As described in section 5.2.4, a comorbidity score was calculated for each patient based on the number of distinct drug classes to which the patient was exposed in the year prior to diagnosis.³⁷⁸

6.2.5. Statistical Analysis

Patient characteristics were tabulated for diabetics according to metformin exposure status (yes/no), and for these groups versus non-diabetics, and differences between the exposure

groups were explored using the Wilcoxon rank-sum test for continuous variables (as the data were non-normally distributed) and Pearson's chi-square test for categorical variables. Crude survival rates for colorectal cancer-specific and overall survival were calculated as deaths per 1,000 person-years.

Within the diabetic subgroup, unadjusted and adjusted Cox Proportional Hazards models (SAS® PROC PHREG) were used to estimate hazard ratios (HR) with 95% confidence intervals (CI) for associations between metformin exposure and colorectal cancer-specific survival. Direct adjusted Kaplan Meier curves were also estimated.⁴¹⁵ These curves, produced using a SAS® macro, are summary survival curves that are adjusted for covariates which are included in the Cox Proportional Hazards model. They are used in place of univariate Kaplan-Meier curves, which may be misleading due to different distributions of confounding risk factors between the exposed and unexposed groups.⁴¹⁵

As described previously (section 5.3.1), prior knowledge, literature review and causal diagrams were used to identify potential covariates from amongst the available patient, tumour and treatment variables for inclusion in the multivariate model of colorectal cancer-specific survival.^{385, 386} The causal diagram, or directed acyclic graph, which depicts possible confounding relationships between the covariates in question, is presented in Appendix Four. Cancer treatment variables were included as time-varying covariates. The final multivariate model was selected using backwards elimination based on a maximum cumulative change in the risk estimates of 10%.^{389, 416} Analyses were also conducted stratifying by metformin dosing intensity (low/high) and by receipt of metformin exclusively or in combination with non-metformin anti-diabetic drugs. This process was repeated for overall (all-cause) survival. Finally, analyses were repeated as above in the full cohort, that is, with the inclusion of non-diabetic patients as the reference group in place of diabetic patients who did not receive metformin.

All analyses were performed using SAS®, version 9.2 (SAS® Institute Inc, Cary, North Carolina). A two-sided *P*-value of <0.05 was considered statistically significant. The proportional hazards assumption was checked by testing for interaction with time within the SAS® model.

6.2.6. Sensitivity Analyses

Sensitivity analyses were carried out to explore the effect of different classifications of recorded cause of death as follows. Analyses of colorectal cancer-specific survival were repeated with the inclusion of: (i) all deaths where colorectal cancer was identified as a secondary/contributory cause of death ('Definition 2'); and (ii) deaths due to malignant

neoplasms of other/ill-defined digestive organs (C26), ill-defined cancer sites (C76.1, C80), secondary cancer sites (C77-79), cancers of uncertain or unknown behaviour (D48.6, D48.9) and unspecified causes of death ('Definition 3'). Finally, sensitivity analyses were carried out including patients with stage IV colorectal cancer or unspecified-stage colorectal cancer.

6.3. Results

6.3.1. Characteristics of the study cohort

A flow chart outlining selection of the study cohort is presented in Figure 6.1. Patient characteristics for the diabetic subgroup, classified as metformin exposed (n=207) or unexposed (n=108) are summarised in Table 6.1. No significant differences were found between the metformin exposed and unexposed diabetic groups in terms of tumour stage, grade or other tumour-related or socio-demographic factors. There was a non-significant higher prevalence of radiation therapy (16% versus 8%, $p=0.06$) and aspirin use (70% versus 60%, $p=0.09$) within the metformin exposed group. Among metformin users, 52% of patients also received a sulfonylurea drug while 72% of metformin unexposed patients received sulfonylurea drugs. Insulin use was also significantly higher in the non-metformin group (28% versus 9%) though use of other anti-diabetic drugs (e.g. thiazolidinediones) was more prevalent in the metformin group.

Details of pre-diagnostic metformin exposure are listed in Table 6.2. The median metformin dosing intensity in the year prior to diagnosis was 92% (IQR 55%, 100%).

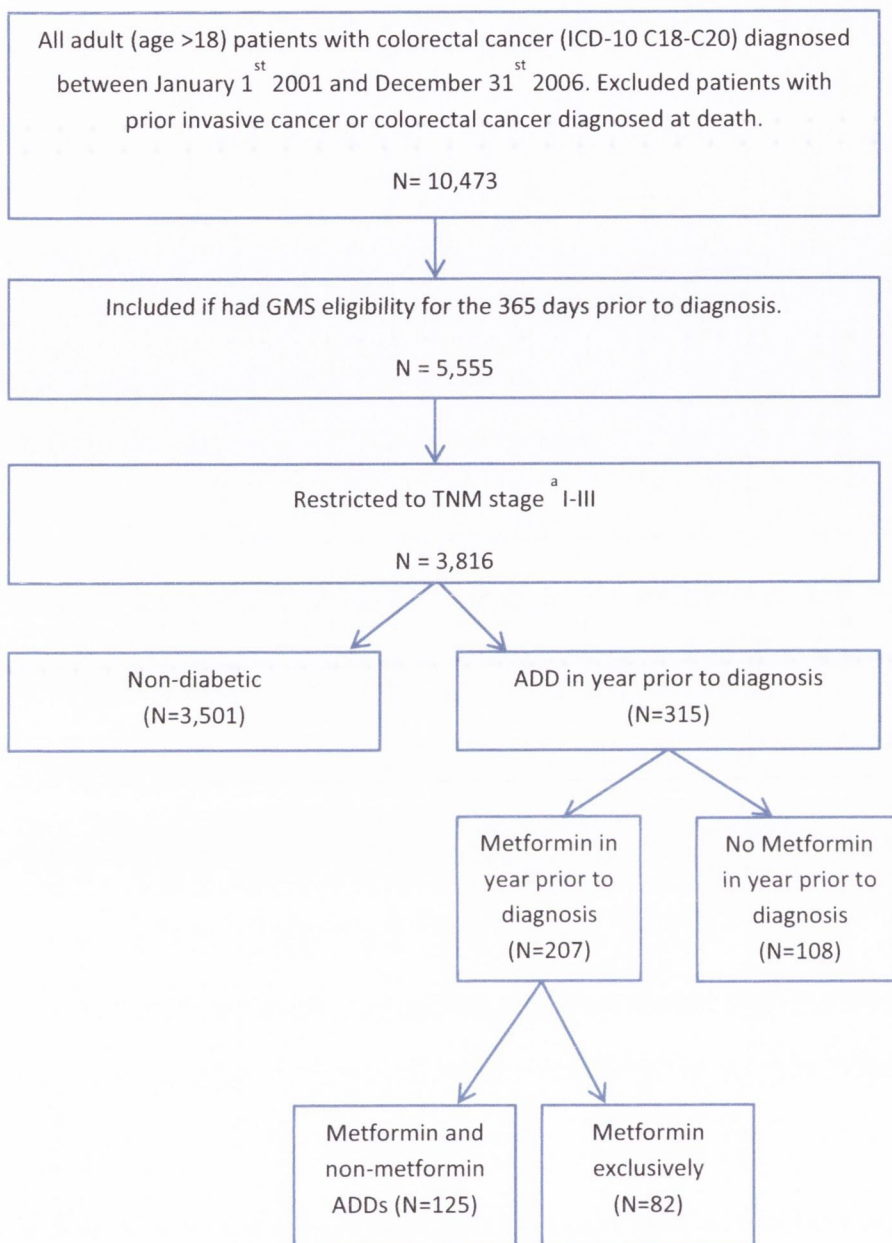
6.3.2. Survival Analyses: diabetic subgroup

The results from analyses of stages I-III colorectal cancer patients with diabetes are presented in Table 6.3. Person-time contributed by the overall diabetic subgroup totalled 1,194 person-years; the crude colorectal cancer-specific mortality rates for metformin exposed and unexposed patients were 70 and 97 deaths per 1,000 person-years, respectively. The main multivariate analysis model included adjustment for age, tumour stage, tumour grade, year of diagnosis, radiation therapy, socioeconomic status, comorbidities, aspirin use, and exposure to non-metformin anti-diabetic drugs (sulfonylureas yes/no, insulin yes/no, other anti-diabetic drugs yes/no). The test for interaction with time for the main analysis was borderline significant ($p=0.44$), indicating that hazards were not proportional and that the association between metformin use and mortality varied over time. Visual examination of the adjusted survival plots (Figure 6.2) indicated that much of the apparent benefit from metformin use was accrued in the first two years after diagnosis.

The results of these multivariate analyses suggested that exposure to metformin, versus no metformin, was associated with a lower risk of colorectal cancer-specific mortality; this approached statistical significance (HR=0.61, p=0.06; Table 6.3, Figure 6.2). Associations of a similar magnitude, although not statistically significant, were observed between metformin exposure and colorectal cancer-specific mortality for high and low exposure intensities (Table 6.3). When deaths from all causes were considered, any metformin exposure was associated with a significantly lower risk of death (HR 0.69, 95% CI 0.49-0.97) and high intensity metformin exposure was associated with a more pronounced significantly lower risk of death (Table 6.3).

In analyses stratified by co-prescription with non-metformin anti-diabetic drugs, metformin exposure, exclusively or co-prescribed, was associated with 39% and 30% lower risk of colorectal cancer-specific mortality respectively, but these estimates were not statistically significant (Table 6.3, Figure 6.3). Significant associations between metformin use and colorectal cancer-specific mortality were observed in analyses stratified by both metformin dosing intensity and co-prescription with non-metformin anti-diabetic drugs. In comparison to diabetics not receiving metformin, the risk of colorectal cancer-specific mortality was significantly lower in patients receiving metformin exclusively at high intensity (HR 0.44, 95% CI 0.20-0.95). Use of metformin exclusively at low intensity was not associated with a lower risk of colorectal cancer-specific mortality (HR 0.81, 95%CI 0.41-1.58). No significant associations were observed for metformin exposure at either high or low intensity when co-prescribed with non-metformin anti-diabetic drugs.

For reference, the milligram dose range of metformin for the exposure categories referred to above is illustrated using boxplots in Figure 6.4. Dosage intensity corresponded to milligram dose as median average daily metformin doses were similar between high metformin groups and also between low metformin intensity groups. The largest average daily dosing range was observed in patients receiving both high intensity metformin and co-prescribed non-metformin anti-diabetic drugs.



a) AJCC Cancer Staging Manual 6th Edition. Springer, 2002.

GMS: General Medical Services Scheme.

ADD: Anti-diabetic drug

Figure 6.1: Flow chart of patient selection into the study

Table 6.1: Characteristics of the study cohort, stages I-III colorectal cancer, according to diabetic status and exposure to metformin

Characteristic	Any Metformin Exposure in Year Prior to Diagnosis			P –value	P –value	
	Non-diabetic (n=3,501)	Diabetic unexposed to metformin (n=108)	Diabetic exposed to metformin (n=207)	Diabetic unexposed v exposed	Non-diabetic v diabetic exposed, unexposed	
Patient Details						
Age – Median (IQR)	Years	75 (69, 80)	76 (71, 79)	74 (71, 80)	0.80	0.74
Comorbidity – Median (IQR)	Number of drug classes	8 (5, 13)	14 (10, 20)	15 (11, 19)	0.39	<0.001
Gender – (%)	Male	1,879 (53.7)	67 (62.0)	127 (61.4)	0.91	0.03
Smoking status – (%)	Current	559 (16.0)	10 (9.3)	17 (8.2)	0.96	0.05
	Former	1,540 (44.0)	49 (45.4)	100 (48.3)		
	Never	724 (20.7)	26 (24.1)	49 (23.7)		
	Unspecified	678 (19.4)	23 (21.3)	41 (19.8)		
Socioeconomic status – (%)	1 (Least deprived)	499 (14.3)	10 (9.3)	21 (10.1)	0.79	0.44
	2	391 (11.2)	13 (12.0)	26 (12.6)		
	3	473 (13.5)	18 (16.7)	26 (12.6)		
	4	617 (17.6)	19 (17.6)	28 (13.5)		
	5 (Most deprived)	1,264 (36.1)	40 (37.0)	89 (43.0)		
	Unspecified	257 (7.3)	8 (7.4)	17 (8.2)		
Tumour Details						
TNM Stage – (%)	I	666 (19.0)	23 (21.3)	36 (17.4)	0.49	0.49
	II	1,505 (43.0)	38 (35.2)	86 (41.6)		
	III	1,330 (38.0)	47 (43.5)	85 (41.1)		
Grade – differentiation (%)	Well/moderate	2,682 (76.6)	75 (69.4)	159 (76.3)	0.41	0.55
	Poorly differentiated	419 (12.0)	17 (15.7)	24 (11.6)		
	Unspecified	400 (11.4)	16 (14.8)	25 (12.1)		
Site – (%)	Colon (v rectum)	2,232 (63.8)	81 (75.0)	147 (71.0)	0.45	0.01
Morphology – (%)	Adenocarcinoma (v other)	2,996 (85.6)	93 (86.1)	179 (86.5)	0.93	0.93

Table 6.1 (continued): Characteristics of the full study cohort, stages I-III colorectal cancer, according to diabetic status and exposure to metformin

Characteristic	Any Metformin Exposure in Year Prior to Diagnosis			P-value	P-value	
	Non-diabetic (n=3,501)	Diabetic unexposed to metformin (n=108)	Diabetic exposed to metformin (n=207)	Diabetic unexposed v exposed	Non-diabetic v diabetic exposed, unexposed	
Tumour Details						
Treatment ^a – (%)						
	Surgery	3,337 (95.3)	104 (96.3)	192 (92.8)	0.21	0.21
	Chemotherapy	1,103 (31.5)	22 (20.4)	59 (28.5)	0.12	0.03
	Radiation	555 (15.9)	9 (8.3)	33 (15.9)	0.06	0.11
Year of Diagnosis – (%)						
	2001	475 (13.6)	15 (13.9)	20 (9.7)	0.46	0.49
	2002	496 (14.2)	18 (16.7)	25 (12.1)		
	2003	608 (17.4)	19 (17.6)	37 (17.9)		
	2004	639 (18.3)	12 (11.1)	38 (18.4)		
	2005	678 (19.4)	21 (19.4)	42 (20.3)		
	2006	605 (17.3)	23 (21.3)	45 (21.7)		
Drug exposures – (%)						
Sulfonylurea ^b	-	78 (72.2)	108 (52.2)	<0.001	-	
Insulin ^b	-	30 (27.8)	18 (8.7)	<0.001	-	
Other ADDs ^b	-	9 (8.3)	31 (15.0)	0.09	-	
Aspirin ^b	1,199 (34.3)	65 (60.2)	144 (69.6)	0.09	<0.001	
Exposure to metformin post cancer diagnosis - (%)	100 (2.9)	20 (18.5)	178 (86.0)			

IQR: Interquartile range ADD: anti-diabetic drug

- a) Refers to treatment received in year post diagnosis.
- b) Exposures in year prior to diagnosis.

Table 6.2: Details of metformin exposure in exposed patients - main analysis cohort.

Metformin exposure information for year prior to colorectal cancer diagnosis			
Number of patients exposed	n	207	
Dosing Intensity – median (IQR) ^a	%	92	(55, 100)
Mean daily dose – median (IQR) ^b	mg/day	1,148	(682, 4318)

IQR: Interquartile range

- a) Exposure intensity calculated as number of days with supply available in year prior to diagnosis, divided by 365 and expressed as a percentage.
- b) Mean daily dose calculated as cumulative dose in year prior to diagnosis, divided by 365.

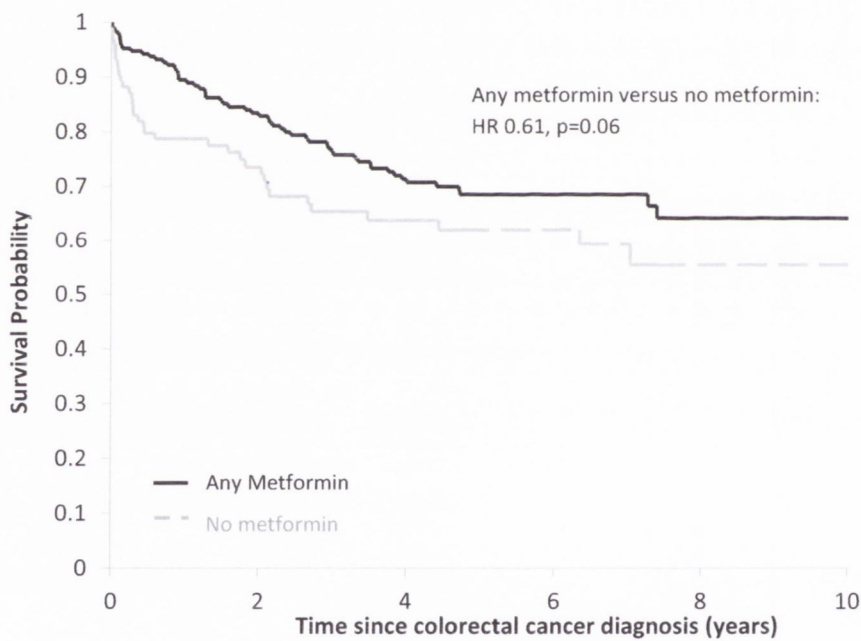


Figure 6.2: Direct adjusted survival curve: Adjusted cumulative incidences of colorectal cancer-specific mortality for metformin users and non-users in diabetic patients with stages I-III colorectal cancer.

Cumulative incidences are adjusted for tumour stage, tumour grade, year of diagnosis, comorbidity score, aspirin use, exposure to non-metformin ADDs (sulfonylureas yes/no, insulin yes/no, other ADDs yes/no), socioeconomic status, radiation therapy. ADD: anti-diabetic drug. HR: hazard ratio.

Table 6.3: Diabetic Subgroup, stages I-III colorectal cancer; unadjusted and adjusted hazard ratios for metformin exposure and mortality.

Exposure Information			Colorectal cancer-specific survival			Overall survival		
Main analysis ^a	N (N=315)	Person -years	Deaths (Crude Rate) ^c	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	Deaths (Crude Rate) ^c	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
No Metformin	108	361	35 (97)	(Ref)	(Ref)	74 (205)	(Ref)	(Ref)
Any Metformin	207	833	58 (70)	0.72 (0.48-1.10)	0.61 (0.37-1.01)	122 (146)	0.73 (0.55-0.97)	0.69 (0.49-0.97)
Stratified Analyses								
Dosing intensity^a								
Any Metformin (Low)	103	406	31 (76)	0.79 (0.49-1.28)	0.62 (0.35-1.10)	68 (168)	0.83 (0.59-1.15)	0.76 (0.52-1.10)
Any Metformin (High)	104	427	27 (63)	0.66 (0.40-1.09)	0.60 (0.33-1.07)	54 (126)	0.64 (0.45-0.90)	0.62 (0.42-0.93)
Co-prescription^b								
Metformin + Co-Rx ADDs	125	483	35 (73)	0.75 (0.47-1.19)	0.70 (0.43-1.15)	76 (157)	0.78 (0.57-1.07)	0.77 (0.55-1.08)
Metformin exclusively	82	350	23 (66)	0.69 (0.41-1.17)	0.61 (0.35-1.06)	46 (131)	0.66 (0.46-0.96)	0.56 (0.38-0.82)
Dosing intensity & Co-prescription^b								
Metformin (Low) + Co-Rx ADDs	65	259	17 (66)	0.68 (0.38-0.22)	0.60 (0.32-1.10)	41 (158)	0.78 (0.53-1.14)	0.74 (0.49-1.12)
Metformin (High) + Co-Rx ADDs	60	223	18 (81)	0.81 (0.46-1.44)	0.82 (0.45-1.49)	35 (157)	0.78 (0.52-1.17)	0.79 (0.52-1.19)
Metformin (Low) exclusively	38	146	14 (96)	0.97 (0.52-1.80)	0.81 (0.41-1.58)	27 (185)	0.91 (0.59-1.42)	0.74 (0.46-1.20)
Metformin (High) exclusively	44	204	9 (44)	0.48 (0.23-1.00)	0.44 (0.20-0.95)	19 (93)	0.48 (0.29-0.79)	0.41 (0.24-0.70)

Ref: reference. ADD: anti-diabetic drug. HR: hazard ratio. CI: confidence interval. Low: low dosing intensity. High: high dosing intensity.

- a) Adjusted for age, tumour stage, tumour grade, year of diagnosis, comorbidity score, aspirin use, exposure to non-metformin ADDs (sulfonylureas yes/no, insulin yes/no, other ADDs yes/no), socioeconomic status, radiation therapy.
- b) Adjusted for age, tumour stage, tumour grade, year of diagnosis, comorbidity score, aspirin use, socioeconomic status, radiation therapy. 'Co-Rx' ADDs include sulfonylureas, insulin and/or other ADDs.
- c) Rate calculated as deaths per 1,000 person-years

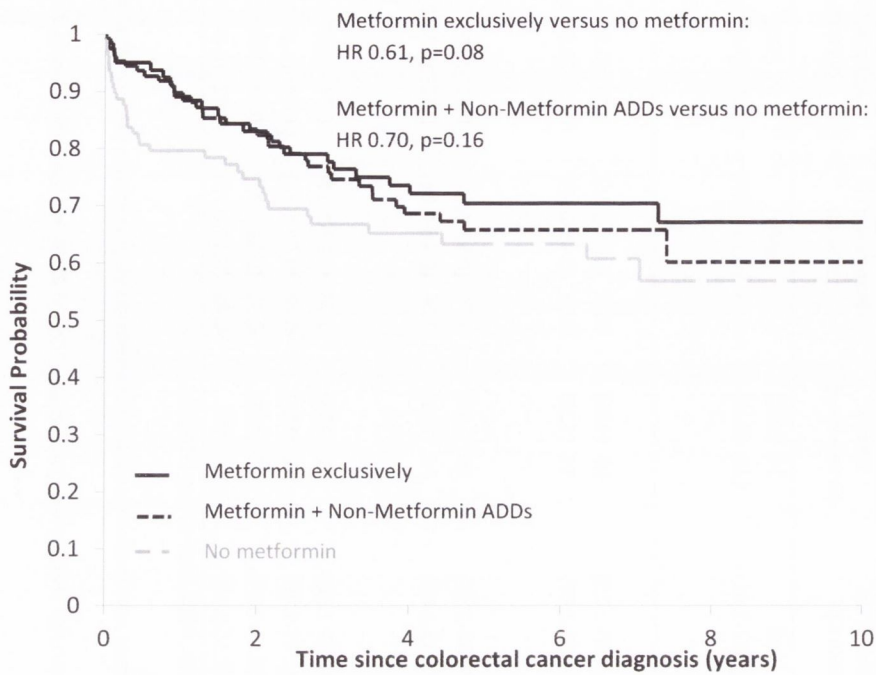


Figure 6.3: Direct adjusted survival curve: Adjusted cumulative incidences of colorectal cancer-specific mortality for metformin users and non-users in diabetic patients with stages I-III colorectal cancer. Stratified by co-prescription with non-metformin ADDs.

Cumulative incidences are adjusted for tumour stage, tumour grade, year of diagnosis, comorbidity score, aspirin use, socioeconomic status, radiation therapy. ADD: anti-diabetic drug. HR: hazard ratio.

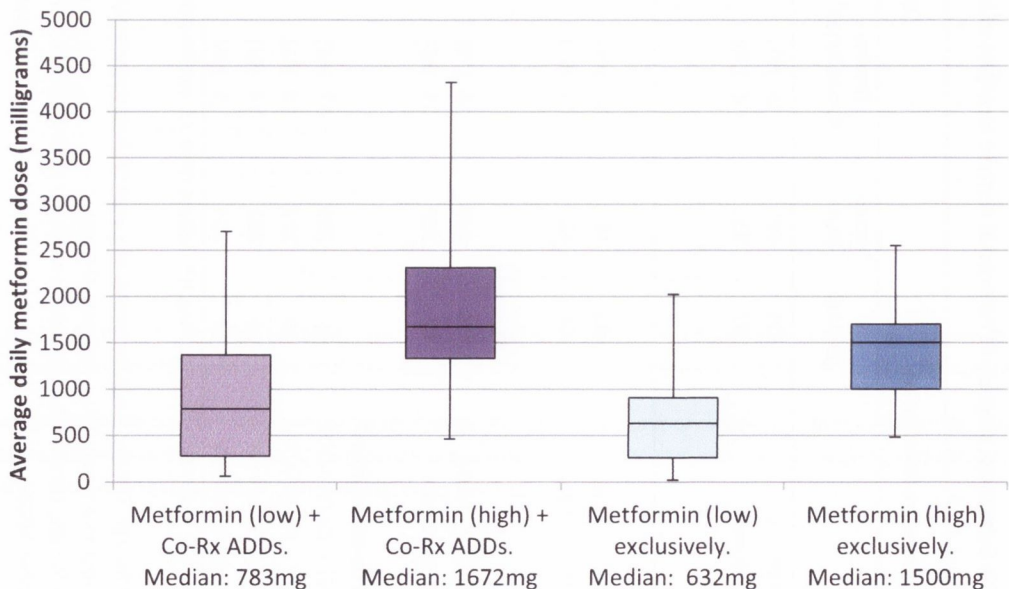


Figure 6.4: Box-plot of metformin dose

ADD: anti-diabetic drug. Co-Rx: 'Co-Rx' ADDs include sulfonylureas, insulin and/or other ADDs.

Low: low dosing intensity. High: high dosing intensity.

6.3.3. Survival Analyses: full cohort

Characteristics of non-diabetic patients, and metformin exposed and unexposed diabetic patients, are compared in Table 6.1. Male gender was more prevalent in diabetic cancer patients than in non-diabetics and slightly greater numbers of diabetic patients had colon cancer (as opposed to rectal cancer) than non-diabetic patients. There was a significant difference in the proportion of patients receiving chemotherapy ($p=0.03$). A higher proportion of non-diabetics received chemotherapy (31.5%) than diabetics (metformin: 28.5%, non-metformin: 20.4%). As would be expected, comorbidity was significantly higher among the diabetic patients than the non-diabetics, as well as aspirin use.

Results from analyses including non-diabetic patients as the reference group are presented in Table 6.4. In these analyses, diabetic patients receiving metformin had a non-significantly lower risk of colorectal cancer-specific mortality compared to non-diabetic patients (HR 0.84, 95% CI 0.58-1.20). Results from analyses stratified by dosing intensity and co-prescription with non-metformin anti-diabetic drugs followed similar trends to those observed in the analyses including only the diabetic subgroup.

6.3.4. Sensitivity Analyses

The results from sensitivity analyses exploring the impact on the result of different classifications of cause of death are presented for the diabetic subgroup and the full cohort in Table 6.5 and in Table 6.6, respectively. Associations between metformin exposure and colorectal cancer-specific mortality did not differ appreciably from those found in the primary analysis when either of the two alternative definitions of colorectal cancer-specific mortality was applied. However, using the broadest definition (Definition 3 in section 6.2.6 above), overall exposure to metformin was associated with a significantly lower risk of colorectal cancer-specific mortality in the diabetic subgroup. This effect was also significant for diabetic patients receiving metformin exclusively or at high dosing intensity, or under both of these conditions.

The results from sensitivity analyses including patients with stage IV colorectal cancer or unspecified staging are presented for the diabetic subgroup and the full cohort in Table 6.7 (also Figure 6.5 and Figure 6.6) and Table 6.8, respectively. In general, associations between metformin exposure and colorectal cancer-specific mortality were closer to the null than those observed in the analyses of stage I-III patients and no results reached statistical significance.

Table 6.4: Full cohort, stages I-III colorectal cancer; unadjusted and adjusted hazard ratios for metformin exposure and mortality.

Exposure Information			Colorectal cancer-specific survival			Overall survival		
Main analysis ^a	N (N=3816)	Person -years	Deaths (Crude Rate) ^c	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	Deaths (Crude Rate) ^c	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
Non-diabetic	3,501	14,717	1,082 (74)	(Ref)	(Ref)	1,897 (129)	(Ref)	(Ref)
Diabetic - No Metformin	108	361	35 (97)	1.27 (0.91-1.78)	1.22 (0.69-2.14)	74 (205)	1.54 (1.22-1.95)	1.19 (0.82-1.74)
Any Metformin	207	833	58 (70)	0.91 (0.70-1.18)	0.84 (0.58-1.20)	122 (146)	1.11 (0.92-1.33)	0.90 (0.69-1.17)
Stratified Analyses								
Dosing intensity^a								
Any Metformin (Low)	103	406	31 (76)	0.99 (0.70-1.42)	0.87 (0.56-1.37)	68 (167)	1.26 (0.99-1.61)	0.99 (0.72-1.35)
Any Metformin (High)	104	427	27 (63)	0.83 (0.56-1.21)	0.80 (0.51-1.26)	54 (126)	0.96 (0.73-1.26)	0.82 (0.60-1.13)
Co-prescription^b								
Metformin + Co-Rx ADDs	125	483	35 (72)	0.93 (0.67-1.31)	0.82 (0.58-1.16)	76 (157)	1.18 (0.94-1.48)	1.04 (0.82-1.32)
Metformin exclusively	82	350	23 (66)	0.87 (0.58-1.32)	0.73 (0.48-1.11)	46 (131)	1.01 (0.75-1.35)	0.82 (0.61-1.10)
Dosing intensity & Co-prescription^b								
Metformin (Low) + Co-Rx ADDs	65	259	17 (66)	0.86 (0.53-1.39)	0.74 (0.45-1.20)	41 (158)	1.20 (0.88-1.63)	1.04 (0.76-1.43)
Metformin (High) + Co-Rx ADDs	60	223	18 (81)	1.01 (0.64-1.62)	0.93 (0.58-1.49)	35 (157)	1.16 (0.83-1.62)	1.04 (0.74-1.45)
Metformin (Low) exclusively	38	146	14 (96)	1.22 (0.72-2.07)	0.93 (0.55-1.59)	27 (185)	1.37 (0.94-2.01)	1.02 (0.69-1.50)
Metformin (High) exclusively	44	204	9 (44)	0.60 (0.31-1.16)	0.54 (0.28-1.05)	19 (93)	0.73 (0.46-1.15)	0.64 (0.41-1.01)

Ref: reference. ADD: anti-diabetic drug. HR: hazard ratio. CI: confidence interval. Low: low dosing intensity. High: high dosing intensity.

- a) Adjusted for age, tumour stage, tumour grade, year of diagnosis, comorbidity score, aspirin use, exposure to non-metformin ADDs (sulfonylureas yes/no, insulin yes/no, other ADDs yes/no).
- b) Adjusted for age, tumour stage, tumour grade, year of diagnosis, comorbidity score, aspirin use. 'Co-Rx' ADDs include sulfonylureas, insulin and/or other ADDs.
- c) Rate calculated as deaths per 1,000 person-years.

Table 6.5: Sensitivity Analysis 1 (different classification of cause of death) – Diabetic Subgroup, stages I-III colorectal cancer.

Exposure Information			Colorectal cancer-specific survival (Definition 2) ^d			Colorectal cancer-specific survival (Definition 3) ^e		
Main analysis ^a	N (N=315)	Person -years	Deaths (Crude Rate) ^c	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	Deaths (Crude Rate) ^c	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
No Metformin	108	361	36 (100)	(Ref)	(Ref)	47 (130)	(Ref)	(Ref)
Any Metformin	207	833	62 (74)	0.75 (0.50-1.13)	0.63 (0.38-1.03)	77 (92)	0.72 (0.50-1.03)	0.59 (0.38-0.92)
Stratified Analyses								
Dosing intensity^a								
Any Metformin (Low)	103	406	33 (81)	0.81 (0.51-1.31)	0.63 (0.36-1.10)	44 (108)	0.83 (0.55-1.25)	0.67 (0.41-1.09)
Any Metformin (High)	104	427	29 (68)	0.69 (0.42-1.13)	0.63 (0.35-1.11)	33 (77)	0.61 (0.39-0.95)	0.52 (0.31-0.87)
Co-prescription^b								
Metformin + Co-Rx ADDs	125	483	37 (77)	0.77 (0.48-1.21)	0.73 (0.45-1.18)	45 (93)	0.72 (0.48-1.08)	0.66 (0.43-1.01)
Metformin exclusively	82	350	25 (71)	0.73 (0.44-1.22)	0.65 (0.38-1.11)	32 (91)	0.72 (0.46-1.13)	0.61 (0.38-0.98)
Dosing intensity & Co-prescription^b								
Metformin (Low) + Co-Rx ADDs	65	259	18 (70)	0.70 (0.40-1.24)	0.61 (0.34-1.12)	24 (93)	0.71 (0.44-1.16)	0.61 (0.36-1.04)
Metformin (High) + Co-Rx ADDs	60	223	19 (85)	0.84 (0.48-1.46)	0.86 (0.48-1.53)	21 (94)	0.72 (0.43-1.21)	0.70 (0.41-1.20)
Metformin (Low) exclusively	38	146	15 (103)	1.01 (0.55-1.84)	0.83 (0.43-1.60)	20 (137)	1.04 (0.62-1.76)	0.88 (0.50-1.56)
Metformin (High) exclusively	44	204	10 (49)	0.52 (0.26-1.05)	0.49 (0.23-1.02)	12 (59)	0.48 (0.25-0.90)	0.40 (0.20-0.78)

Ref: reference. ADD: anti-diabetic drug. HR: hazard ratio. CI: confidence interval. Low: low dosing intensity. High: high dosing intensity.

- a) Adjusted for age, tumour stage, tumour grade, year of diagnosis, comorbidity score, aspirin use, exposure to non-metformin ADDs (sulfonylureas yes/no, insulin yes/no, other ADDs yes/no), socioeconomic status, radiation therapy.
- b) Adjusted for age, tumour stage, tumour grade, year of diagnosis, comorbidity score, aspirin use, socioeconomic status, radiation therapy. 'Co-Rx' ADDs include sulfonylureas, insulin and/or other ADDs.
- c) Rate calculated as deaths per 1,000 person-years
- d) Includes deaths where colorectal cancer was identified as a secondary/contributory cause of death.
- e) Includes deaths due to malignant neoplasms of other/ill-defined digestive organs (C26), ill-defined cancer sites (C76.1, C80), secondary cancer sites (C77-79), cancers of uncertain or unknown behaviour (D48.6, D48.9) and unspecified causes of death.

Table 6.6: Sensitivity Analysis 1 (different classification of cause of death) – Full cohort, stages I-III colorectal cancer.

Exposure Information			Colorectal cancer-specific survival (Definition 2) ^d			Colorectal cancer-specific survival (Definition 3) ^e		
Main analysis ^a	N (N=3,816)	Person -years	Deaths (Crude Rate) ^c	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	Deaths (Crude Rate) ^c	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
Non-diabetic	3,501	14,717	1130 (77)	(Ref)	(Ref)	1,136 (91)	(Ref)	(Ref)
Diabetic - No Metformin	108	361	36 (100)	1.25 (0.90-1.74)	1.19 (0.69-2.07)	47 (130)	1.38 (1.03-1.85)	1.34 (0.82-2.19)
Any Metformin	207	833	62 (74)	0.93 (0.72-1.20)	0.85 (0.60-1.20)	77 (92)	0.98 (0.78-1.23)	0.92 (0.67-1.25)
Stratified Analyses								
Dosing intensity^a								
Any Metformin (Low)	103	406	33 (81)	1.01 (0.68-1.31)	0.87 (0.56-1.34)	44 (108)	1.14 (0.85-1.54)	1.02 (0.70-1.49)
Any Metformin (High)	104	427	29 (68)	0.85 (0.61-1.35)	0.83 (0.54-1.28)	33 (77)	0.82 (0.58-1.16)	0.82 (0.55-1.22)
Co-prescription^b								
Metformin + Co-Rx ADDs	125	483	37 (77)	0.94 (0.68-1.31)	0.83 (0.59-1.16)	45 (93)	0.97 (0.72-1.31)	0.86 (0.64-1.17)
Metformin exclusively	82	350	25 (71)	0.91 (0.61-1.35)	0.75 (0.50-1.12)	32 (91)	0.98 (0.69-1.40)	0.82 (0.58-1.17)
Dosing intensity & Co-prescription^b								
Metformin (Low) + Co-Rx ADDs	65	259	18 (69)	0.87 (0.55-1.39)	0.73 (0.46-1.17)	24 (93)	0.98 (0.66-1.47)	0.84 (0.56-1.27)
Metformin (High) + Co-Rx ADDs	60	223	19 (85)	1.02 (0.65-1.61)	0.94 (0.60-1.49)	21 (94)	0.96 (0.62-1.48)	0.89 (0.58-1.38)
Metformin (Low) exclusively	38	146	15 (103)	1.25 (0.75-2.08)	0.93 (0.55-1.56)	20 (137)	1.41 (0.91-2.20)	1.06 (0.68-1.67)
Metformin (High) exclusively	44	204	10 (49)	0.64 (0.35-1.20)	0.58 (0.31-1.09)	12 (59)	0.65 (0.37-1.15)	0.60 (0.34-1.05)

Ref: reference. **ADD:** anti-diabetic drug. **HR:** hazard ratio. **CI:** confidence interval. **Low:** low dosing intensity. **High:** high dosing intensity.

- a) Adjusted for age, tumour stage, tumour grade, year of diagnosis, comorbidity score, aspirin use, exposure to non-metformin ADDs (sulfonylureas yes/no, insulin yes/no, other ADDs yes/no).
- b) Adjusted for age, tumour stage, tumour grade, year of diagnosis, comorbidity score, aspirin use. 'Co-Rx' ADDs include sulfonylureas, insulin and/or other ADDs.
- c) Rate calculated as deaths per 1,000 person-years
- d) Includes deaths where colorectal cancer was identified as a secondary/contributory cause of death.
- e) Includes deaths due to malignant neoplasms of other/ill-defined digestive organs (C26), ill-defined cancer sites (C76.1, C80), secondary cancer sites (C77-79), cancers of uncertain or unknown behaviour (D48.6, D48.9) and unspecified causes of death.

Table 6.7: Sensitivity Analysis 2 (inclusion of stage IV and unspecified-stage patients) – Diabetic subgroup.

Exposure Information			Colorectal cancer-specific survival			Overall survival		
Main analysis ^a	N (N=444)	Person -years	Deaths (Crude Rate) ^c	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	Deaths (Crude Rate) ^c	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
No Metformin	159	416	72 (173)	(Ref)	(Ref)	123 (296)	(Ref)	(Ref)
Any Metformin	285	918	111 (121)	0.72 (0.54-0.97)	0.84 (0.60-1.18)	194 (211)	0.74 (0.59-0.93)	0.87 (0.68-1.13)
Stratified Analyses								
Dosing intensity^a								
Any Metformin (Low)	144	445	59 (133)	0.78 (0.55-1.10)	0.93 (0.64-1.36)	107 (240)	0.83 (0.64-1.08)	0.99 (0.75-1.31)
Any Metformin (High)	141	472	52 (110)	0.66 (0.47-0.95)	0.75 (0.50-1.13)	87 (184)	0.65 (0.50-0.86)	0.75 (0.55-1.02)
Co-prescription^b								
Metformin + Co-Rx ADDs	178	547	71 (130)	0.76 (0.55-1.05)	0.88 (0.63-1.24)	125 (229)	0.79 (0.61-1.01)	0.91 (0.70-1.18)
Metformin exclusively	107	371	40 (108)	0.66 (0.45-0.98)	0.86 (0.58-1.28)	69 (186)	0.67 (0.50-0.90)	0.78 (0.58-1.06)
Dosing intensity & Co-prescription^b								
Metformin (Low) + Co-Rx ADDs	93	290	35 (121)	0.71 (0.48-1.07)	0.84 (0.55-1.26)	68 (234)	0.81 (0.60-1.09)	0.93 (0.69-1.26)
Metformin (High) + Co-Rx ADDs	85	258	36 (140)	0.81 (0.54-1.20)	0.94 (0.62-1.43)	57 (221)	0.76 (0.56-1.05)	0.88 (0.63-1.21)
Metformin (Low) exclusively	51	156	24 (154)	0.90 (0.57-1.43)	1.26 (0.78-2.02)	39 (250)	0.87 (0.61-1.24)	1.09 (0.75-1.59)
Metformin (High) exclusively	56	215	16 (74)	0.48 (0.28-0.82)	0.58 (0.33-1.01)	30 (140)	0.51 (0.35-0.77)	0.56 (0.37-0.85)

Ref: reference. ADD: anti-diabetic drug. HR: hazard ratio. CI: confidence interval. Low: low dosing intensity. High: high dosing intensity.

- a) Adjusted for age, tumour stage, tumour grade, year of diagnosis, comorbidity score, aspirin use, exposure to non-metformin ADDs (sulfonylureas yes/no, insulin yes/no, other ADDs yes/no).
- b) Adjusted for age, tumour stage, tumour grade, year of diagnosis, comorbidity score, aspirin use. 'Co-Rx' ADDs include sulfonylureas, insulin and/or other ADDs.
- c) Rate calculated as deaths per 1,000 person-years

Table 6.8: Sensitivity Analysis 2 (inclusion of stage IV and unspecified-stage patients) – Full cohort.

Exposure Information			Colorectal cancer-specific survival			Overall survival		
Main analysis ^a	N (N=5,555)	Person -years	Deaths (Crude Rate) ^c	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	Deaths (Crude Rate) ^c	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
Non-diabetic	5,111	17108	2204 (129)	(Ref)	(Ref)	3,352 (196)	(Ref)	(Ref)
Diabetic - No Metformin	159	416	72 (173)	1.24 (0.98-1.57)	1.10 (0.73-1.65)	123 (296)	1.41 (1.18-1.69)	1.07 (0.79-1.45)
Any Metformin	285	918	111 (121)	0.89 (0.75-1.25)	0.91 (0.69-1.21)	194 (211)	1.04 (0.90-1.20)	0.94 (0.76-1.17)
Stratified Analyses								
Dosing intensity^a								
Any Metformin (Low)	144	445	59 (133)	0.97 (0.75-1.25)	0.96 (0.68-1.35)	107 (240)	1.17 (0.96-1.42)	1.03 (0.79-1.34)
Any Metformin (High)	141	472	52 (110)	0.82 (0.62-1.08)	0.87 (0.63-1.22)	87 (184)	0.91 (0.74-1.13)	0.87 (0.67-1.13)
Co-prescription^b								
Metformin + Co-Rx ADDs	178	547	71 (130)	0.94 (0.74-1.19)	0.89 (0.70-1.13)	125 (229)	1.10 (0.92-1.32)	1.04 (0.87-1.25)
Metformin exclusively	107	371	40 (108)	0.82 (0.60-1.13)	0.86 (0.63-1.18)	69 (186)	0.94 (0.74-1.19)	0.92 (0.72-1.17)
Dosing intensity & Co-prescription^b								
Metformin (Low) + Co-Rx ADDs	93	290	35 (121)	0.89 (0.63-1.24)	0.80 (0.57-1.13)	68 (234)	1.15 (0.90-1.46)	1.04 (0.81-1.33)
Metformin (High) + Co-Rx ADDs	85	258	36 (140)	0.99 (0.71-1.38)	0.99 (0.71-1.38)	57 (221)	1.05 (0.81-1.37)	1.05 (0.80-1.36)
Metformin (Low) exclusively	51	156	24 (154)	1.12 (0.75-1.67)	1.14 (0.76-1.72)	39 (250)	1.21 (0.88-1.66)	1.16 (0.84-1.60)
Metformin (High) exclusively	56	215	16 (74)	0.59 (0.36-0.97)	0.62 (0.38-1.02)	30 (140)	0.73 (0.51-1.04)	0.72 (0.50-1.04)

Ref: reference. **ADD:** anti-diabetic drug. **HR:** hazard ratio. **CI:** confidence interval. **Low:** low dosing intensity. **High:** high dosing intensity.

- a) Adjusted for age, tumour stage, tumour grade, year of diagnosis, comorbidity score, aspirin use, exposure to non-metformin ADDs (sulfonylureas yes/no, insulin yes/no, other ADDs yes/no).
- b) Adjusted for age, tumour stage, tumour grade, year of diagnosis, comorbidity score, aspirin use. 'Co-Rx' ADDs include sulfonylureas, insulin and/or other ADDs.
- c) Rate calculated as deaths per 1,000 person-years

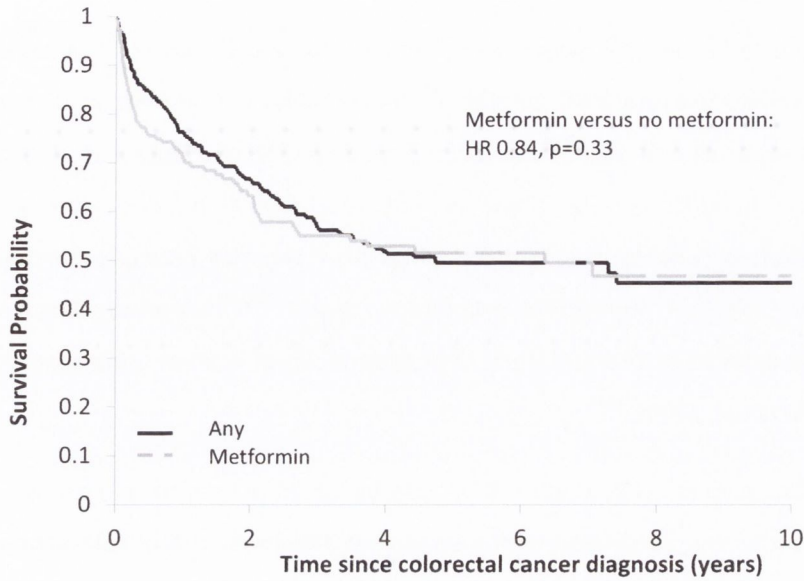


Figure 6.5: Direct adjusted survival curve, all stages colorectal cancer: Adjusted cumulative incidences of colorectal cancer-specific mortality for metformin users and non-users in diabetic patients with stages I-IV and unspecified-stage colorectal cancer.

Cumulative incidences are adjusted for tumour stage, tumour grade, year of diagnosis, comorbidity score, aspirin use, socioeconomic status, radiation therapy. **ADD**: anti-diabetic drug. **HR**: hazard ratio.

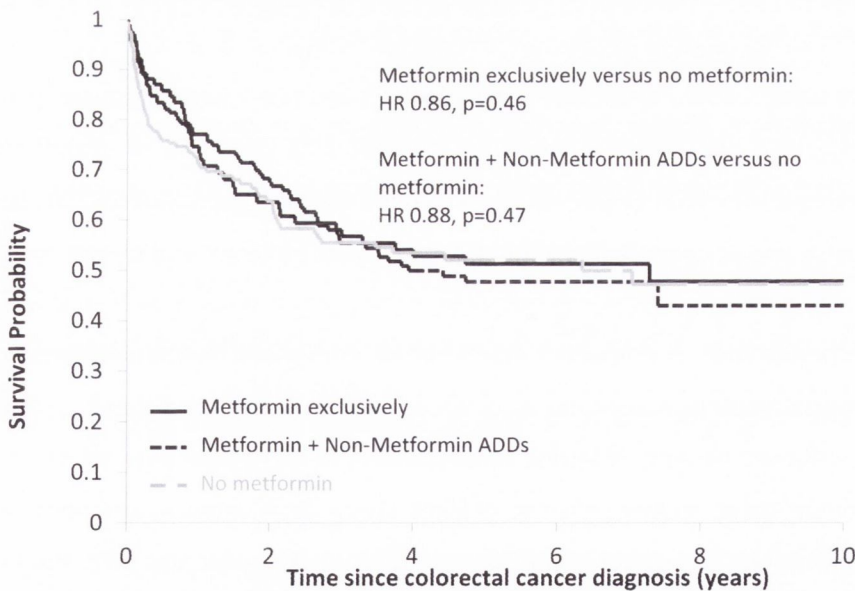


Figure 6.6: Direct adjusted survival curve, all stages colorectal cancer: Adjusted cumulative incidences of colorectal cancer-specific mortality for metformin users and non-users in diabetic patients with stages I-IV and unspecified-stage colorectal cancer. Stratified by co-prescription with non-metformin ADDs.

Cumulative incidences are adjusted for tumour stage, tumour grade, year of diagnosis, comorbidity score, aspirin use, socioeconomic status, radiation therapy. **ADD**: anti-diabetic drug. **HR**: hazard ratio.

6.4. Discussion

This study examined associations between metformin exposure and colorectal cancer-specific mortality among patients with stage I-III colorectal cancer receiving treatment for diabetes. For overall metformin exposure, the risk of colorectal cancer-specific mortality was 39% lower in metformin treated diabetics, versus diabetics not receiving metformin, although non-significant. This result is consistent with the findings from the two previous (single-centre) studies of metformin exposure and survival in colorectal cancer.^{250, 251} A non-significant survival improvement was observed in a recent study of postmenopausal women using data from the Women's health Initiative cohort.²⁵²

In a study of diabetic patients with stage I-IV disease by Lee et al., metformin exposure for a minimum of six months was associated with a significant 34% lower risk of colorectal cancer-specific mortality.²⁵⁰ The results from this study have, however, been questioned due to the possible presence of immortal time bias. As the exposure time window in the study included metformin use both before and after cancer diagnosis, patients in the study may be required to be alive for a certain period of follow up in order to meet cohort entry criteria. Immortal time is therefore introduced to the analysis and may over-estimate the effect of metformin.¹⁴⁶ As the exposure window in the present study is solely prior to diagnosis, this bias was not introduced.

In the study by Garrett et al., of diabetic patients with stage I-IV colorectal cancer, metformin exposure at diagnosis was associated with a significant 40% lower risk of overall mortality (analyses examining colorectal cancer-specific mortality were not performed).²⁵¹ A significant association – of a similar magnitude – between metformin exposure and overall mortality in patients with colorectal cancer was also observed in the present study. However, these results should be interpreted with caution as the risk of non-cancer related deaths is likely to be lower in diabetic patients receiving metformin, a common first-line choice for diabetes treatment, in comparison to diabetic patients receiving second and third line treatments, which served as the comparator in these analyses. In the present study, attenuated associations between metformin exposure and colorectal cancer-specific survival were observed after the inclusion of patients with stage IV disease in the study cohort. This is consistent with the studies by Lee et al. and Garrett et al., both of which reported no association between metformin exposure and mortality in unadjusted analyses of patients with stage IV colorectal cancer.^{250, 251} Cossor et al. did not observe a significant association between metformin exposure and colorectal cancer survival in diabetic postmenopausal women.²⁵² However, this study was limited by a

number of important factors including small sample size, lack of information on timing or extent of metformin exposure, and lack of covariates such as receipt of cancer treatments.²⁵²

The present study and prior studies^{250, 251} have examined associations between metformin exposure and outcomes in colorectal cancer patients with diabetes. However, it has been suggested that the results from studies nested within a diabetic population may be biased due to differences in the severity of diabetes or the effectiveness of diabetes control between patients receiving metformin versus non-metformin anti-diabetic drugs.^{394, 410} Additionally it has also been suggested that any apparent benefit of metformin exists only relative to potential harmful effects of comparator diabetes treatments.²⁰⁰ To explore this possibility, we also assessed associations between metformin exposure and colorectal cancer-specific mortality with reference to non-diabetic patients. Although associations were non-significant, the risk of colorectal cancer-specific mortality in diabetic patients treated with metformin, particularly high intensity, exclusive metformin therapy, was lower in comparison to non-diabetics. While reassuring, these results should not be taken to suggest that associations between metformin and cancer survival in diabetics are generalizable to a non-diabetic population. Neither Cossor et al. nor Currie et al. observed associations between metformin exposure, relative to non-diabetic patients, and survival from colorectal cancer in previous studies.^{234, 252} However, the results of the study by Currie et al. for overall metformin exposure versus non-diabetics (HR=0.89, 95% CI 0.67-1.19) were similar to those observed in the present study for this comparison (HR=0.91, 95% CI 0.69-1.21, Table 8); stratification of results by dosing intensity or co-prescription was not performed by Currie et al.²³⁴ On-going and upcoming clinical trials of metformin in non-diabetic cancer patients will explore the possibility of an effect of metformin in improving survival in this setting.²⁵⁴

This study is the first, to the authors' knowledge, to assess the presence of an exposure response effect between increasing metformin use and colorectal cancer outcomes. In analyses stratified by metformin exposure intensity there was little difference in associations between low and high intensity metformin exposure and colorectal cancer-specific mortality. However, there was a suggestion that a stronger association was present for patients receiving high intensity metformin among those patients receiving metformin exclusively. In this subgroup, low and high metformin exposure intensity were associated with a 19% and 56% lower risk of colorectal cancer-specific mortality respectively. The latter of these was statistically significant and approached significance in analyses with non-diabetics as the reference group. Healthy adherer bias could be suggested as one explanation for the greater effect seen among patients receiving consistent, exclusive metformin use.³⁹² However, a

significant survival benefit was not observed among patients receiving both consistent metformin use and other anti-diabetic drugs also. It should be noted, however, that the number of patients in these subgroup analyses was small; hence these results require further confirmation in larger studies.

This study has a number of additional strengths. It is the first study of associations between metformin exposure and colorectal cancer survival using national, prospectively-collected linked cancer and prescribing data. Access to pharmacy claims data in these analyses provided detailed, objective, longitudinal exposure data which is not influenced by recall bias. While non-compliance with received treatment (about which information is not available) will have resulted in exposure misclassification, this would usually bias results towards the null. Finally, this study was conducted using an intention-to-treat based analysis, with metformin exposure defined prior to the beginning of follow-up. This study design is not influenced by time-related biases or time-varying confounding due to changes in metformin prescribing associated with prognosis,¹⁴⁶ though it should be noted that the results of intention-to-treat analyses may be biased towards the null due to post-diagnostic treatment crossover. Of patients who did not receive metformin prior to diagnosis, 18.5% received the drug following their diagnosis. Of those patients who received metformin prior to diagnosis, 14% discontinued therapy following colorectal cancer diagnosis.

The results from this study should be interpreted with consideration of the fact that the test for interaction with time was borderline significant and that the associations between metformin and survival may have varied over time. Due to limitations in the sample size available for analysis, a full exploration of this could not be performed. Another concern is that many patients had both pre-and post- diagnostic metformin use. It is therefore difficult to discern in our study whether possible benefits from metformin exposure are attributable to only pre-diagnosis, or to pre-diagnosis followed by post-diagnostic exposure. Because of this, it is unclear whether these results are applicable to de-novo use of metformin after a diagnosis of colorectal cancer. Additional study limitations include a limited sample size which restricted the power to detect significant differences in survival. However, the two previous studies of metformin and colorectal cancer survival, which included patients with all stages of colorectal cancer, had similar sample sizes (Lee et al., N=494,²⁵⁰ Garrett et al., N=424²⁵¹) to that achieved in this study (stages I-III, N=315; all stages, N=444). Data regarding obesity, such as BMI, were also not available in this study, as discussed in section 5.5.5. However, previous research has suggested that BMI is not a strong predictor of colorectal cancer-specific survival^{12, 407, 417} and

it has not been shown to be a confounder in previous studies of metformin and colorectal cancer outcomes.²⁵¹

In conclusion, this study examined varying levels of metformin exposure and associations with colorectal cancer specific mortality. Evidence for a significant association between overall metformin exposure and colorectal cancer specific mortality was inconclusive, which is broadly consistent with prior studies. However, significant associations were observed in stratified analyses of high intensity exclusive metformin use and the results also suggest that metformin exposure may potentially improve survival relative to non-diabetic patients. Additional studies in larger population-based cohorts are required to further explore the influence of varying exposure levels and timing and to determine which patient subgroups are most likely to benefit from metformin.

7. Metformin exposure and risk of lymph node positive or metastatic disease in patients with colorectal cancer

7.1. Introduction

There is a large body of evidence, as discussed in chapter three, which suggests that exposure to metformin, versus other anti-diabetic treatments, is associated with lower incidence of colorectal cancer. Also, some studies have suggested that metformin exposure may be associated with improved colorectal cancer survival,^{250, 251} this association having been explored in analyses described in chapter six. These observations follow preclinical studies which have found that metformin inhibits the growth of colon cancer cells in vitro and in vivo.^{161, 168}

As discussed previously in section 3.2.3, mechanisms proposed for the anti-cancer effect of metformin include reduction of hyperinsulinaemia and inhibition of mTOR, both of which actions inhibit cancer cell proliferation.⁴⁷ Recently a number of studies have also suggested that metformin may have a specific role in inhibiting tumour dissemination and metastasis in various cell lines.⁴¹⁸⁻⁴²⁰ Mechanisms suggested for such effects include the suppression of metastasis-associated proteins,^{421, 422} the inhibition of tumour stem cell function⁴²³ and the reduction of angiogenesis via mTOR inhibition.^{20, 424} Indeed, the importance of mTOR in tumour metastasis has recently been extensively described.⁴²⁵ These in vitro observations suggest the hypothesis that metformin may function to inhibit metastasis of tumours to lymph nodes or distant sites in a clinical setting.

The presence of lymph node and/or distant metastases is one of the most important predictors of outcome in colorectal cancer, determining both treatment decisions and survival prognosis.⁴²⁶ In this study we sought to investigate, for the first time, associations between metformin exposure prior to colorectal cancer diagnosis and the risk of presenting with lymph node positive disease or distant metastases at the time of diagnosis.

7.2. Methods

7.2.1. Study design

Patients eligible for this study comprised the basic study cohort as described in section 5.2.1, i.e., patients over the age of 18 diagnosed with colorectal cancer (ICD-10 C18-C20) between

January 1st 2001 and December 31st 2006 inclusive. Patients were excluded from the cohort if they had a prior history of an invasive cancer other than non-melanoma skin cancer, or if they did not have GMS eligibility for the full year prior to colorectal cancer diagnosis. Unlike in chapter six, this study also included patients who were diagnosed at autopsy as survival time was not relevant to this analysis. Patients with known stage I-IV disease (pathologic or clinical staging¹⁹,) were selected for inclusion in analyses.

Cohort members were divided into two groups: “diabetic” and “non-diabetic”. Individuals were classed as diabetic if they were identified through the pharmacy claims data to have received a supply of at least one anti-diabetic drug (ADD: WHO ATC therapeutic subgroup A10³⁷⁰) in the year prior to colorectal cancer diagnosis. All other cohort members were classified as non-diabetic.

7.2.2. Exposure Definition

Metformin exposure was identified from linked prescription refill data using WHO-ATC drug codes (Appendix Three). As in chapter six, exposure (yes/no) was defined according to whether or not the patient had a supply of metformin available to them at any point in the year prior to colorectal cancer diagnosis. Metformin dosing intensity was calculated as per section 5.2.3.³⁷⁶ This was presented as a percentage and stratified as ‘low’ or ‘high’ at the median.

7.2.3. Outcomes and comparisons

The study outcome was the presence of lymph node metastases and/or distant metastases at the time of colorectal cancer diagnosis (henceforth ‘disseminated colorectal cancer’). Information on pathological and clinical assessment of nodal/distant metastatic involvement was taken from the NCRI database. Patients were classified as having ‘disseminated colorectal cancer’ (yes/no) if they had a nodal status of N1 or N2 at diagnosis or a metastatic status of M1. In these analyses, patients with unknown nodal status (11.6% in both metformin exposed and unexposed groups) were also classified as having ‘disseminated colorectal cancer’; sensitivity analyses were conducted around this (see below).

The primary analyses were conducted within the diabetic subgroup; outcomes were compared between diabetics receiving metformin and a reference group comprising diabetics not receiving metformin. Secondary analyses were conducted such that diabetics receiving metformin were compared to non-diabetics. As in chapter six, this was to address the concern that studies among diabetics may be biased due to differences in the severity of diabetes or

the effectiveness of diabetes control between patients receiving metformin versus non-metformin anti-diabetic drugs.^{394, 410}

7.2.4. Covariates

Covariates related to patients' socio-demographic information, tumour details and cancer treatment status were collected as described in section 6.2.4. Tumour details included: AJCC stage (I, II, III, IV), T-stage (1/2, 3, 4),¹⁹ tumour grade (well/moderately differentiated, poorly differentiated, unspecified), site (colon, rectum; Appendix Three), morphology (adenocarcinoma, other; Appendix Three), and year of diagnosis (categorical). Linked prescription refill data was used to identify exposure (yes/no; Appendix Three) to non-metformin anti-diabetic drugs (sulfonylureas, insulin, other anti-diabetic drugs (thiazolidinediones, DPP4 inhibitors, meglitinides and alpha glucosidase inhibitors)) in the year prior to diagnosis. Exposure to aspirin was also considered due to recent evidence suggesting a link between aspirin exposure and reduced development of metastases.²⁹⁴ A comorbidity score was calculated for each patient as described in section 5.2.4.³⁷⁸

7.2.5. Statistical Analysis

Patient characteristics were tabulated for diabetics according to metformin exposure status (yes/no), and for these groups versus non-diabetics, and possible differences between the exposure groups were explored using the Wilcoxon rank-sum test for continuous variables (as the data were non-normally distributed) and Pearson's chi-squared test for categorical variables.

Within the diabetic subgroup, univariate and adjusted logistic regression models (SAS® PROC LOGISTIC) were used to estimate odds ratios (OR) with 95% confidence intervals (CI) for associations between metformin exposure (versus no metformin exposure) and 'disseminated colorectal cancer'. Prior knowledge, literature review and causal diagrams were used to identify potential covariates for inclusion in the multivariate model.^{385, 386, 426} The causal diagram, or directed acyclic graph, which depicts possible confounding relationships between the covariates in question, is presented in Appendix Five. The final multivariate model was then selected using backwards elimination based on a maximum cumulative change in the risk estimate of 10%.^{389, 416} Analyses were also conducted stratifying by metformin dosing intensity and by receipt of metformin exclusively or in combination with non-metformin anti-diabetic drugs. Finally, analyses were repeated as above in the full cohort with non-diabetic patients as the reference group.

T-stage was held fixed in the multivariate model as a specific measure to reduce the potential for detection bias due to possible increased colonoscopy use by metformin users. It was previously reported that, among treated diabetic patients, metformin users are slightly more likely to undergo a colonoscopy than sulfonylurea users,³⁹¹ though it is unclear whether this association holds in other healthcare systems. Where analyses are adjusted for T-stage, which reflects the depth of invasion, or size, of a tumour, it is likely that any early detection bias due to colonoscopy use would be minimised; previous research in other cancers has found that, for a given tumour size, the risk of disseminated disease is not significantly affected by detection method.⁴²⁷

All analyses were performed using SAS®, version 9.2 (SAS® Institute Inc, Cary, North Carolina). A two-sided *P*-value of <0.05 was considered statistically significant.

7.2.6. Sensitivity Analysis

As patients with unknown nodal status were coded as 'disseminated disease' in the primary analysis, analyses were carried out to test the sensitivity of the results to this assumption. The outcome was reclassified such that only patients with known positive nodal status and/or known positive distant metastases were classed as having 'disseminated disease' and all analyses within the diabetic subgroup were repeated using this outcome definition.

7.3. Results

7.3.1. Characteristics of the study cohort

A flow chart illustrating selection of the cohort is presented in Figure 7.1. Patient characteristics for the diabetic subgroup, classified as metformin exposed (n=241) or unexposed (n=129), are compared in Table 7.1. No significant differences were observed between the metformin exposed and unexposed patients with respect to tumour-related or socio-demographic characteristics. Significant differences were only observed for drug exposures; as would be expected, sulfonylurea use and insulin use was higher in the metformin unexposed group. There was a non-significant higher prevalence of aspirin use in the metformin exposed group (66% versus 57%, *p*=0.06). The median dosing intensity for metformin in the year prior to diagnosis was 92% (IQR 59, 100).

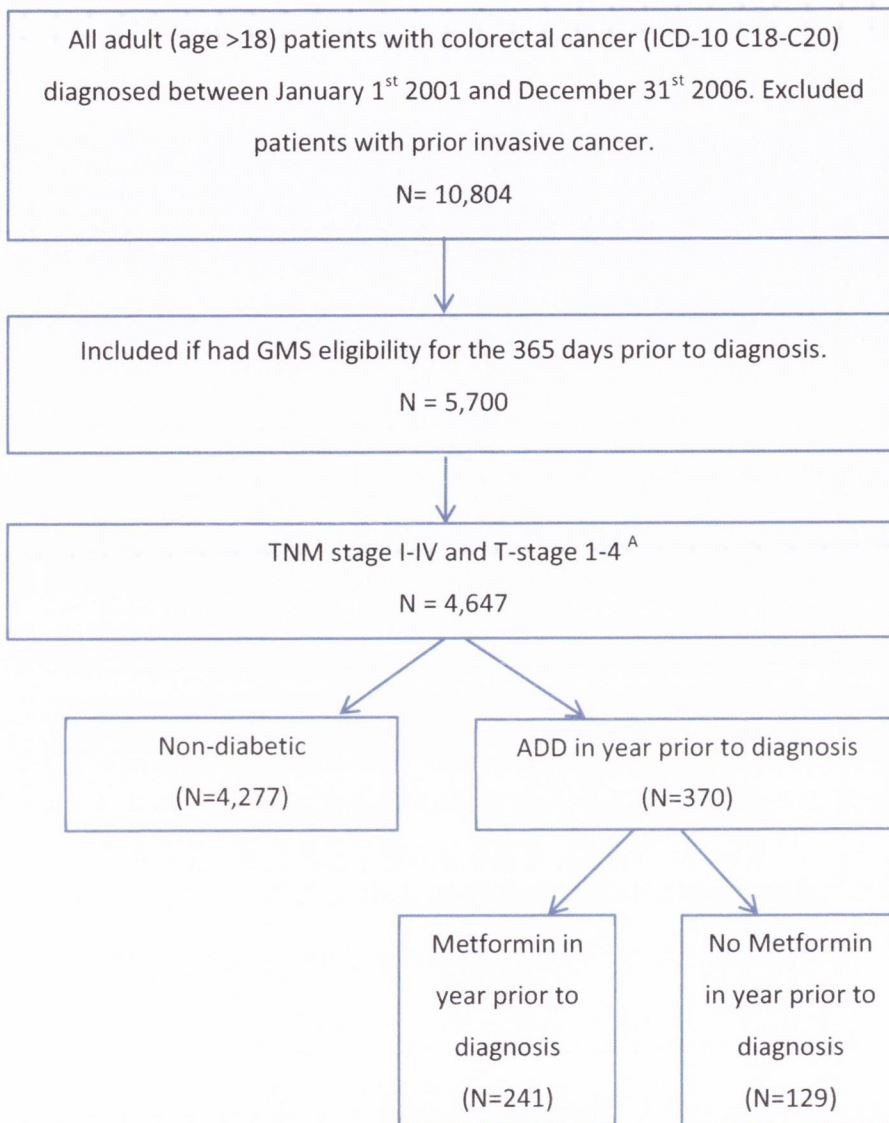
Details of metformin exposure in the year prior to diagnosis are listed in Table 7.2.

7.3.2. Metformin and disseminated disease: diabetic subgroup

The results from multivariate analyses within the diabetic subgroup are presented in Table 7.3. Exposure to metformin, versus non-metformin anti-diabetic drugs, was associated with a one

third reduction in the odds of disseminated disease (OR=0.66, 95% CI 0.39-1.12), though the association was not statistically significant. When stratified by high and low dosing intensities, by co-prescription with non-metformin anti-diabetic drugs, or by both of these factors, associations remained non-significant. However, the reduction in odds of disseminated disease was more pronounced within the high intensity metformin exposure strata (high intensity metformin versus non-metformin diabetic medication: OR=0.60, 95% CI 0.33 – 1.09), and particularly among those who received high intensity metformin and no other anti-diabetic medications (OR=0.52, 95% CI 0.25 – 1.10).

For reference, the milligram dosage range of metformin for the exposure categories referred to above is illustrated using boxplots in Figure 7.2. As in chapter six, dosage intensity corresponded to milligram dosage as median average daily metformin dosages were similar between high metformin groups and also between low metformin intensity groups.



a) AJCC Cancer Staging Manual 6th Edition. Springer, 2002.

GMS: General Medical Services Scheme.

ADD: Anti-diabetic drug

Figure 7.1: Flow chart of patient selection into the study

Table 7.1: Characteristics of the study cohort according to diabetic status and exposure to metformin

Characteristic	Any Metformin Exposure in Year Prior to Diagnosis			P-value	P-value	
	Non-diabetic	Diabetic unexposed to metformin	Diabetic exposed to metformin			
	(n=4,277)	(n=129)	(n=241)	Diabetic unexposed v exposed	Non-diabetic v diabetic exposed, unexposed	
Patient Details						
Age – Median (IQR)	Years	75 (69, 80)	75 (70, 79)	74 (70, 79)	0.48	0.59
Comorbidity – Median (IQR)	N drug classes	8 (5, 13)	14 (9, 19)	15 (19, 35)	0.34	<0.001
Gender – (%)	Male	2,315 (54.1)	84 (65.1)	155 (64.3)	0.88	<0.001
Smoking status – (%)	Current	698 (16.3)	12 (9.3)	21 (8.7)	0.69	0.01
	Former	1,863 (43.6)	56 (43.4)	119 (49.4)		
	Never	879 (20.6)	35 (27.1)	54 (22.4)		
	Unspecified	837 (19.6)	26 (20.2)	47 (19.5)		
Socioeconomic status – (%)	1 (Least deprived)	589 (13.8)	13 (10.1)	26 (10.8)	0.69	0.66
	2	485 (11.3)	17 (13.2)	30 (12.5)		
	3	570 (13.3)	22 (17.1)	30 (12.5)		
	4	749 (17.5)	23 (17.8)	36 (14.9)		
	5 (Most deprived)	1,574 (36.8)	47 (36.4)	100 (41.5)		
	Unspecified	310 (7.3)	7 (5.4)	19 (7.9)		
Tumour Details						
TNM Stage – (%)	1	668 (15.6)	23 (17.8)	36 (14.9)	0.45	0.57
	2	1,519 (35.5)	38 (29.5)	87 (36.1)		
	3	1,334 (31.2)	44 (34.1)	84 (34.9)		
	4	756 (17.7)	24 (18.6)	34 (14.1)		
T-stage – (%)	1	270 (6.3)	6 (4.7)	6 (2.5)	0.27	0.12
	2	586 (13.7)	25 (19.4)	39 (16.2)		
	3	2,386 (55.8)	66 (51.2)	147 (61.0)		
	4	1,035 (24.2)	32 (24.8)	49 (20.3)		

Table 7.1 (Continued)

Characteristic	Any Metformin Exposure in Year Prior to Diagnosis			P-value	P-value	
	Non-diabetic	Diabetic unexposed to metformin	Diabetic exposed to metformin			
	(n=4,277)	(n=129)	(n=241)			
Tumour Details						
N-stage – (%)	0	2,018 (47.2)	56 (43.4)	111 (46.1)	0.24	0.49
	1	1,140 (26.7)	41 (31.8)	56 (23.2)		
	2	663 (15.5)	17 (13.2)	46 (19.1)		
	Unspecified	456 (10.7)	15 (11.6)	28 (11.6)		
M-stage – (%)	0	2,349 (54.9)	65 (50.4)	139 (57.7)	0.35	0.53
	1	756 (17.7)	24 (18.6)	34 (14.1)		
	Unspecified	1,172 (27.4)	40 (31.0)	68 (28.2)		
Grade – (%)	Well/moderate	3,145 (73.5)	85 (65.9)	177 (73.4)	0.28	0.21
	Poorly differentiated	563 (13.2)	18 (14.0)	29 (12.0)		
	Unspecified	569 (13.3)	26 (20.2)	35 (14.5)		
Site – (%)	Colon (v rectum)	2,760 (64.5)	95 (73.6)	167 (69.3)	0.38	0.04
Morphology – (%)	Adenocarcinoma	3,630 (84.9)	108 (83.7)	202 (83.8)	0.98	0.85
	Other	647 (15.1)	21 (16.3)	39 (16.2)		
Year of Diagnosis – (%)	2001	565 (13.2)	17 (13.2)	24 (10.0)	0.47	0.45
	2002	627 (14.7)	20 (15.5)	31 (12.9)		
	2003	728 (17.0)	24 (18.6)	39 (16.2)		
	2004	789 (18.5)	15 (11.6)	46 (19.1)		
	2005	828 (19.4)	24 (18.6)	50 (20.8)		
	2006	740 (17.3)	29 (22.5)	51 (21.2)		
Drug exposures ^A – (%)	Sulfonylureas	-	99 (76.7)	130 (53.9)	<0.001	-
	Insulin	-	29 (22.5)	19 (7.9)	<0.001	-
	Other ADDs	-	11 (8.5)	35 (14.5)	0.10	-
	Aspirin	1,446 (33.8)	73 (56.6)	160 (66.4)	0.06	<0.001

IQR: Interquartile range

ADD: anti-diabetic drug

(a) Exposures in year prior to diagnosis.

Table 7.2: Details of metformin exposure in exposed patients - main analysis cohort.

Metformin exposure information for year prior to colorectal cancer diagnosis				
Number of patients exposed			241	
Dosing Intensity – median (IQR) ^a	%		92	(59, 100)
Mean daily dose – median (IQR) ^b	mg/day	1,290		(685, 1,700)

IQR: Interquartile range

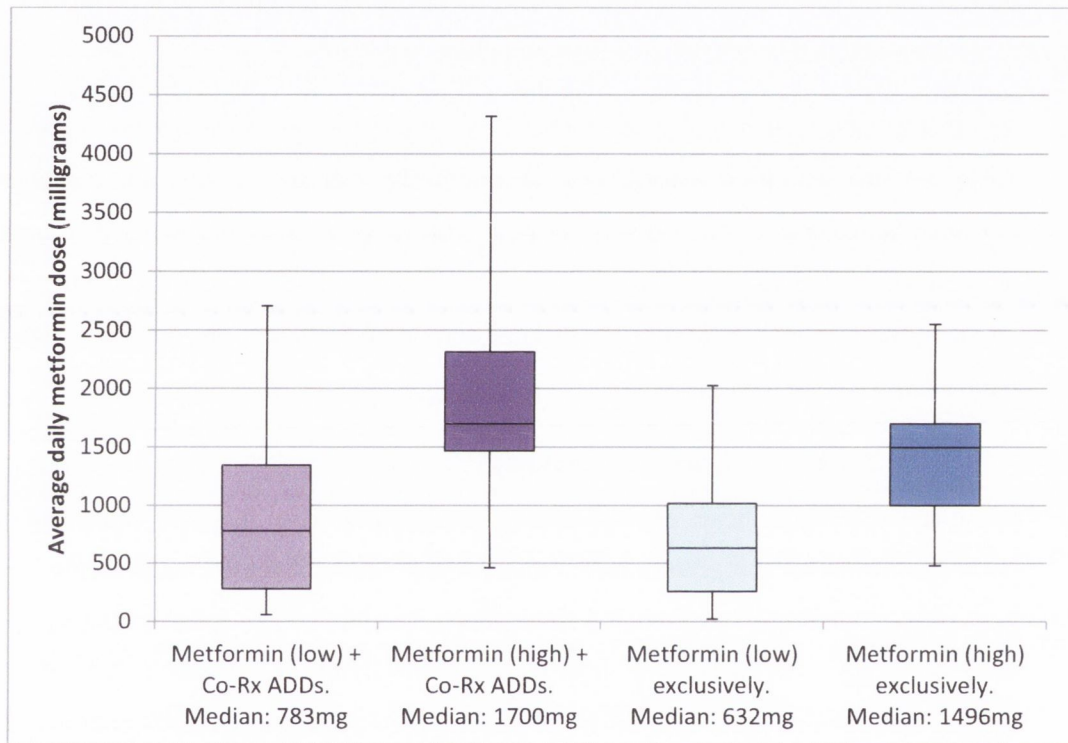
- a) Exposure intensity calculated as number of days with supply available in year prior to diagnosis, divided by 365 and expressed as a percentage.
- b) Mean daily dose calculated as cumulative dose in year prior to diagnosis, divided by 365.

Table 7.3: Diabetic subgroup; Unadjusted and adjusted odds ratios for metformin exposure and disseminated colorectal cancer

Exposure Information		Odds of Disseminated Disease		
Main Analysis ^A	N (N=370)	N Disseminated (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
No Metformin	129	78 (60.5)	(Ref)	(Ref)
Any Metformin	241	135 (56.0)	0.83 (0.54-1.29)	0.66 (0.39-1.12)
Stratified Analyses				
Dosing Intensity^A				
Any Metformin (Low)	119	69 (58.0)	0.90 (0.54-1.50)	0.73 (0.40-1.34)
Any Metformin (High)	122	66 (54.1)	0.77 (0.47-1.27)	0.60 (0.33-1.09)
Co-prescription^B				
Metformin + Co-Rx ADDs	149	88 (59.1)	0.94 (0.58-1.53)	0.91 (0.53-1.54)
Metformin exclusively	92	47 (51.1)	0.68 (0.40-1.17)	0.67 (0.37-1.21)
Dosing Intensity & Co-prescription^B				
Metformin (Low) + Co-Rx ADDs	77	46 (59.7)	0.97 (0.55-1.73)	0.95 (0.50-1.80)
Metformin (High) + Co-Rx ADDs	72	42 (58.3)	0.92 (0.51-1.65)	0.87 (0.46-1.65)
Metformin (Low) exclusively	42	23 (54.8)	0.79 (0.39-1.60)	0.88 (0.41-1.91)
Metformin (High) exclusively	50	24 (48.0)	0.60 (0.31-1.17)	0.52 (0.25-1.10)

Ref: reference. ADD: anti-diabetic drug. OR: odds ratio. CI: confidence interval. Low: low dosing intensity. High: high dosing intensity.

- a) Adjusted for T-stage, tumour grade, year of diagnosis, socioeconomic status, aspirin use, exposure to non-metformin ADDs (sulfonylureas yes/no, insulin yes/no, other ADDs yes/no).
- b) Adjusted for T-stage, tumour grade, year of diagnosis, socioeconomic status, aspirin use. 'Co-Rx' ADDs include sulfonylureas, insulin and/or other ADDs.



ADD: anti-diabetic drug. 'Low' and 'high' refer to dosage intensity. Co-Rx ADDs include sulfonylureas, insulin and/or other ADDs.

Figure 7.2: Box-plot of metformin dose

7.3.3. Metformin and disseminated disease: full cohort

Characteristics of non-diabetic patients (n=4,277) as compared to diabetic subgroup (metformin exposed and unexposed) are compared in Table 7.1. Significant differences were observed between the diabetic and non-diabetic groups for gender, comorbidity level, smoking status, tumour site and aspirin use. As observed in chapter six, male gender was more prevalent in diabetic cancer patients than in non-diabetics and comorbidity and aspirin use were higher among the diabetic patients than the non-diabetics. More non-diabetics than diabetics were recorded as 'current' smokers. Colon cancer (as opposed to rectal cancer) was more prevalent in diabetic patients than non-diabetic patients.

Results from analyses including non-diabetic patients as the reference group are presented in Table 7.4. No significant associations for metformin exposure versus non-diabetics were observed across any strata in these analyses; odds ratios for patients exposed to metformin were all close to unity, with the exception of the odds ratio for exclusive exposure to high intensity metformin, which was reduced by one-quarter but did not approach significance (Table 7.4).

7.3.4. Sensitivity Analyses

Sensitivity analyses using an altered outcome classification such that only confirmed positive nodal status/distant metastases represented 'disseminated disease' are presented in Table 7.5(diabetic subgroup) and Table 7.6 (full cohort). Results broadly followed the same trends observed in the main analysis. However, a statistically significant association was observed in the case of patients who received high intensity metformin and no additional anti-diabetic treatment, as compared to diabetic patients who did not receive metformin OR 0.40, 95% CI 0.19 – 0.85) (Table 7.5). Results from analyses where non-diabetics served as the reference group did not show any significant associations between metformin and disseminated disease (Table 7.6), although, relative to non-diabetics, those receiving high intensity metformin and no other anti-diabetic treatment had lower odds of disseminated disease, approaching significance (OR 0.58, 95% CI 0.32-1.08).

Table 7.4: Full cohort; Unadjusted and adjusted odds ratios for metformin exposure and disseminated colorectal cancer

Exposure Information		Odds of Disseminated Disease		
Main Analysis ^A	N (N=4,647)	N Disseminated (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Non-diabetic	4,277	2,385 (55.8)	(Ref)	(Ref)
No Metformin	129	78 (60.5)	1.21 (0.85-1.74)	1.41 (0.78-2.54)
Any Metformin	241	135 (56.0)	1.01 (0.78-1.31)	1.07 (0.71-1.61)
Stratified Analyses				
Dosing Intensity^A				
Any Metformin (Low)	119	69 (58.0)	1.10 (0.76-1.58)	1.19 (0.72-1.97)
Any Metformin (High)	122	66 (54.1)	0.94 (0.65-1.34)	0.97 (0.61-1.57)
Co-prescription^B				
Metformin + Co-Rx ADDs	149	88 (59.1)	1.14 (0.82-1.60)	1.25 (0.88-1.78)
Metformin exclusively	92	47 (51.1)	0.83 (0.55-1.25)	0.88 (0.57-1.35)
Dosing Intensity & Co-prescription^B				
Metformin (Low) + Co-Rx ADDs	77	46 (59.7)	1.18 (0.74-1.86)	1.35 (0.83-2.18)
Metformin (High) + Co-Rx ADDs	72	42 (58.3)	1.11 (0.69-1.78)	1.15 (0.70-1.90)
Metformin (Low) exclusively	42	23 (54.8)	0.96 (0.52-1.77)	1.08 (0.57-2.04)
Metformin (High) exclusively	50	24 (48.0)	0.73 (0.42-1.28)	0.73 (0.41-1.32)

Ref: reference. **ADD:** anti-diabetic drug. **OR:** odds ratio. **CI:** confidence interval. **Low:** low dosing intensity. **High:** high dosing intensity.

- a) Adjusted for T-stage, tumour grade, year of diagnosis, aspirin use, exposure to non-metformin ADDs (sulfonylureas yes/no, insulin yes/no, other ADDs yes/no).
- b) Adjusted for T-stage, tumour grade, year of diagnosis, aspirin use. 'Co-Rx' ADDs include sulfonylureas, insulin and/or other ADDs.

Table 7.5: Sensitivity Analysis (altered outcome classification). Diabetic subgroup; Unadjusted and adjusted odds ratios for metformin exposure and disseminated colorectal cancer

Exposure Information		Odds of Disseminated Disease ^c		
Main Analysis ^A	N (N=370)	N Disseminated (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
No Metformin	129	68 (52.7)	(Ref)	(Ref)
Any Metformin	241	118 (49.0)	0.86 (0.56-1.32)	0.69 (0.41-1.16)
Stratified Analyses				
Dosing Intensity^A				
Any Metformin (Low)	119	62 (52.1)	0.98 (0.59-1.61)	0.78 (0.43-1.40)
Any Metformin (High)	122	56 (45.9)	0.76 (0.46-1.25)	0.61 (0.34-1.11)
Co-prescription^B				
Metformin + Co-Rx ADDs	149	78 (52.4)	0.99 (0.62-1.58)	0.97 (0.57-1.62)
Metformin exclusively	92	40 (43.5)	0.69 (0.40-1.18)	0.62 (0.34-1.12)
Dosing Intensity & Co-prescription^B				
Metformin (Low) + Co-Rx ADDs	77	40 (52.0)	0.97 (0.55-1.71)	0.94 (0.50-1.75)
Metformin (High) + Co-Rx ADDs	72	38 (52.8)	1.00 (0.56-1.79)	1.01 (0.53-1.90)
Metformin (Low) exclusively	42	22 (52.4)	0.99 (0.49-1.98)	1.00 (0.47-2.16)
Metformin (High) exclusively	50	18 (36.0)	0.51 (0.26-0.99)	0.40 (0.19-0.85)

Ref: reference. **ADD:** anti-diabetic drug. **OR:** odds ratio. **CI:** confidence interval. **Low:** low dosing intensity. **High:** high dosing intensity.

- a) Adjusted for T-stage, tumour grade, year of diagnosis, aspirin use, exposure to non-metformin ADDs (sulfonylureas yes/no, insulin yes/no, other ADDs yes/no).
- b) Adjusted for T-stage, tumour grade, year of diagnosis, aspirin use. 'Co-Rx' ADDs include sulfonylureas, insulin and/or other ADDs.
- c) 'Disseminated disease' includes N-stage of 1 or 2, or M-stage of 1.

Table 7.6: Sensitivity Analysis (altered outcome classification). Full cohort; Unadjusted and adjusted odds ratios for metformin exposure and disseminated colorectal cancer.

Exposure Information		Odds of Disseminated Disease		
Main Analysis ^A	N (N=4,647)	N Disseminated (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Non-diabetic	4,277	2,090 (48.9)	(Ref)	(Ref)
No Metformin	129	68 (52.7)	1.17 (0.82-1.66)	1.38 (0.76-2.50)
Any Metformin	241	118 (49.0)	1.00 (0.78-1.30)	1.00 (0.67-1.51)
Stratified Analyses				
Dosing Intensity^A				
Any Metformin (Low)	119	62 (52.1)	1.14 (0.79-1.64)	1.13 (0.69-1.86)
Any Metformin (High)	122	56 (45.9)	0.89 (0.62-1.28)	0.91 (0.56-1.46)
Co-prescription^B				
Metformin + Co-Rx ADDs	149	78 (52.4)	1.15 (0.83-1.60)	1.23 (0.87-1.75)
Metformin exclusively	92	40 (43.5)	0.81 (0.53-1.22)	0.83 (0.53-1.29)
Dosing Intensity & Co-prescription^B				
Metformin (Low) + Co-Rx ADDs	77	40 (52.0)	1.13 (0.72-1.78)	1.22 (0.76-1.98)
Metformin (High) + Co-Rx ADDs	72	38 (52.8)	1.17 (0.73-1.87)	1.24 (0.76-2.04)
Metformin (Low) exclusively	42	22 (52.4)	1.15 (0.63-2.12)	1.23 (0.65-2.33)
Metformin (High) exclusively	50	18 (36.0)	0.59 (0.33-1.05)	0.58 (0.32-1.08)

Ref: reference. ADD: anti-diabetic drug. OR: odds ratio. CI: confidence interval. Low: low dosing intensity. High: high dosing intensity.

- a) Adjusted for T-stage, tumour grade, year of diagnosis, aspirin use, exposure to non-metformin ADDs (sulfonylureas yes/no, insulin yes/no, other ADDs yes/no).
- b) Adjusted for T-stage, tumour grade, year of diagnosis, aspirin use. 'Co-Rx' ADDs include sulfonylureas, insulin and/or other ADDs.
- c) 'Disseminated disease' includes N-stage of 1 or 2, or M-stage of 1.

7.4. Discussion

This study examined associations between metformin exposure prior to diagnosis and likelihood of presenting with disseminated colorectal cancer, that is, lymph node positive or metastatic disease. In primary analyses of diabetic patients only, the association for overall metformin exposure (versus non-metformin anti-diabetic drugs) was not statistically significant, though the odds of disseminated disease were reduced by a third. Associations observed in the secondary analysis, including non-diabetics as the reference group, were also non-significant. Results from analyses stratified by metformin dosing intensity, co-prescription with non-metformin anti-diabetic drugs, or a combination of these factors, suggested the possibility of a greater reduction in odds of disseminated disease in patients with high intensity exclusive metformin use. The results were broadly unchanged in sensitivity analyses, although the association between high-intensity exclusive metformin use and disseminated colorectal cancer among diabetes reached statistical significance.

The potential of metformin to inhibit tumour spread is supported by a number of preclinical studies;²⁰ in vitro evidence has found that metformin can inhibit colon cancer cell migration¹⁶² and a range of potential mechanisms for such anti-metastatic effects have been proposed.²⁰ Also, a recent study found that establishment of colorectal cancer metastasis in vivo was completely abolished with targeted inhibition of mTORC1 and mTORC2.⁴²⁸ As metformin is a recognised inhibitor of mTORC1,⁴²⁹ these recent findings suggest a potential mechanism of action for the drug in the preclinical setting. However, the plausibility of such effects of metformin as a clinical treatment has been questioned due to the fact that concentrations of the drug in preclinical studies often far exceed those achieved in diabetes patients.⁴⁷ In light of this, it is therefore noteworthy, that the strongest suggestions of associations between metformin use and reduced risk of disseminated disease in this study were observed at high dosing intensities. Thus, the possibility of a benefit in patients exposed to high intensity metformin monotherapy cannot be excluded.

This study is the first, to the authors' knowledge, to formally assess the presence of an association between metformin use and disseminated disease at diagnosis among colorectal cancer patients with diabetes. Previous studies have examined associations between metformin exposure and prostate cancer characteristics.^{430, 431} Hitron et al. studied the influence of metformin and other anti-diabetic therapies on grade of prostate cancer at diagnosis.⁴³⁰ Patients receiving any of metformin, thiazolidinediones and/or low-dose insulin were compared to patients receiving sulfonylureas and/or high dose insulin, and were also

compared to non-diabetics. Those in the group including metformin patients were found to have a non-significant lower risk of presenting with a high grade tumour when compared to either of the latter groups (versus sulfonylureas/high-dose insulin: OR=0.61, p=0.32; versus non-diabetics: OR=0.61, p=0.35).⁴³⁰ Margel et al. also studied the influence of metformin, versus other anti-diabetic therapies, on prostate cancer grade, and found no association.⁴³¹ Additional studies have examined associations between metformin and metastasis by performing univariate comparisons; a recent study examining diabetic patients with breast cancer reported that metformin-treated patients had a higher rate of lymph node positivity than non-metformin treated diabetics.²²³ In this study, a lack of lymph node metastasis was found in 49% of metformin-treated patients, 55% of non-metformin treated patients, and 57% of non-diabetics (p<0.001).²²³ Similarly a study of diabetic patients with lung cancer found that those exposed to metformin prior to diagnosis were more likely to present with metastatic disease than those not receiving metformin.²²⁸ However, the effect of metformin exposure on tumour progression following cancer diagnosis has also been examined in a number of observational studies, all of which suggested lower risks of progression or distant metastasis following cancer diagnosis among those exposed to metformin versus other drugs.^{220, 226, 227, 430, 432} The present study adds to the knowledge base by providing data from a comprehensive analysis of associations between metformin and colorectal cancer metastasis at diagnosis.

This study has a number of strengths. It assessed associations between metformin exposure and disseminated colorectal cancer at diagnosis using high-quality, national, prospectively-collected cancer data collected by trained tumour registration officers, who follow standard international protocols for identification of cases and abstraction of data. Access to linked prospective pharmacy claims data in these analyses provided detailed, objective, longitudinal exposure data which is not influenced by recall bias, and this strength has enabled the examination of the effects of continuous exposure to metformin. While non-compliance with received treatment may have resulted in exposure misclassification, this would usually be expected to bias results towards the null. Study limitations that must be considered include the possibility of early detection bias, due to potential increased colonoscopy use by metformin users, as discussed in section 7.2.5. However, adjustment of analyses for T-stage within this study is expected to have minimised the likelihood of this bias occurring. Remaining limitations of the study include the absence of information on the number of evaluated lymph nodes; insufficient lymph node evaluation has been associated with misclassification of nodal status.⁴³³ Also, the absence of information relating to diabetes duration or severity necessitated defining diabetic patients by the receipt of anti-diabetic drug therapy. Finally, the

results from this study should be interpreted with caution given the small sample size within subgroup analyses and possible limited statistical power for some associations.

In conclusion, this study was unable to confirm whether previously observed preclinical benefits of metformin in preventing colorectal tumour dissemination are demonstrable among diabetic patients taking metformin. Odds of disseminated disease were moderately reduced among metformin users, particularly in the case of high intensity metformin monotherapy, compared to those using other diabetic medications. However, associations in the primary analyses were not statistically significant. Given the importance of lymph node status and the presence of distant metastases at diagnosis in colorectal cancer prognosis, further investigation of this potential association among larger patient groups is warranted.

8. Aspirin exposure and survival in patients with non-metastatic colorectal cancer

8.1. Introduction

As discussed in section 4.13, evidence has emerged in recent years to suggest that aspirin exposure is associated with a survival benefit among patients with colorectal cancer. Analyses of randomised clinical trials of aspirin for cardiovascular disease have found a significant reduction in the long-term risk of fatal colorectal cancer among patients exposed to aspirin.³¹⁷ Additionally, several observational studies have found an association between aspirin exposure and improved survival among patients with established colorectal cancer (Table 4.1 and Table 4.2, section 4.13).

The mechanisms of action by which aspirin may reduce cancer mortality are not fully understood.²⁹⁵ However, it is increasingly thought that the anti-platelet effect of aspirin is responsible for reduced development of distant metastases.²⁹⁵ Data from cardiovascular trials have been studied to determine associations between aspirin exposure and risk of metastasis development following colorectal cancer diagnosis. Among patients who presented with non-metastatic colorectal cancer (stage I-III), previous exposure to aspirin was found to decrease the risk of developing distant metastases after diagnosis.²⁹⁴ As the vast majority of cancer deaths result from metastases,^{434, 435} we hypothesised that exposure to aspirin prior to diagnosis may reduce the risk of death from colorectal cancer in patients with non-metastatic disease at the time of diagnosis.

In this study, we investigated associations between pre-diagnostic aspirin exposure and colorectal cancer-specific mortality primarily in a population with non-metastatic colorectal cancer. We also examined how such associations were affected by the following factors: (i) tumour location (proximal colon, distal colon or rectum); (ii) aspirin dosage; (iii) frequency of aspirin exposure in the year prior to diagnosis.

8.2. Methods

8.2.1. Study design

Patients eligible for this study comprised the basic study cohort as described in section 5.2.1: patients over the age of 18 diagnosed with colorectal cancer (ICD-10 C18-C20) between January 1st 2001 and December 31st 2006 inclusive. Patients were excluded from the cohort if their colorectal cancer was diagnosed at autopsy, if they had a prior history of an invasive cancer other than non-melanoma skin cancer, or if they did not have GMS eligibility for the full year prior to colorectal cancer diagnosis. Additionally, the main study was restricted to patients with a diagnosis of TNM stage I-III cancer (pathologic or clinical staging, ¹⁹), for reasons described above, and to patients between the ages of 45 and 80 inclusive. Aspirin has been recommended as a cardiopreventive agent in patients aged 45 to 80 who are at increased risk of ischaemic heart disease.⁴³⁶ Therefore, this analysis was restricted to patients within this age-group in order to provide a sample of aspirin patients who would most likely represent the appropriate prescribing of the drug. Sensitivity analyses were carried out with expansion of the cohort to adult patients of all ages, as described below.

8.2.2. Exposure Definition

Aspirin exposure was identified from linked prescription refill data using WHO ATC drug codes (Appendix Three). Exposure (yes/no) was defined according to whether or not the individual had a supply of aspirin available at any point in the year prior to colorectal cancer diagnosis. Aspirin dosing intensity was calculated as described in section 5.2.3. Stratification by aspirin dose was also performed. Patients were divided into two aspirin dose categories based on the highest single aspirin dose received in the year prior to colorectal cancer diagnosis. These included: (i) patients who received only aspirin doses of 75mg or less, and (ii) patients who received a dose of aspirin greater than 75mg in the year prior to cancer diagnosis.

8.2.3. Outcomes

Outcomes were defined as described in section 6.2.3; the primary outcome was colorectal cancer-specific survival and overall survival was examined in secondary analyses.⁴¹¹ Survival time was calculated from the date of colorectal cancer diagnosis to the first of death or end of follow-up (31st December 2010). Patients were censored if they were alive on the latter date.

8.2.4. Covariates

Covariates related to patients' socio-demographic information, tumour details and cancer treatment status were collected as described in section 6.2.4. Linked prescription refill data was used to identify exposure (yes/no; Appendix Three) to non-aspirin NSAIDs, statins, anti-diabetic drugs, and biguanides (as an individual category) in the year prior to diagnosis. A comorbidity score was calculated for each patient as described in section 5.2.4.

8.2.5. Statistical Analysis

Patient characteristics were tabulated according to aspirin exposure status (yes/no). Potential differences between the exposure groups were explored using the Wilcoxon rank-sum test for continuous variables (as these data were non-normally distributed) and Pearson's chi-square test for categorical variables. Crude survival rates for colorectal cancer-specific and overall survival were calculated as deaths per 1000 person-years.

A similar analysis approach was adopted to that described in section 6.2.5. Unadjusted and adjusted Cox Proportional Hazards models (SAS® PROC PHREG) were used to estimate hazard ratios (HR) with 95% confidence intervals (CI) for associations between aspirin exposure and colorectal cancer-specific survival. Direct adjusted Kaplan Meier curves were also estimated, as described in section 6.2.5. Covariates were identified for potential inclusion in the multivariate model, as described previously. The causal diagram, or directed acyclic graph, which illustrates potential confounding relationships among the covariates in question, is presented in Appendix Six. As described previously (sections 5.3.1 and 6.2.5), the final multivariate model was selected using backwards elimination based on a maximum cumulative change in the risk estimates of 10%. Analyses were conducted stratified by tumour site (proximal/distal/rectal) (Appendix Three). Analyses were also performed with stratification by aspirin dosage ($\leq 75\text{mg}$, $>75\text{mg}$), by dosing intensity, and by a combination of these factors.

As described previously, all analyses were performed using SAS®, version 9.2 (SAS® Institute Inc, Cary, North Carolina) and a two-sided *P*-value of <0.05 was considered statistically significant. The proportional hazards assumption for the main analysis was checked by testing for interaction with time within the SAS® model.

8.2.6. Sensitivity Analyses

As similarly performed in Chapter Six, sensitivity analyses were carried out to explore the effect of different classifications of recorded cause of death. Analyses were repeated using

'Definition 2' and 'Definition 3' of colorectal-cancer specific survival, as detailed in section 6.2.6.

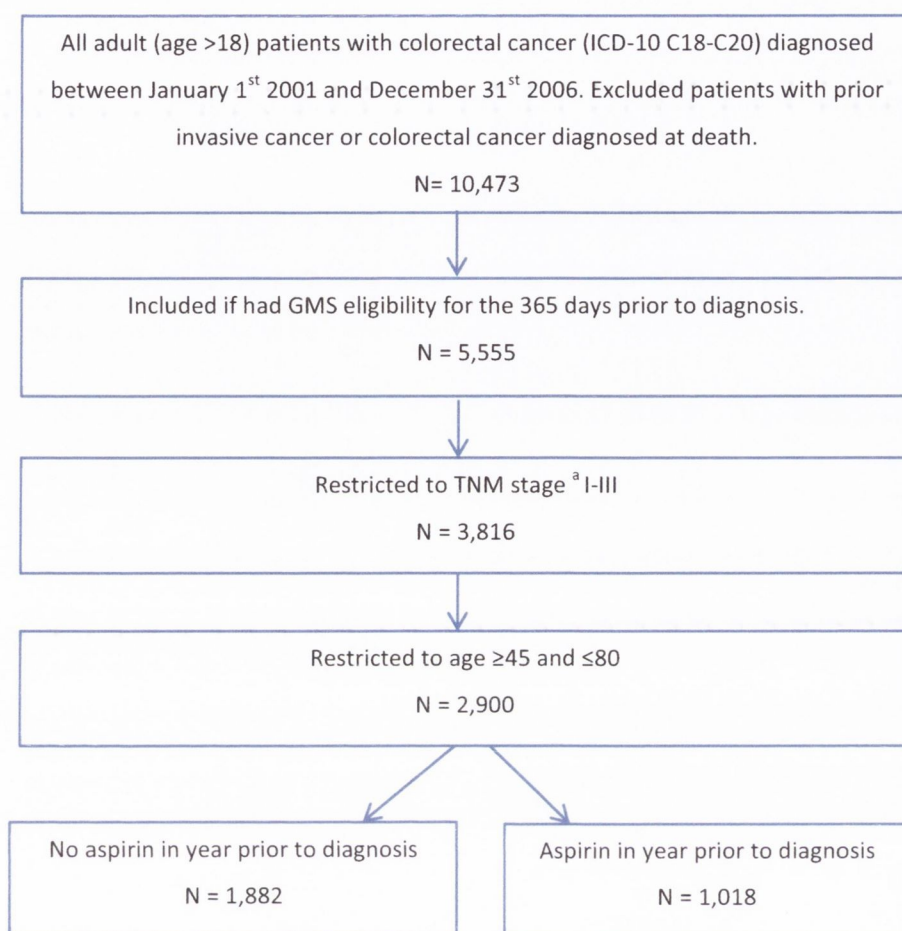
Sensitivity analyses were also carried out to explore whether associations would differ with inclusion of adult patients of all ages and all stages of colorectal cancer, as opposed to the age and stage-restricted main analysis cohort. The analyses described in section 8.2.5 were repeated in this expanded cohort. The previously described procedure for selection of covariates for the multivariate model was repeated in order to design a multivariate model for these analyses.

8.3. Results

8.3.1. Characteristics of the Study Cohort

A flow chart illustrating selection of the main analysis cohort is presented in Figure 8.1. Patient characteristics are presented according to pre-diagnostic aspirin exposure status (unexposed n=1,882; exposed n=1,018) in Table 8.1. Significant differences were found between the exposed and unexposed groups for a number of covariates. Patients exposed to aspirin, compared to unexposed patients, were more likely to be male (61% male versus 55%, $p=0.002$), and were slightly older (median age 74 years versus 72, $p<0.001$), with greater comorbidity ($p<0.001$). Differences also existed between the groups in relation to smoking status and socioeconomic status (Table 8.1). Regarding tumour-related characteristics, patients exposed to aspirin had a higher proportion of TNM stage I tumours (versus stage II or III) than unexposed patients (23% versus 19%). Correspondingly, the proportion of patients receiving chemotherapy and/or radiotherapy was lower in those exposed to aspirin than those unexposed. There were also slight differences in the year of diagnosis for patients exposed and unexposed to aspirin (Table 8.1). Regarding drug exposures, there was no difference in use of non-aspirin NSAIDs between the groups but statin use was significantly higher in the aspirin exposed group (49% versus 13%, $p<0.001$). Patients exposed to aspirin were also more likely to be receiving anti-diabetic drugs (17% versus 4%, $p<0.001$). Among aspirin exposed patients, 14% did not receive aspirin following their cancer diagnosis. Among patients without pre-diagnostic aspirin exposure, 25% went on to receive aspirin at some point between cancer diagnosis and end of follow-up.

Details of pre-diagnostic exposure are listed in Table 8.2. The median aspirin dosing intensity was 85% (IQR 41, 98). The majority of aspirin prescriptions (85%) were for doses ≤ 75 mg.



a) AJCC Cancer Staging Manual 6th Edition. Springer, 2002.

GMS: General Medical Services Scheme.

Figure 8.1: Flow chart of patient selection into the study

Table 8.1: Characteristics of the main analysis cohort

Characteristic		Aspirin in Year Prior to Diagnosis		P-value
		Unexposed (n=1,882)	Exposed (n=1,018)	
Patient Details				
Age – Median (IQR)	Years	72 (65, 76)	74 (71, 77)	<0.001
Comorbidity – Median (IQR)	N drug classes	7 (3, 11)	11 (8, 16)	<0.001
Gender – (%)	Male	1,029 (54.7)	619 (60.8)	0.002
Smoking status – (%)	Current	361 (19.2)	134 (13.2)	<0.001
	Former	831 (44.2)	428 (42.0)	
	Never	373 (19.8)	248 (24.4)	
	Unspecified	317 (16.8)	208 (20.4)	
Socioeconomic status – (%)	1 (Least deprived)	232 (12.3)	155 (15.2)	0.04
	2	203 (10.8)	124 (12.2)	
	3	245 (13.0)	139 (13.7)	
	4	319 (17.0)	180 (17.7)	
	5 (Most deprived)	747 (39.7)	344 (33.8)	
	Unspecified	136 (7.2)	76 (7.5)	
Tumour Details				
TNM Stage – (%)	I	353 (18.8)	236 (23.2)	0.01
	II	782 (41.6)	416 (40.9)	
	III	747 (39.7)	366 (36.0)	
Grade – differentiation (%)	Well/moderate	1,467 (78.0)	783 (76.9)	0.56
	Poor	206 (11.0)	125 (12.3)	
	Unspecified	209 (11.1)	110 (10.8)	
Site – (%)	Proximal colon	471 (25.0)	280 (27.5)	0.34
	Distal colon	639 (34.0)	337 (33.1)	
	Rectal	697 (37.0)	370 (36.4)	
	Unspecified	75 (4.0)	31 (3.1)	
Morphology – (%)	Adenocarcinoma	1,611 (85.6)	887 (87.1)	0.25
	Other	271 (14.4)	131 (12.9)	
Treatment ^a – (%)	Surgery	1,801 (95.7)	969 (95.2)	0.53
	Chemotherapy	799 (42.5)	297 (29.2)	<0.001
	Radiotherapy	373 (19.8)	143 (14.1)	<0.001
Year of Diagnosis – (%)	2001	295 (15.7)	116 (11.4)	0.01
	2002	251 (13.3)	148 (14.5)	
	2003	333 (17.7)	181 (17.8)	
	2004	348 (18.5)	193 (19.0)	
	2005	363 (19.3)	186 (18.3)	
	2006	292 (15.5)	194 (19.1)	
Drug exposures – (%)	NSAID ^b	701 (37.3)	411 (40.4)	0.10
	Statins ^b	245 (13.0)	499 (49.0)	<0.001
	Anti-diabetic ^b	80 (4.3)	168 (16.5)	<0.001
	Biguanide ^b	50 (2.7)	112 (11.0)	<0.001
	Aspirin post diagnosis ^c	464 (24.7)	871 (85.6)	

IQR: Interquartile range

- a) Refers to treatment received in year post diagnosis.
- b) Exposures in year prior to diagnosis.
- c) Any exposure between diagnosis and end of follow-up.

Table 8.2: Details of aspirin exposure in exposed patients: main analysis cohort

Aspirin exposure information for year prior to colorectal cancer diagnosis			
Number of patients exposed		1,018	
Number of prescriptions dispensed		9,193	
Prescription strength category – (%)	75mg	7,474	(81.3)
	300mg	1,131	(12.3)
	Other	588	(6.4)
Prescription strength category – (%)	Low (≤75mg)	7,854	(85.4)
	High (>75mg)	1,339	(14.6)
Dosing Intensity – median (IQR) ^a	%	85	(41, 98)
Mean daily dose – median (IQR) ^b	mg/day	67.3	(33.1, 75)

IQR: Interquartile range

- a) Exposure intensity calculated as number of days with supply available in year prior to diagnosis, divided by 365 and expressed as a percentage.
- b) Mean daily dose calculated as cumulative dose in year prior to diagnosis, divided by 365.

8.3.2. Survival Analyses

The results from analyses of the main study cohort (stage I-III, age ≥45 and ≤80) are presented in Table 8.3. Person-time contributed by the cohort totalled 13,130 person-years; the crude colorectal-cancer specific mortality rates for aspirin exposed and unexposed patients were 64 and 61 deaths per 1,000 person-years, respectively.

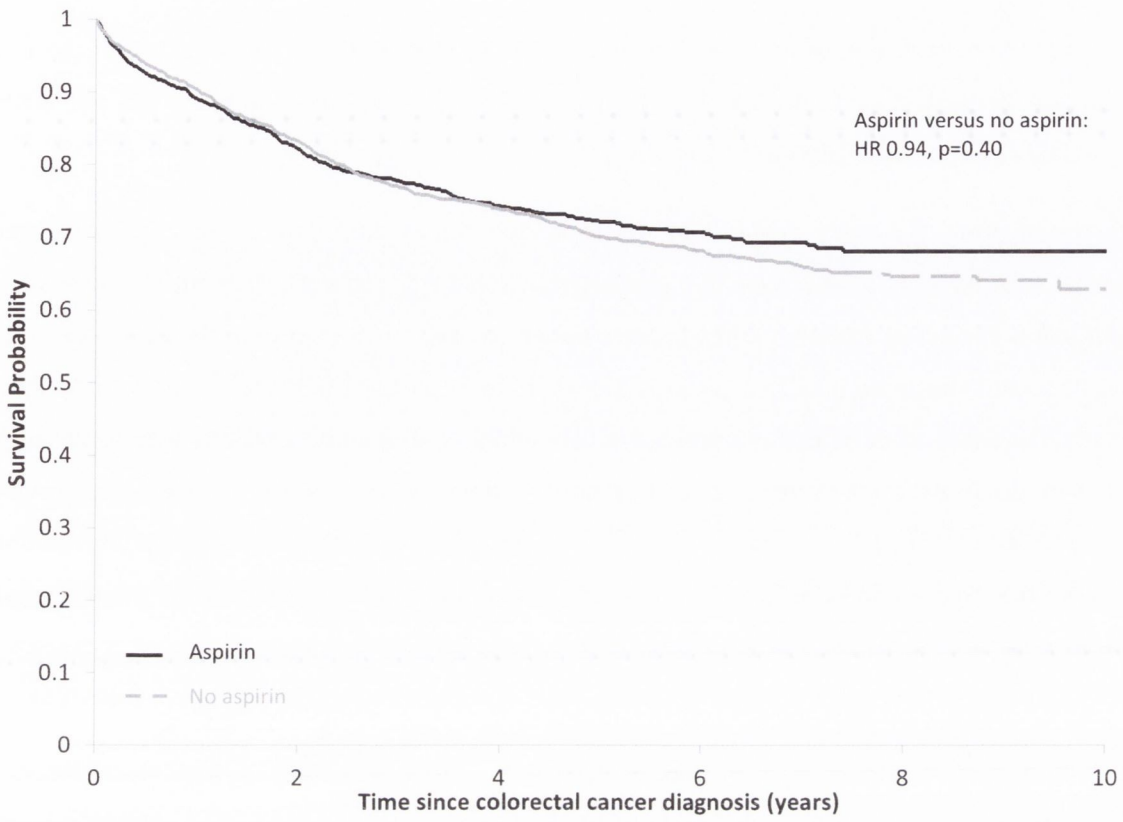
In multivariate analyses of effects on colorectal cancer-specific survival, no association was observed for overall exposure to aspirin and colorectal cancer-specific mortality (Table 8.3, Figure 8.2). Neither was an association observed for effects on overall mortality (Table 8.3). Analyses stratified by tumour site suggested that aspirin was associated with a significant colorectal cancer-specific survival improvement among patients with tumours of the proximal colon (HR=0.73, 95% CI 0.54-0.99); no association was observed among patients with distal colon or rectal tumours (Table 8.3). However, the interaction between aspirin and tumour site was not found to be statistically significant when tested in the model ($P_{interaction}=0.61$). Analyses stratified by dosage of aspirin received, by aspirin dosing intensity, or by a combination of these factors, did not result in significant associations and hazard ratios were close to unity (Table 8.3). The test for interaction with time for the main analysis was not significant, indicating that hazards were proportional.

Table 8.3: Main analysis cohort; Unadjusted and adjusted hazard ratios for aspirin exposure and mortality.

Exposure Information			Colorectal cancer-specific survival			Overall survival		
Aspirin exposure	N	Person-years	Deaths (Crude Rate) ^a	Unadjusted HR (95% CI)	Adjusted HR (95% CI) ^b	Deaths (Crude Rate) ^a	Unadjusted HR (95% CI)	Adjusted HR (95% CI) ^b
Unexposed	1,882	8,766	536 (61.1)	(Ref)	(Ref)	896 (102.2)	(Ref)	(Ref)
Exposed	1,018	4,364	280 (64.2)	1.02 (0.88-1.18)	0.94 (0.80-1.09)	534 (122.4)	1.18 (1.06-1.31)	0.97 (0.87-1.09)
Stratified Analyses: Site								
Proximal								
Unexposed	471	2,130	138 (64.8)	(Ref)	(Ref)	227 (106.6)	(Ref)	(Ref)
Exposed	280	1,162	67 (57.7)	0.86 (0.64-1.15)	0.73 (0.54-0.99)	140 (120.5)	1.11 (0.90-1.37)	0.87 (0.70-1.08)
Distal								
Unexposed	639	2,998	162 (54.0)	(Ref)	(Ref)	292 (97.4)	(Ref)	(Ref)
Exposed	337	1,419	95 (66.9)	1.20 (0.93-1.54)	1.11 (0.86-1.44)	185 (130.4)	1.31 (1.09-1.57)	1.09 (0.91-1.32)
Rectal								
Unexposed	697	3,278	214 (65.3)	(Ref)	(Ref)	344 (104.9)	(Ref)	(Ref)
Exposed	370	1,632	115 (70.5)	1.06 (0.84-1.33)	1.01 (0.80-1.28)	198 (121.3)	1.14 (0.96-1.36)	0.97 (0.81-1.16)
Stratified Analyses: Dosage and dosing intensity								
Unexposed	1,882	8,766	536 (61.1)	(Ref)	(Ref)	896 (102.2)	(Ref)	(Ref)
Aspirin dosage received								
≤75mg	811	3,447	221 (64.1)	1.01 (0.86-1.18)	0.92 (0.78-1.09)	411 (119.2)	1.14 (1.01-1.28)	0.94 (0.83-1.07)
>75mg	207	916	59 (64.4)	1.06 (0.81-1.39)	0.99 (0.75-1.31)	123 (134.3)	1.31 (1.09-1.59)	1.09 (0.89-1.32)
Aspirin dosing intensity								
1%-85% (low)	512	2,227	138 (62.0)	0.99 (0.82-1.20)	0.90 (0.74-1.09)	277 (124.4)	1.20 (1.05-1.37)	0.98 (0.85-1.12)
86%-100% (high)	506	2,137	142 (66.4)	1.05 (0.87-1.26)	0.98 (0.81-1.19)	257 (120.3)	1.15 (1.00-1.32)	0.97 (0.84-1.12)
Aspirin dosage received & dosing intensity								
Intensity 1%-85% (low)								
≤75mg	378	1,647	102 (61.9)	0.99 (0.80-1.22)	0.88 (0.71-1.10)	196 (119.0)	1.15 (0.98-1.34)	0.93 (0.79-1.09)
>75mg	134	580	36 (62.1)	1.01 (0.72-1.41)	0.94 (0.67-1.32)	81 (139.7)	1.36 (1.08-1.70)	1.11 (0.88-1.40)
Intensity 86%-100% (high)								
≤75mg	433	1,800	119 (66.1)	1.03 (0.84-1.26)	0.96 (0.78-1.18)	215 (119.4)	1.13 (0.98-1.32)	0.96 (0.82-1.11)
>75mg	73	336	23 (68.5)	1.15 (0.76-1.74)	1.10 (0.72-1.67)	42 (125.0)	1.24 (0.91-1.68)	1.04 (0.76-1.42)

Ref: reference. HR: hazard ratio. CI: confidence interval.

a) Rate calculated as deaths per 1,000 person-years



HR: hazard ratio

Figure 8.2: Direct adjusted survival curve - Adjusted cumulative incidence of colorectal cancer-specific mortality for aspirin users and non-users in patients with stages I-III colorectal cancer, age ≥ 45 and ≤ 80 .

Cumulative incidences are adjusted for age, gender, tumour stage, tumour grade, comorbidity score.

8.3.3. Sensitivity Analyses

The results from sensitivity analyses exploring the impact of using different classifications of cause of death are presented in Table 8.4. Results from overall and stratified analyses did not differ appreciably from those displayed in Table 8.3.

The results from sensitivity analyses in the expanded cohort, including all adult patients, and patients with tumours of any stage, are presented in Table 8.5, Table 8.6, (patient characteristics) and Table 8.7 (survival analyses). Differences in patient characteristics between the aspirin exposed (n=2,008) and unexposed (n=3,547) patients followed similar patterns to those for the main analysis cohort (Table 8.1) as described in section 8.3.1. In addition, exposure to NSAIDs was significantly higher in aspirin exposed patients versus unexposed patients in the expanded cohort (42% versus 37%, $p < 0.001$) (Table 8.5). Similar to the main analysis cohort, differences in stage distribution between the exposed and unexposed groups were apparent ($p < 0.001$, Table 8.5); in the expanded cohort, fewer aspirin exposed patients than unexposed patients had metastatic disease at diagnosis (stage IV disease: 21% aspirin exposed patients versus 24% aspirin unexposed patients, Table 8.5).

Unlike in the main analysis cohort, survival analyses in the expanded cohort resulted in a number of significant associations between aspirin exposure and improved colorectal cancer-specific survival (Table 8.7). Overall aspirin exposure was associated with a slight survival improvement (HR=0.92, 95% CI 0.83-0.99) (Table 8.7). When stratified by tumour site, an association between aspirin and survival was observed only for proximal colon tumours (HR=0.84, 95% CI 0.71-0.99), similar to results in the main analysis cohort. Neither interactions with tumour site ($P_{\text{interaction}} = 0.83$) nor tumour stage ($P_{\text{interaction}} = 0.56$) were significant.

Analyses in the expanded cohort that were stratified by dose resulted in a significant cancer-specific survival improvement for doses of aspirin above 75mg (HR=0.84, 95% CI 0.72-1.00, $p = 0.04$) (Table 8.7). For doses ≤ 75 mg, a slight, non-significant survival improvement was observed (HR=0.92, 95% CI 0.84-1.01). When stratified by dosing intensity, a significant survival benefit was found in patients with low intensity (1%-85%) aspirin exposure (HR=0.86, 95% CI 0.77-0.96) (Table 8.7). No association was observed for patients with high intensity aspirin exposure (86%-100%). When the effects of aspirin dosage and dosage intensity were considered together, a significant association between aspirin exposure and survival was only observed for patients with doses of aspirin > 75 mg and low dosing intensity (HR=0.78, 95% CI 0.63-0.95) (Table 8.7). A small, non-significant survival improvement was observed with low aspirin doses (≤ 75 mg) at low dosing intensity (HR=0.89, 95% CI 0.79-1.01) (Table 8.7).

Table 8.4: Sensitivity analysis 1 (different classification of cause of death). Unadjusted and adjusted hazard ratios for aspirin exposure and mortality.

Exposure Information			Colorectal cancer-specific survival (definition 2) ^c			Colorectal cancer-specific Survival (definition 3) ^d		
Aspirin exposure	N	Person-years	Deaths (Crude Rate) ^a	Unadjusted HR (95% CI)	Adjusted HR (95% CI) ^b	Deaths (Crude Rate) ^a	Unadjusted HR (95% CI)	Adjusted HR (95% CI) ^b
Unexposed	1,882	8,766	553 (63.08)	(Ref)	(Ref)	655 (74.72)	(Ref)	(Ref)
Exposed	1,018	4,364	295 (67.60)	1.04 (0.90-1.20)	0.95 (0.81-1.10)	352 (80.66)	1.05 (0.92-1.20)	0.96 (0.83-1.10)
Stratified Analyses: Site								
Proximal								
Unexposed	471	2,130	144 (67.6)	(Ref)	(Ref)	168 (78.9)	(Ref)	(Ref)
Exposed	180	1,162	70 (60.2)	0.86 (0.65-1.15)	0.73 (0.54-0.98)	85 (73.1)	0.90 (0.69-1.17)	0.77 (0.59-1.00)
Distal								
Unexposed	639	2,998	166 (55.4)	(Ref)	(Ref)	204 (68.0)	(Ref)	(Ref)
Exposed	337	1,419	99 (69.8)	1.22 (0.95-1.56)	1.11 (0.86-1.44)	119 (83.9)	1.19 (0.95-1.50)	1.09 (0.87-1.38)
Rectal								
Unexposed	697	3,278	220 (67.1)	(Ref)	(Ref)	256 (78.1)	(Ref)	(Ref)
Exposed	370	1,632	123 (75.4)	1.10 (0.88-1.37)	1.04 (0.83-1.31)	143 (87.6)	1.10 (1.35-0.82)	1.04 (0.85-1.29)
Stratified Analyses: Dosage and dosing intensity								
Unexposed	1,882	8,766	553 (63.08)	(Ref)	(Ref)	655 (74.72)	(Ref)	(Ref)
Aspirin dosage received								
≤75mg	811	3,447	231 (67.01)	1.02 (0.88-1.19)	0.92 (0.79-1.09)	273 (79.20)	1.02 (0.89-1.18)	0.93 (0.80-1.08)
>75mg	207	916	64 (69.87)	1.11 (0.86-1.44)	1.03 (0.79-1.35)	79 (86.24)	1.16 (0.92-1.46)	1.07 (0.84-1.37)
Aspirin dosing intensity								
1%-85% (low)	512	2,227	146 (65.56)	1.02 (0.85-1.22)	0.91 (0.75-1.10)	181 (81.28)	1.07 (0.91-1.26)	0.96 (0.80-1.13)
86%-100% (high)	506	2,137	149 (69.72)	1.06 (0.89-1.28)	0.98 (0.81-1.19)	171 (80.02)	1.03 (0.87-1.22)	0.96 (0.80-1.14)
Aspirin dosage received & dosing intensity								
Intensity 1%-85% (low)								
≤75mg	378	1,647	106 (64.36)	0.99 (0.81-1.22)	0.88 (0.71-1.09)	128 (77.72)	1.02 (0.84-1.23)	0.90 (0.74-1.10)
>75mg	134	580	40 (68.97)	1.09 (0.79-1.50)	1.00 (0.72-1.38)	53 (91.38)	1.22 (0.92-1.61)	1.12 (0.84-1.49)
Intensity 86%-100% (high)								
≤75mg	433	1,800	125 (69.44)	1.05 (0.86-1.27)	0.97 (0.79-1.18)	145 (80.56)	1.03 (0.86-1.23)	0.95 (0.79-1.15)
>75mg	73	336	24 (71.43)	1.16 (0.77-1.75)	1.09 (0.72-1.65)	26 (77.38)	1.06 (0.71-1.56)	0.99 (0.67-1.48)

a) Rate calculated as deaths per 1000 person-years

b) Adjusted for age, gender, tumour stage, tumour grade, comorbidity score.

c) Includes deaths where colorectal cancer was identified as a secondary/contributory cause of death.

d) Includes deaths due to malignant neoplasms of other/ill-defined digestive organs (C26), ill-defined cancer sites (C76.1, C80), secondary cancer sites (C77-79), cancers of uncertain or unknown behaviour (D48.6, D48.9) and unspecified causes of death.

Ref: reference. **HR:** hazard ratio. **CI:** confidence interval

Table 8.5: Sensitivity Analysis 2 (expanded study cohort). Characteristics of the expanded study cohort.

Characteristic		Aspirin in Year Prior to Diagnosis		P-value
		Unexposed (n=3,547)	Exposed (n=2,008)	
Patient Details				
Age – Median (IQR)	Years	74 (67, 79)	77 (72, 82)	<0.001
Comorbidity – Median (IQR)	N drug classes	7 (4, 12)	12 (8, 17)	<0.001
Gender – (%)	Male	1,882 (53.1)	1,170 (58.3)	<0.001
Smoking status – (%)	Current	638 (18.0)	261 (13.0)	<0.001
	Former	1,554 (43.8)	827 (41.2)	
	Never	686 (19.3)	446 (22.2)	
	Unspecified	669 (18.9)	474 (23.6)	
Socioeconomic status – (%)	1 (Least deprived)	446 (12.6)	305 (15.2)	0.02
	2	402 (11.3)	230 (11.5)	
	3	457 (12.9)	272 (13.6)	
	4	610 (17.2)	365 (18.2)	
	5 (Most deprived)	1,373 (38.7)	697 (34.7)	
	Unspecified	259 (7.3)	139 (6.9)	
Tumour Details				
TNM Stage – (%)	I	415 (11.7)	310 (15.4)	<0.001
	II	1,026 (28.9)	603 (30.0)	
	III	966 (27.2)	496 (24.7)	
	IV	833 (23.5)	419 (20.9)	
	Unspecified	307 (8.7)	180 (9.0)	
Grade – differentiation (%)	Well/moderate	2,384 (67.2)	1,349 (67.2)	0.99
	Poor	436 (12.3)	245 (12.2)	
	Unspecified	727 (20.5)	414 (20.6)	
Site – (%)	Proximal colon	929 (26.2)	554 (27.6)	0.29
	Distal colon	1,118 (31.5)	648 (32.3)	
	Rectal	1,308 (36.9)	690 (34.4)	
	Unspecified	192 (5.4)	116 (5.8)	
Morphology – (%)	Adenocarcinoma	2,867 (80.8)	1,649 (82.1)	0.24
	Other	680 (19.2)	359 (17.9)	
Treatment ^a – (%)	Surgery	3,210 (90.5)	1,804 (89.8)	0.43
	Chemotherapy	1,246 (35.1)	486 (24.2)	<0.001
	Radiotherapy	574 (16.2)	237 (11.8)	<0.001
Year of Diagnosis – (%)	2001	518 (14.6)	228 (11.4)	0.01
	2002	512 (14.4)	287 (14.3)	
	2003	628 (17.7)	344 (17.1)	
	2004	625 (17.6)	384 (19.1)	
	2005	669 (18.9)	384 (19.1)	
	2006	595 (16.8)	381 (19.0)	
Drug exposures ^b – (%)	NSAID ^b	1,302 (36.7)	846 (42.1)	<0.001
	Statins ^b	403 (11.4)	834 (41.5)	<0.001
	Anti-diabetic ^b	164 (4.6)	281 (14.0)	<0.001
	Biguanide ^b	93 (2.6)	193 (9.6)	<0.001
	Aspirin post diagnosis ^c	669 (18.9)	1,651 (82.2)	

IQR: Interquartile range

- a) Refers to treatment received in year post diagnosis.
- b) Exposures in year prior to diagnosis.
- c) Any exposure between diagnosis and end of follow-up.

Table 8.6: Sensitivity Analysis 2 (expanded study cohort). Details of aspirin exposure in exposed patients.

Aspirin exposure information for year prior to colorectal cancer diagnosis			
Number of patients exposed		2,008	
Number of prescriptions dispensed		18,220	-
Prescription strength category – (%)	75mg	14,986	(82.3)
	300mg	2,163	(11.9)
	Other	1,071	(5.9)
Prescription strength category – (%)	Low (≤ 75 mg)	15,710	(86.2)
	High (> 75 mg)	2,510	(13.8)
Dosing Intensity – median (IQR) ^a	%	84	(44, 98)
Mean daily dose – median (IQR) ^b	mg/day	66.8	(35.0, 75.0)

IQR: Interquartile range

- a) Exposure intensity calculated as number of days with supply available in year prior to diagnosis, divided by 365.
- b) Mean daily dose calculated as cumulative dose in year prior to diagnosis, divided by 365.

Table 8.7: Sensitivity Analysis 2 (expanded study cohort). Unadjusted and adjusted hazard ratios for aspirin exposure and mortality.

Exposure Information			Colorectal cancer-specific survival			Overall survival		
Aspirin exposure	N	Person-years	Deaths (Crude Rate) ^a	Unadjusted HR (95% CI)	Adjusted HR (95% CI) ^b	Deaths (Crude Rate) ^a	Unadjusted HR (95% CI)	Adjusted HR (95% CI) ^b
Unexposed	3,547	12,168	1,536 (126.2)	(Ref)	(Ref)	2,294 (188.5)	(Ref)	(Ref)
Exposed	2,008	6,274	851 (135.6)	1.03 (0.95-1.12)	0.91 (0.83-0.99)	1,375 (219.2)	1.12 (1.05-1.20)	0.93 (0.87-1.00)
Stratified Analyses: Site								
Proximal								
Unexposed	929	3,035	408 (134.4)	(Ref)	(Ref)	615 (202.6)	(Ref)	(Ref)
Exposed	554	1,751	221 (126.2)	0.92 (0.78-1.08)	0.84 (0.71-0.99)	364 (207.9)	1.01 (0.89-1.15)	0.86 (0.75-0.98)
Distal								
Unexposed	1,118	4,014	450 (112.1)	(Ref)	(Ref)	686 (170.9)	(Ref)	(Ref)
Exposed	648	2,098	266 (126.8)	1.07 (0.92-1.25)	0.91 (0.78-1.06)	440 (209.7)	1.18 (1.05-1.33)	0.95 (0.84-1.08)
Rectal								
Unexposed	1,308	4,628	586 (126.6)	(Ref)	(Ref)	853 (184.3)	(Ref)	(Ref)
Exposed	690	2,135	318 (148.9)	1.11 (0.97-1.27)	1.03 (0.90-1.19)	488 (228.6)	1.18 (1.06-1.32)	1.03 (0.92-1.15)
Stratified Analyses: Dosage and dosing intensity								
Unexposed	3,547	12,168	1,536 (126.2)	(Ref)	(Ref)	2,294 (188.5)	(Ref)	(Ref)
Aspirin dosage received								
≤75mg	1,620	4,993	684 (137.0)	1.03 (0.94-1.13)	0.92 (0.84-1.01)	1,095 (219.3)	1.12 (1.04-1.20)	0.94 (0.87-1.01)
>75mg	388	1,282	167 (130.3)	1.02 (0.87-1.19)	0.84 (0.72-1.00)	280 (218.4)	1.14 (1.01-1.30)	0.89 (0.78-1.02)
Aspirin dosing intensity								
1%-85% (low)	1,038	3,300	428 (129.7)	0.99 (0.89-1.10)	0.86 (0.77-0.96)	719 (217.9)	1.12 (1.03-1.22)	0.92 (0.84-1.00)
86%-100% (high)	970	2,974	423 (142.2)	1.07 (0.96-1.19)	0.96 (0.85-1.07)	656 (220.6)	1.12 (1.03-1.22)	0.95 (0.87-1.04)
Aspirin dosage received & dosing intensity								
Intensity 1%-85% (low)								
≤75mg	784	2,449	325 (132.7)	1.01 (0.89-1.14)	0.89 (0.79-1.01)	537 (219.3)	1.12 (1.02-1.24)	0.93 (0.85-1.03)
>75mg	254	852	103 (120.9)	0.95 (0.77-1.15)	0.78 (0.63-0.95)	182 (213.6)	1.12 (0.96-1.30)	0.86 (0.74-1.00)
Intensity 86%-100% (high)								
≤75mg	836	2,544	359 (141.1)	1.05 (0.94-1.18)	0.95 (0.84-1.07)	558 (219.3)	1.11 (1.01-1.22)	0.95 (0.86-1.04)
>75mg	134	430	64 (148.8)	1.16 (0.90-1.49)	0.98 (0.76-1.26)	98 (227.9)	1.19 (0.97-1.46)	0.96 (0.78-1.18)

Ref: reference. HR: hazard ratio. CI: confidence interval

a) Rate calculated as deaths per 1000 person-years

b) Adjusted for age, gender, tumour stage, tumour grade, comorbidity score

8.4. Discussion

This study examined associations between pre-diagnostic aspirin exposure and colorectal cancer-specific mortality primarily among patients with stage I-III colorectal cancer at diagnosis. For overall aspirin use in this cohort of patients aged 45-80 years, no survival benefit was observed between the exposed and unexposed groups. This result did not differ appreciably with expanded definitions of colorectal cancer mortality, or with expansion of the cohort to include patients of all stages of colorectal cancer and of any age-group.

As discussed in section 4.13, a number of previous studies have examined associations between pre-diagnostic aspirin exposure and colorectal cancer-specific survival. In one such study by Zell et al., no association was observed for patients who reported less than five years of aspirin use. However, a significant reduced mortality risk of 67% was found for patients who reported at least five years of aspirin exposure.³⁴² Three studies of pre-diagnostic aspirin exposure were carried out by Coghill et al. with varying results (section 4.13.1).³⁴³⁻³⁴⁵ One of these, a small study conducted only in female subjects, did not find an effect of aspirin,³⁴⁴ while a larger study in males and females found a significant overall hazard reduction of 24% with use of aspirin at any stage prior to diagnosis.³⁴³ The most recent study from this group, performed in post-menopausal women, again did not find an association between aspirin use and colorectal cancer mortality.³⁴⁵ Other groups have observed associations between post-diagnostic exposure and colorectal cancer survival, but not pre-diagnostic exposure (section 4.13.1).^{349, 351}

Few previous studies have performed analyses of the effects of aspirin specifically in relation to patients with non-metastatic (stage I-III) colorectal cancer at diagnosis. Chan et al. examined non-metastatic colorectal cancer patients but did not observe an association between pre-diagnostic aspirin exposure and survival.³⁴⁹ Coghill et al., adopted a similar approach to the present study, analysing patients with all stages of colorectal cancer as well as patients with non-metastatic disease.³⁴³ As in the present study, a significant association was observed between aspirin use and mortality in patients with all stages of cancer, though no effect was found in patients with non-advanced colorectal cancer at diagnosis.³⁴³ It is possible that the associations observed in all stages of colorectal cancer were significant simply due to the greater number of patients available for these analyses.

The primary analyses of the present study were stratified by tumour location in order to determine whether associations between aspirin use and survival could differ by site. A significant survival benefit was observed with aspirin use among cases of proximal colon

tumours, but not distal colon or rectal tumours. Both sensitivity analyses showed a similar pattern, that is, a significant association in proximal tumours but not in distal colon or rectal tumours. These results are in line with previous studies which found that survival differences following aspirin exposure varied according to exact site of colorectal tumour. Rothwell et al. found that the greatest reduction in risk of fatal colorectal cancer following aspirin exposure was observed among tumours of the proximal colon while little effect was observed for distal colon or rectal tumours.³¹⁷ When studying the effects of aspirin solely among cases of colorectal cancer, further analyses by Rothwell et al. found there was a non-significant reduction in relative risk of mortality with aspirin exposure; this was more pronounced for proximal colon tumours, was absent for rectal tumours, and was described as an 'intermediate' risk reduction in the case of distal colon tumours.³¹⁷ Cohort studies of aspirin and colorectal cancer survival have examined individual effects for colon and rectal tumour sites^{347, 351} but few have stratified analyses by proximal colon, distal colon and rectal sites.^{343, 344} As detailed in section 4.13.1, Table 4.2, results of these studies have tended to suggest a benefit for aspirin in colon tumours,^{342, 347, 351} particularly in proximal colon tumours,^{343, 344} but not for tumours of the rectum. This differential effect has been discussed by Chia et al., who proposed that effects of aspirin differing with tumour site may occur due to variation in biological phenotypes between different segments of the bowel; tumours of the proximal colon may display molecular characteristics that are more responsive to aspirin than the distal colon and rectum.²⁹⁵

Analyses in this study were also stratified by dosage of aspirin ($\leq 75\text{mg}$, 'low'; $>75\text{mg}$, 'high'), and intensity of exposure, as measured by the proportion of days in the year prior to diagnosis for which exposure occurred (1-85%, 'low'; 85-100%, 'high'). In the main study cohort, no associations were observed between aspirin exposure and survival for any of these dosing combinations. Sensitivity analyses in the expanded study cohort suggested associations between aspirin exposure and cancer survival in the case of (i) high dose aspirin, and (ii) low aspirin dosing intensity. The strongest association was observed for a combination of these factors, that is, high dose aspirin at a low frequency of exposure. As discussed in section 4.13.1, few previous observational studies have specifically examined aspirin dosing parameters and there is as yet no clear consensus regarding the effects of dosage or dosing frequency on survival associations. Post-diagnostic aspirin has been associated with improved survival at low doses³⁴⁷ and particularly with more frequent use.^{347, 349} In the case of pre-diagnostic exposure, Coghill et al., did not find that average daily dose was associated with survival.³⁴³ A recent meta-analysis of cohort studies examined effects of aspirin on colorectal

cancer incidence according to dosage regime; this study suggested that greater effects on incidence were observed with higher doses of aspirin and regular aspirin exposure (2-7 times per week).³¹⁹ The finding in the present study that an association with survival existed for aspirin at low dosing intensity, as opposed to exposure for the majority of the pre-diagnostic period, is therefore difficult to interpret as it might be expected that more frequent exposure to aspirin would result in a greater association. Chia et al., in explaining results from a study showing no survival benefit of aspirin (pre or post-diagnosis), suggested that tumours which succeed in developing following continuous exposure to aspirin may simply be less susceptible to the effects of aspirin.²⁹⁵ This concept of resistance to aspirin may explain the lower effect observed in this study in patients with regular exposure to aspirin throughout the year prior to diagnosis.

This study is the first, to the author's knowledge, to use cancer registry data and pharmacy claims records to examine associations between pre-diagnostic aspirin exposure and colorectal cancer mortality in a national-level dataset. The use of pharmacy claims data to ascertain exposure to aspirin, rather than patient questionnaires, was described as a key strength of a recent study of post-diagnostic aspirin exposure which used the Eindhoven cancer registry and PHARMO prescription database.^{295, 347} Pharmacy claims data provides detailed, objective, longitudinal exposure information in these analyses which is not subject to recall bias. While compliance information was not available, non-compliance with prescribed aspirin would be likely to bias results towards the null. Detailed cancer registry data enabled stratification of analyses by exact tumour site, and adjustment for factors affecting prognosis such as stage at diagnosis and tumour grade.

This study examined associations only for pre-diagnostic exposure to aspirin. As discussed in section 5.4.2, post-diagnostic exposure was not examined as it was considered that such analyses are likely to be subject to time-varying confounding.³⁵³ Remaining limitations of this study include the lack of available data on duration of aspirin exposure beyond one year prior to diagnosis, and the lack of information regarding the indication for aspirin prescription. Additionally, aspirin or other NSAIDs purchased 'over-the-counter' are not recorded in this dataset. However, patients who regularly use such medications can be expected to be more likely to acquire them on prescription; this is because prescription medications may be obtained free of charge, as opposed to paying out-of-pocket for the items. Also, 'low-dose' 75mg aspirin may not be purchased without a prescription in Ireland. Therefore, very few cases of aspirin exposure are expected to have been unrecorded. Clinical data regarding side effects of aspirin, such as gastrointestinal bleeding, were also unavailable. Patients in the

aspirin exposure group in this study were found to have a higher proportion of stage I tumours (versus more advanced tumours) than patients unexposed to aspirin. It is possible that some of these patients may have been diagnosed with colorectal cancer following investigation of gastrointestinal bleeding, as potentially caused by aspirin. However, the stage distribution of aspirin exposed patients is unlikely to have influenced the results of this study, as stage at diagnosis was adjusted for in all multivariate analyses. There was also imbalance between the aspirin exposed and unexposed groups for a number of additional patient characteristics, including smoking status and NSAID exposure; these imbalances could theoretically have introduced confounding. This potential for confounding is not expected to have an important effect in these analyses as all covariates which were imbalanced between the groups had been considered a priori for inclusion in the multivariate analysis model. These covariates did not remain in the final model as they were not found to affect the point estimate for survival during backwards deletion.

In the absence of examination of post-diagnostic exposure effects, it is difficult to discern whether associations observed in this study between aspirin exposure and colorectal cancer survival (particularly in proximal colon tumours) are attributable to only pre-diagnostic exposure, or to pre-diagnostic exposure followed by post-diagnostic exposure. It has been suggested that, among patients with established colorectal cancer, associations between pre-diagnostic aspirin exposure and colorectal cancer survival may reflect the development of tumours that are relatively benign and have a lower incidence of micrometastasis.²⁹⁵ Patients in this study who were exposed to aspirin had a higher proportion of stage I tumours than unexposed patients, which may indicate a biological effect of aspirin in promoting a less aggressive tumour phenotype (if not the result of earlier diagnosis following gastrointestinal bleeding, as suggested above). Alternatively, effects observed following pre-diagnostic aspirin use may represent post-diagnostic exposure effects on the development of metastases following surgery.²⁹⁵ A recently commenced clinical trial of aspirin in patients with established Duke's stage B or C colorectal cancer is expected to provide evidence for the effects of aspirin when administered in the post-diagnostic setting, specifically as an adjuvant treatment.⁴³⁷

The results of our study did not suggest a benefit of overall aspirin exposure in reducing colorectal cancer mortality among patients who did not have advanced disease at diagnosis. However, in analyses stratified by site, improved survival following aspirin exposure was observed among patients with proximal colon tumours, which was consistent with previous studies. Sensitivity analyses including all stages of colorectal cancer and all ages suggested a

minor survival gain with aspirin in patients with overall colorectal cancer, particularly among patients exposed to high doses of aspirin and at low exposure frequency.

9. Conclusion

9.1. Introduction

*“Finally, the aspirin and colorectal cancer story also shows that where basic laboratory observations raise the possibility of effects... ..the best approach to ‘translational research’ is often to jump straight to population-based observational epidemiology.”*²⁸⁹

Colorectal cancer mortality remains high despite advances in surgical methods and chemotherapy.^{2, 30, 295} Both aspirin and metformin have been identified through preclinical research and pharmacoepidemiological studies as having a potential role in the prevention and/or treatment of colorectal cancer.^{47, 295} The studies described in this thesis have sought to further elucidate the effects of aspirin and metformin in patients with established colorectal cancer in an Irish population. These studies used national-level data sources and rigorous methodology, and investigated the influence of particular exposure factors (e.g. frequency of exposure prior to diagnosis) on associations between these two drugs and colorectal cancer outcomes. Three main pharmacoepidemiological studies were performed using data from the National Cancer Registry Ireland linked to prescription claims information from the HSE-PCRS database. The study aims were to examine:

1. associations between metformin exposure and colorectal cancer survival,
2. associations between metformin exposure and presence of disseminated disease in patients with colorectal cancer, and
3. associations between aspirin exposure and colorectal cancer survival.

9.2. Research findings

Analyses of metformin exposure (versus non-metformin anti-diabetic drugs) suggested potential improvements in colorectal cancer survival following metformin exposure but associations were, for the most part, not statistically significant (Table 6.3). These associations were sensitive to stratification by dosing intensity and co-prescription with other anti-diabetic drugs; a significant 56% survival improvement was observed with high intensity, exclusive metformin use but not with other categories of metformin exposure. Secondary analyses examining metformin where non-diabetics served as the comparator group also suggested improved survival among metformin exposed patients, though associations were not

significant. Relative to non-diabetics, high intensity, exclusive metformin use was linked to non-significant survival improvements of 46% and 38% in stage I-III and stage I-IV colorectal cancer, respectively (Table 6.4 and Table 6.8).

These results contrast with associations observed between aspirin and colorectal cancer survival; hazard ratios were close to unity for the overall association between aspirin and colorectal cancer survival in both stage I-III and all stages colorectal cancer (Table 8.3 and Table 8.7). However, stratification by dosage and dosing intensity suggested a possible modest improvement in survival with high dose aspirin in patients with all stages colorectal cancer (Table 8.7). Stratification by tumour site resulted in a significant association between aspirin exposure and survival among patients with proximal colon tumours, but not distal colon or rectal cancer (Table 8.3).

Due to the low number of patients receiving anti-diabetic drugs, the effect of metformin on colorectal cancer survival was not studied according to tumour site; analyses were focused on examining the effects of metformin according to co-prescription with other anti-diabetic drugs, as well as dosing intensity. In order to further explore the potential effects of metformin, associations with disseminated colorectal cancer were investigated. For overall metformin exposure, no association was observed between metformin and disseminated (versus localized) colorectal cancer.

High intensity, exclusive metformin exposure was linked to reduced odds of disseminated disease at diagnosis; this association was non-significant in the main analysis (Table 7.3) but was found to be significant in sensitivity analyses (**Error! Reference source not found.**).

9.3. Contribution of findings to the existing literature

As detailed in the individual chapters, the overall results of the studies investigating effects of metformin and aspirin on survival are broadly consistent with existing literature evidence.

The results of the study described in Chapter Six contribute to existing literature by suggesting that high intensity exposure may be required for an effect of metformin on colorectal cancer survival.

The study of metformin and odds of disseminated disease (Chapter Seven) provides novel findings that high intensity metformin exposure, when compared to non-metformin anti-diabetic drugs, may have the effect of reducing tumour spread to the lymph nodes and/or the development of distant metastases. This potential effect may explain the improved survival observed in patients with high intensity exclusive metformin exposure in Chapter Six.

Associations with improved outcomes (increased survival and lack of disseminated disease) were not observed among patients who received both metformin and non-metformin anti-diabetic drugs. Such drugs primarily include insulin and sulfonylureas, which increase insulin levels in the bloodstream. These results may, therefore, suggest that the mechanism of action of metformin in colorectal cancer could include a relative reduction in insulin levels, which is supported by the literature.^{47, 169} The finding of potential effects of high intensity, exclusive metformin use, when compared to non-diabetics, on survival (significant association) and tumour dissemination (non-significant) provide some indication that an anti-cancer activity of metformin may not simply reflect the reversal of the effects of insulin-releasing anti-diabetic drugs.

Results from Chapter Eight, which examined aspirin exposure and colorectal cancer survival, have provided evidence that pre-diagnostic aspirin exposure may result in a survival benefit particularly in proximal colon tumours. Prior studies specifically examining pre-diagnostic aspirin exposure have not provided stratified estimates based on intensity of exposure prior to diagnosis and have not provided individual estimates for each of proximal, distal and rectal disease (Table 4.2). Studies of post-diagnostic aspirin exposure have found a similar effect; that is, improved survival of patients exposed to aspirin for patients with colon tumours but not rectal tumours.^{347, 351} Results from Chapter Eight examining the effects of aspirin dosage and dosing intensity on survival were inconclusive but suggested an association between relatively high doses of aspirin (>75mg) and colorectal cancer survival in patients with all stages of colorectal cancer. In summary, this study provided novel data on associations between aspirin and survival for different aspirin dosage categories and frequencies of exposure, and specific sites of colorectal cancer. Such detailed analyses have been called for by Rothwell et al. in their recent review of the observational study evidence on aspirin and cancer incidence.³¹⁸

The studies performed within this thesis are the first pharmacoepidemiological analyses of colorectal cancer outcomes that have been performed using data from the National Cancer Registry Ireland linked to the HSE-PCRS pharmacy claims database. Similar studies examining other types of drugs in prostate cancer and breast cancer have also recently been carried out using this data source.^{63, 64} The present studies, which provide detailed evidence of effects of metformin and aspirin on survival, demonstrate the potential of this data source in pharmacoepidemiological outcomes research examining colorectal cancer.

9.4. Limitations and suggestions for future research

Limitations of the studies performed within this thesis are detailed in Chapter Five and in the individual study chapters. The question of generalisability of results to the overall population is a concern common to the three studies. While the National Cancer Registry Ireland may be viewed as fully representative of colorectal cancer cases in Ireland, the information regarding prescription drug ascertainment was limited to a certain portion of the Irish population which primarily comprises patients of a lower sociodemographic status or more elderly patients. Therefore, it is possible that results presented may not be generalisable to younger patients or patients of a higher socioeconomic status. Also, it should be noted that the population of the Republic of Ireland is predominantly “white”. Colorectal tumour characteristics (e.g. p53 mutation, microsatellite instability) and levels of hyperinsulinaemia have been found to vary with race and extrapolating the results to a more ethnically diverse population may therefore not be possible.^{438, 439}

An important limitation was the relatively small sample size available for study. Data linkage for colorectal cancer patients diagnosed beyond the year 2006 is currently on-going between the National Cancer Registry Ireland and the HSE-PCRS. The completion of this linkage and the resulting greater number of patients available for study will provide the opportunity for more extensive study of the effects of metformin and aspirin; for example, further exploration of the influence of both dosage parameters and colorectal tumour location could be performed. Interestingly, aspirin and metformin have recently been observed to exert an apparent synergistic effect in colorectal cancer cells *in vitro*;³¹⁵ greater numbers of diabetic patients available for analysis could potentially permit the study of the effects of exposure to both metformin and aspirin in colorectal cancer.

Data for some covariates, e.g. smoking and grade, was 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. The linked data available for study in this thesis also lacked certain important clinical parameters, as discussed in section 5.5. Markers of diabetes severity, obesity, and information on long-term duration of use of the drugs of interest were not available for adjustment in the analyses presented. As diagnostic codes were not available, the exact prescribing indication for the drugs was unknown. Also, information on potential side effects of aspirin or metformin (e.g. hospital admissions due to gastrointestinal side effects) and diagnoses of comorbidities were not

available. These would have been of benefit in determining whether patients exposed these drugs had a higher likelihood of early diagnosis of colorectal cancer, and could thereby have provided information on potential confounding. It is recommended that the results of the studies in this thesis be confirmed and validated in other datasets with access to such information.

In addition to clinical data relating to the exposures in these studies, future studies with access to more detailed tumour-related data could provide further information regarding the effects of metformin or aspirin in colorectal cancer. With accurate capture of tumour recurrence, study of the effect of post-diagnostic aspirin/metformin exposure could potentially be performed.³⁵³ Capture of data on pathologic complete response could enable study of the effects of these drugs in the neoadjuvant setting of rectal cancer treatment.²²¹ Also, future linkage of pathology data detailing the molecular profile of individual patients' tumours could enable the study of potential biomarkers of drug response. Previous analyses using the Nurses' Health Study and Health Professionals' Follow-Up Study data, which examined post-diagnostic aspirin exposure, had access to tumour molecular profile information obtained from patient tissue samples. These studies found that associations between aspirin use and colorectal cancer survival varied according to the expression status of various biomarkers, including COX-2 expression and PIK3CA mutation status.^{349, 361, 362} Similarly, preclinical studies examining metformin, and reviews of the existing research, have highlighted the need for future studies to include evaluation of biomarkers which may predict responses.^{47, 168, 169} The potential of pharmacoepidemiological analyses of data which incorporate information from tumour tissue samples has recently been recognised in a collaborative grant funded by the Irish Cancer Society. This programme, 'Breast-Predict', will include the pharmacoepidemiological study of aspirin in patients with breast cancer and will incorporate information gathered from breast tissue samples in analyses.⁴⁴⁰

The benefits of linking the pharmacy claims database used in the present studies to further databases, such as hospital admissions data, were previously discussed in a thesis published in 2002 on the use of this data source for pharmacoepidemiological studies in Ireland.³⁶⁹ Williams discussed the need for a national individual identity number in Ireland, which would permit record linkage across a variety of health information sources.³⁶⁹ The introduction of such an identifier is to be legislated for in the forthcoming Health Information Bill, which is expected to be published in early 2014.^{441, 442} As well as incorporating general hospital admissions data, the use of a national patient identity number could permit the linkage of NCRI data to other prescription claims information sources, including the Drugs Payment Scheme or the Long

Term Illness scheme. 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. For example, the Northern Ireland Cancer Registry is due to be linked to the Enhanced Prescribing Database, a database of all prescription items dispensed to patients within Northern Ireland.⁴⁴³ Pooling of data such as this, using meta-analytical approaches, could increase the statistical power for identifying associations between prescribed medications and cancer outcomes.

9.5. Potential of metformin and aspirin in colorectal cancer

The importance of research into the effects of both aspirin and metformin in cancer was highlighted in the list of 'Provocative Questions' posed by the National Cancer Institute at the end of 2012. This list of research questions cited aspirin and metformin in particular as examples of drugs which may protect against cancer incidence and mortality, and identified the study of the mechanism of action of such drugs as an important research topic.⁴⁴⁴ Furthermore, a recent review of the potential use of aspirin in the adjuvant setting stated that the role of aspirin in established colorectal cancer "*...has emerged as one of the most important and urgent research priority areas for colorectal cancer*".²⁹⁵ Both aspirin and metformin have been suggested to have potential 'indirect' effects on cancer cells at relatively low doses; it is hypothesised that the anti-platelet effect of aspirin may reduce metastasis development while the relative reduction in insulin levels associated with metformin may reduce the growth of insulin-responsive tumours.^{47, 274} At higher doses, 'direct' effects on colorectal cancer cells have been observed with both of these agents, as discussed in Chapters Three and Four. Aspirin and metformin are both the most commonly used drugs in cardiovascular disease prevention and type 2 diabetes, respectively. The highly prevalent use of these drugs is an indication of their relative safety. However, dose-dependent toxicity may limit the application of these drugs in the colorectal cancer setting. Clinical trials of these drugs in patients with diagnosed cancer will help to clarify this concern.

A phase III clinical trial of aspirin in the adjuvant setting is currently underway. This multi-centre trial taking place in Asia will randomise patients with stage II or stage III colorectal cancer to 200mg aspirin or matching placebo once daily for 3 years ; it is due for completion in 2021.^{437, 445} The authors of the trial protocol acknowledged the on-going uncertainty surrounding the dose of aspirin necessary for an effect and adopted a 200mg dose schedule as

a midway point between 'high' and 'low' doses.⁴³⁷ Results from this thesis which were stratified by dose found no effect of aspirin at doses $\leq 75\text{mg}$ but did observe a small survival benefit with doses $>75\text{mg}$ (section 8.3.2). The trial protocol also plans to stratify results according to site.⁴³⁷ This approach is supported by the present study, which found a survival difference only in proximal colon tumours.

Regarding the potential use of metformin in colorectal cancer, it is as yet unknown whether it could be of benefit in patients who do not have diabetes. In a recent review of metformin in cancer, Quinn et al. identified this as 'the most important unanswered question'.¹⁶⁹ As described in section 3.2.9, a pilot clinical trial in non-diabetics found that metformin treatment, at low doses, was associated with a reduction in rectal aberrant crypt foci.²⁵⁸ These results have led to a multi-centre study which is currently being brought to completion in Japan and is examining colorectal polyp recurrence in non-diabetic patients exposed to 250mg metformin once daily.⁴⁴⁶ Smaller clinical trials of short duration are also underway to investigate biological effects (e.g. effects on markers of proliferation) of metformin in non-diabetic colorectal cancer patients or in non-diabetic patients with precancerous lesions.¹⁶⁹ A previously published study of metformin in non-diabetic patients with breast cancer did not find an overall reduction in proliferation markers following treatment.²⁵⁷ However, proliferation markers were found to decrease among subgroups of women with high BMI or insulin resistance, suggesting that metformin may have a potential effect in these settings.²⁵⁷ Some have argued that clinical trials of metformin are premature as the observational evidence supporting their initiation is not sufficiently robust.^{146, 447} Results from the study described in Chapter Six suggested a possible survival improvement, though not statistically significant, in colorectal cancer patients exposed to high intensity metformin relative to non-diabetics. However, even if this result reflects a true association, this does not necessarily suggest that metformin may exert an effect in non-diabetic patients. Nonetheless, these results provide some support for the clinical study of metformin in non-diabetics.

In the event of aspirin and/or metformin displaying a benefit in future clinical trials, these drugs may be used as adjuvant treatment in combination with conventional chemotherapy, or in the neoadjuvant setting to reduce tumour burden prior to surgery. Alternatively, if the effects of one or both of these drugs are more apparent in the prevention of cancer, they could be developed as chemopreventive agents. It is also possible that emerging pharmacoepidemiological and laboratory evidence will encourage the development of improved chemical entities, or the identification of novel targets, based on the effects of aspirin and metformin. In the case of metformin, novel derivatives have recently already been

described which appear to inhibit proliferation and invasion of breast cancer cells in a much more potent manner than metformin.⁴⁴⁸

9.6. Overall conclusion

The identification of metformin and aspirin as possible candidates in colorectal cancer prevention and/or treatment is an exciting development. Metformin and aspirin are well-characterised and inexpensive treatments used in common conditions; if they are found to improve outcomes in colorectal cancer, these drugs may be of considerable benefit to public health. The studies within this thesis contribute detailed analyses of potential associations between metformin and aspirin, individually, and colorectal cancer outcomes. Results obtained should be considered as hypothesis-generating but suggest that both metformin and aspirin may individually reduce cancer mortality under certain conditions. Metformin, at a high level of exposure, was also associated with reduced colorectal cancer dissemination. Associations in the case of metformin were found to depend on the level of drug exposure and on co-prescription with other anti-diabetic agents. Associations between aspirin and colorectal cancer survival differed according to the colorectal tumour site and the dose received. These studies demonstrate the potential for using Irish data to perform further pharmacoepidemiological outcomes research in the area of colorectal cancer. Future research efforts would benefit from a greater overall sample size and the incorporation of additional relevant clinical information and tumour details; these may be achieved with on-going and potential future data linkage projects. Overall, the studies herein described support the continued investigation of the potential clinical uses of metformin and aspirin in colorectal cancer.

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Appendix One

'DailyDrug' macro SAS® code

```
%MACRO DAILYDRUG
(drugname=,
 startdate=,
 enddate=,
 atc1=XXXXXXXX,
 atc2=XXXXXXXX,
 atc3=XXXXXXXX,
 atc4=XXXXXXXX,
 atc5=XXXXXXXX,
 atc6=XXXXXXXX,
 atc7=XXXXXXXX,
 atc8=XXXXXXXX,
 atc9=XXXXXXXX,
 atc10=XXXXXXXX,
 atc11=XXXXXXXX,
 atc12=XXXXXXXX,
 atc13=XXXXXXXX,
 atc14=XXXXXXXX,
 atc15=XXXXXXXX,
 atc16=XXXXXXXX,
 atc17=XXXXXXXX,
 atc18=XXXXXXXX,
 atc19=XXXXXXXX,
 atc20=XXXXXXXX,
 lib1=work,
 lib2=work);
```

```
/*SUBSET DRUG*/
DATA &lib2..&drugname;
SET &lib1..drug;

/*SUBSET DRUG*/
IF SUBSTR(atcdma,1,%LENGTH(&atc1)) = "&atc1"
OR SUBSTR(atcdma,1,%LENGTH(&atc2)) = "&atc2"
OR SUBSTR(atcdma,1,%LENGTH(&atc3)) = "&atc3"
OR SUBSTR(atcdma,1,%LENGTH(&atc4)) = "&atc4"
OR SUBSTR(atcdma,1,%LENGTH(&atc5)) = "&atc5"
OR SUBSTR(atcdma,1,%LENGTH(&atc6)) = "&atc6"
OR SUBSTR(atcdma,1,%LENGTH(&atc7)) = "&atc7"
OR SUBSTR(atcdma,1,%LENGTH(&atc8)) = "&atc8"
OR SUBSTR(atcdma,1,%LENGTH(&atc9)) = "&atc9"
OR SUBSTR(atcdma,1,%LENGTH(&atc10)) = "&atc10"
OR SUBSTR(atcdma,1,%LENGTH(&atc11)) = "&atc11"
OR SUBSTR(atcdma,1,%LENGTH(&atc12)) = "&atc12"
OR SUBSTR(atcdma,1,%LENGTH(&atc13)) = "&atc13"
OR SUBSTR(atcdma,1,%LENGTH(&atc14)) = "&atc14"
OR SUBSTR(atcdma,1,%LENGTH(&atc15)) = "&atc15"
OR SUBSTR(atcdma,1,%LENGTH(&atc16)) = "&atc16"
OR SUBSTR(atcdma,1,%LENGTH(&atc17)) = "&atc17"
OR SUBSTR(atcdma,1,%LENGTH(&atc18)) = "&atc18"
```

```
OR SUBSTR(atcdma,1,%LENGTH(&atc19)) = "&atc19"  
OR SUBSTR(atcdma,1,%LENGTH(&atc20)) = "&atc20"  
THEN DO; drug=atcdma; strength=str; END;
```

```
IF SUBSTR(drug,1,%LENGTH(&atc1))="&atc1"  
OR SUBSTR(drug,1,%LENGTH(&atc2))="&atc2"  
OR SUBSTR(drug,1,%LENGTH(&atc3))="&atc3"  
OR SUBSTR(drug,1,%LENGTH(&atc4))="&atc4"  
OR SUBSTR(drug,1,%LENGTH(&atc5))="&atc5"  
OR SUBSTR(drug,1,%LENGTH(&atc6))="&atc6"  
OR SUBSTR(drug,1,%LENGTH(&atc7))="&atc7"  
OR SUBSTR(drug,1,%LENGTH(&atc8))="&atc8"  
OR SUBSTR(drug,1,%LENGTH(&atc9))="&atc9"  
OR SUBSTR(drug,1,%LENGTH(&atc10))="&atc10"  
OR SUBSTR(drug,1,%LENGTH(&atc11))="&atc11"  
OR SUBSTR(drug,1,%LENGTH(&atc12))="&atc12"  
OR SUBSTR(drug,1,%LENGTH(&atc13))="&atc13"  
OR SUBSTR(drug,1,%LENGTH(&atc14))="&atc14"  
OR SUBSTR(drug,1,%LENGTH(&atc15))="&atc15"  
OR SUBSTR(drug,1,%LENGTH(&atc16))="&atc16"  
OR SUBSTR(drug,1,%LENGTH(&atc17))="&atc17"  
OR SUBSTR(drug,1,%LENGTH(&atc18))="&atc18"  
OR SUBSTR(drug,1,%LENGTH(&atc19))="&atc19"  
OR SUBSTR(drug,1,%LENGTH(&atc20))="&atc20";
```

```
IF patient_id = " then delete;
```

*/*IDENTIFY HOSPITAL EMERGENCY PRESCRIPTIONS*/*

```
FORMAT hosp BEST5.;  
IF dnum=61559 OR pdoc=61559 THEN hosp=1;
```

*/*CALCULATE DAYS SUPPLY*/*

```
FORMAT days BEST5.;  
IF qnty <= 40 AND hosp ^= 1 THEN days = qnty/1;  
IF qnty > 40 AND hosp ^= 1 THEN days = qnty/1.5;  
IF qnty > 50 AND hosp ^= 1 THEN days = qnty/2;  
IF qnty > 70 AND hosp ^= 1 THEN days = qnty/3;  
IF qnty > 105 AND hosp ^= 1 THEN days = qnty/4;  
IF qnty > 130 AND hosp ^= 1 THEN days = qnty/5;  
IF qnty > 160 AND hosp ^= 1 THEN days = qnty/6;  
IF qnty > 190 AND hosp ^= 1 THEN days = qnty/7;  
IF qnty > 220 AND hosp ^= 1 THEN days = qnty/8;  
IF qnty > 250 AND hosp ^= 1 THEN days = qnty/9;  
IF qnty > 280 AND hosp ^= 1 THEN days = qnty/10;  
  
IF qnty <= 9 AND hosp = 1 THEN days = qnty/1;  
IF qnty > 9 AND hosp = 1 THEN days = qnty/2;  
IF qnty > 19 AND hosp = 1 THEN days = qnty/3;  
IF qnty > 25 AND hosp = 1 THEN days = qnty/4;  
IF qnty > 32 AND hosp = 1 THEN days = qnty/5;  
IF qnty > 39 AND hosp = 1 THEN days = qnty/6;  
IF qnty > 46 AND hosp = 1 THEN days = qnty/7;  
IF qnty > 53 AND hosp = 1 THEN days = qnty/8;  
IF qnty > 60 AND hosp = 1 THEN days = qnty/9;  
IF qnty > 67 AND hosp = 1 THEN days = qnty/10;
```

*/*CALCULATE DOSE*/*

```

FORMAT dose BEST5.;
IF qnty <=      40      AND hosp ^= 1 THEN dose = strength*1;
IF qnty >       40      AND hosp ^= 1 THEN dose = strength*1.5;
IF qnty >       50      AND hosp ^= 1 THEN dose = strength*2;
IF qnty >       70      AND hosp ^= 1 THEN dose = strength*3;
IF qnty >      105      AND hosp ^= 1 THEN dose = strength*4;
IF qnty >      130      AND hosp ^= 1 THEN dose = strength*5;
IF qnty >      160 AND hosp ^= 1 THEN dose = strength*6;
IF qnty >      190      AND hosp ^= 1 THEN dose = strength*7;
IF qnty >      220      AND hosp ^= 1 THEN dose = strength*8;
IF qnty >      250      AND hosp ^= 1 THEN dose = strength*9;
IF qnty >      280      AND hosp ^= 1 THEN dose = strength*10;

IF qnty <=      9       AND hosp =    1 THEN dose = strength*1;
IF qnty >       9       AND hosp =    1 THEN dose = strength*2;
IF qnty >      19       AND hosp =    1 THEN dose = strength*3;
IF qnty >      25       AND hosp =    1 THEN dose = strength*4;
IF qnty >      32       AND hosp =    1 THEN dose = strength*5;
IF qnty >      39       AND hosp =    1 THEN dose = strength*6;
IF qnty >      46       AND hosp =    1 THEN dose = strength*7;
IF qnty >      53       AND hosp =    1 THEN dose = strength*8;
IF qnty >      60       AND hosp =    1 THEN dose = strength*9;
IF qnty >      67       AND hosp =    1 THEN dose = strength*10;

RUN;

/*COMBINE SAME DRUG ON SAME RX*/
/*SORT BY IDIARC DATE CLAIM ATCDMA*/
PROC SORT DATA=&lib2..&drugname;
BY patient_id rxdate claim drug;
RUN;
/*COLLAPSE WITHIN IDIARC & DATE & CLAIM & ATCDMA*/
PROC MEANS NOPRINT DATA=&lib2..&drugname;
VAR dose days;
BY patient_id rxdate claim drug;
OUTPUT OUT=&lib2..&drugname (KEEP=patient_id rxdate drug dose days)
SUM(dose)=dose MAX(days)=days;
RUN;
/*SAVE DOSE FILE*/
DATA &lib2..&drugname._dose;
SET &lib2..&drugname;
KEEP patient_id rxdate drug dose;
RUN;
/*SORT BY PATIENT_ID RXDATE*/
PROC SORT DATA=&lib2..&drugname;
BY patient_id rxdate;
RUN;
/*ADD SEQUENCE NUMBERS AND TRANSPOSE*/
DATA &lib2..&drugname;

SET &lib2..&drugname;
BY patient_id rxdate;
IF FIRST.patient_id THEN sequence=1;
ELSE sequence+1;
RUN;
PROC TRANSPOSE DATA=&lib2..&drugname

```

```

OUT= rxdate (DROP=_name_) PREFIX=rxdate;

VAR rxdate;
BY patient_id;
ID sequence;
RUN;
PROC TRANSPOSE DATA=&lib2..&drugname
OUT= drug (DROP=_name_) PREFIX=drug;

VAR drug;
BY patient_id;
ID sequence;
RUN;
PROC TRANSPOSE DATA=&lib2..&drugname
OUT= days (DROP=_name_) PREFIX=days;

VAR days;
BY patient_id;
ID sequence;
RUN;
PROC TRANSPOSE DATA=&lib2..&drugname
OUT= dose (DROP=_name_) PREFIX=dose;

VAR dose;
BY patient_id;
ID sequence;
RUN;
/*NUMBER OF OBSERVATIONS AND VARIABLES*/
%LET dsname= rxdate;
%LET dsid=%SYSFUNC(OPEN(&dsname));
%LET nvar=%SYSFUNC(ATTRN(&dsid,NVARS));;
%LET nvar=%EVAL(&nvar-1);
%LET nobs=%SYSFUNC(ATTRN(&dsid,NOBS));;
%LET rc=%SYSFUNC(CLOSE(&dsid));;
%PUT &nvar;
%PUT &nobs;
/*MERGE TRANSPOSED FILES*/
DATA &lib2..&drugname;
MERGE rxdate drug days dose;
BY patient_id;
RUN;
/*CREATE MACRO VARIABLES FOR START AND END AS SASDATE*/
DATA _NULL_;
start="&startdate"d;
end="&enddate"d;
CALL SYMPUT('startnum',TRIM(LEFT(start)));
CALL SYMPUT('endnum',TRIM(LEFT(end)));
RUN;
/*ASSIGN TO DAILY DATA VARIABLES*/
DATA &lib2..&drugname;
SET &lib2..&drugname;

FORMAT      &drugname._days&startnum - &drugname._days&endnum BEST5.;
FORMAT      &drugname._drug&startnum - &drugname._drug&endnum $8.;
FORMAT      &drugname._dose&startnum - &drugname._dose&endnum BEST5.;

```

```

ARRAY  rxdate (1:&nvar)          rxdate1 - rxdate&nvar;

ARRAY  days (1:&nvar)            days1 - days&nvar;

ARRAY  drug (1:&nvar)            drug1 - drug&nvar;

ARRAY  dose (1:&nvar)            dose1 - dose&nvar;

ARRAY  &drugname._days (&startnum:&endnum)  &drugname._days&startnum -
&drugname._days&endnum;
ARRAY  &drugname._drug (&startnum:&endnum)  &drugname._drug&startnum -
&drugname._drug&endnum;

ARRAY  &drugname._dose (&startnum:&endnum)  &drugname._dose&startnum -
&drugname._dose&endnum;

DO i = 1 TO &nvar;
    IF rxdate(i) ^= .                                /*IF
PRESCRIPTION DISPENSED*/
    THEN DO;
    /*THEN*/
        DO j = &startnum TO &endnum;                /*RUN
THROUGH ALL DAILY SUPPLY DATES FROM START TO END OF FOLLOWUP*/
        IF rxdate(i) = j
        /*IF DAILY SUPPLY DATE MATCHES PRESCRIPTION DATE*/
        THEN DO;
        /*THEN*/
            IF &drugname._drug(j) ^= ''                /*IF
PREVIOUS DAILY SUPPLY AT THAT DATE*/
            AND &drugname._drug(j) = drug(i)          /*AND
PRESCRIPTION DRUG IS THE SAME AS THE DAILY SUPPLY AT THAT DATE*/
            THEN DO;
            /*THEN*/
                IF days(i) > 0
                /*IF SUPPLY OF PRESCRIPTION DRUG LEFT TO ASSIGN TO DAILY SUPPLY AT
THAT DATE*/
                THEN DO;
                /*THEN*/
                    rxdate(i) = rxdate(i)+ 1;
                /*ADD 1 TO PRESCRIPTION DATE TO MOVE START DATE TO NEXT DAY*/
                END;
                /*OR*/
                IF days(i) = 0
                /*IF NO SUPPLY OF PRESCRIPTION DRUG LEFT TO ASSIGN TO DAILY SUPPLY
AT THAT DATE*/
                THEN DO;
                /*THEN*/
                    rxdate(i) = rxdate(i)+ 1;
                /*ADD 1 TO PRESCRIPTION DATE TO MOVE START DATE TO NEXT DAY*/
                END;
            END;
            /*OR*/
            IF &drugname._drug(j) = ''                /*IF
NO PREVIOUS DAILY SUPPLY AT THAT DATE*/
            THEN DO;
            /*THEN*/

```

```

                                IF days(i) = 0
                                /*IF NO SUPPLY OF PRESCRIPTION DRUG LEFT TO ASSIGN TO DAILY SUPPLY
AT THAT DATE*/
                                THEN DO;
                                /*THEN*/
                                        &drugname._drug(j) = "";
                                /*CHANGE DAILY SUPPLY DRUG TO NULL*/
                                        &drugname._days(j) = .;
                                /*CHANGE DAILY SUPPLY TO NULL*/
                                        &drugname._dose(j) = .;
                                /*CHANGE DAILY SUPPLY DOSE NULL*/
                                        rxdate(i) = rxdate(i)+ 1;
                                /*ADD 1 TO PRESCRIPTION DATE TO MOVE START DATE TO NEXT DAY*/
                                END;
                                /*OR*/
                                IF days(i) > 0
                                /*IF SUPPLY OF PRESCRIPTION DRUG LEFT TO ASSIGN TO DAILY SUPPLY AT
THAT DATE*/
                                THEN DO;
                                        &drugname._drug(j) = drug(i);
                                /*CHANGE DAILY SUPPLY DRUG TO PRESCRIPTION DRUG*/
                                        &drugname._days(j) = 1;
                                /*CHANGE DAILY SUPPLY TO 1*/
                                        &drugname._dose(j) = dose(i);
                                /*CHANGE DAILY SUPPLY DOSE TO PRESCRIPTION DOSE*/
                                        rxdate(i) = rxdate(i)+ 1;
                                /*ADD 1 TO PRESCRIPTION DATE TO MOVE START DATE TO NEXT DAY*/
                                        days(i) = days(i) - 1;
                                /*SUBTRACT 1 FROM PRESCRIPTION DAYS SUPPLY FOR NEXT DAY*/
                                END;
                                END;
                                /*OR*/
                                IF &drugname._drug(j) ^= ""                                /*IF
PREVIOUS DAILY SUPPLY AT THAT DATE*/
                                AND &drugname._drug(j) ^= drug(i)                                /*AND
PRESCRIPTION DRUG IS NOT THE SAME AS THE DAILY SUPPLY AT THAT DATE*/
                                THEN DO;
                                /*THEN*/
                                        IF days(i) = 0
                                        /*IF NO SUPPLY OF PRESCRIPTION DRUG LEFT TO ASSIGN TO DAILY SUPPLY
AT THAT DATE*/
                                        THEN DO;
                                                /*THEN*/
                                                        &drugname._drug(j) = "";
                                                /*CHANGE DAILY SUPPLY DRUG TO NULL*/
                                                        &drugname._days(j) = .;
                                                /*CHANGE DAILY SUPPLY TO NULL*/
                                                        &drugname._dose(j) = .;
                                                /*CHANGE DAILY SUPPLY DOSE NULL*/
                                                        rxdate(i) = rxdate(i)+ 1;
                                                /*ADD 1 TO PRESCRIPTION DATE TO MOVE START DATE TO NEXT DAY*/
                                                END;
                                                /*OR*/
                                                IF days(i) > 0
                                                /*IF SUPPLY OF PRESCRIPTION DRUG LEFT TO ASSIGN TO DAILY SUPPLY AT
THAT DATE*/
                                                THEN DO;

```



```

&drugname._drug(j) = drug(i);
/*CHANGE DAILY SUPPLY DRUG TO PRESCRIPTION DRUG*/
&drugname._days(j) = 1;
/*CHANGE DAILY SUPPLY TO 1*/
&drugname._dose(j) = dose(i);
/*CHANGE DAILY SUPPLY DOSE TO PRESCRIPTION DOSE*/
rxdate(i) = rxdate(i)+ 1;
/*ADD 1 TO PRESCRIPTION DATE TO MOVE START DATE TO NEXT DAY*/
days(i) = days(i) - 1;
/*SUBTRACT 1 FROM PRESCRIPTION DAYS SUPPLY FOR NEXT DAY*/
END;
END;
END;
END;
END;

/*DROP VARIABLES*/
DROP rxdate1-rxdate&nvar;
DROP days1-days&nvar;
DROP drug1-drug&nvar;
DROP dose1-dose&nvar;
DROP i j;

RUN;
%MEND DAILYDRUG;

```

Appendix Two

'DrugExposure' macro SAS® code

%MACRO DRUGEXPOSURE2

/*

DRUGNAME = NAME OF FILE OUTPUT FROM DAILYDRUG MACRO (USUALLY A DRUG NAME OR CLASS)

THIS FILE SHOULD BE IN LIB2 (SEE BELOW)

STARTDATE = DATE OF FIRST DAY OF STUDY DATA

ENDDATE = DATE OF LAST DAY OF STUDY DATA

INDEX = REFERENCE DATE FROM WHICH TO CALCULATE EXPOSURES (USUALLY DATE OF INCIDENCE)

INDEXFILE = FILE IN WHICH INDEX VARIABLE CAN BE FOUND

THIS FILE SHOULD BE IN LIB1 (SEE BELOW)

INDEXSTART = DATE PRIOR TO THE INDEX (EXPOSURE WILL BE CALCULATED FROM THIS DATE UP TO THE INDEX)

THIS CAN BE A VARIABLE DATE FOR EACH PATIENT (I.E.

DATE OF DRUG INITIATION PRE INDEX)

INDEXSTARTFILE = FILE IN WHICH INDEXSTART VARIABLE CAN BE FOUND

THIS FILE SHOULD BE IN LIB1 (SEE BELOW)

INDEXSTOP = DATE POST THE INDEX (EXPOSURE WILL BE CALCULATED FROM THE INDEX UP TO THIS DATE)

THIS CAN BE A VARIABLE DATE FOR EACH PATIENT (I.E. END

OF FOLLOW UP)

INDEXSTOPFILE = FILE IN WHICH INDEXSTART VARIABLE CAN BE FOUND

THIS FILE SHOULD BE IN LIB1 (SEE BELOW)

OFFSET = NUMBER OF DAYS PRIOR TO THE INDEX STOP DATE THAT EXPOSURE SHOULD BE CALCULATED UP TO

THIS VARIABLE EXCLUDES THE NUMBER OF OFFSET DAYS PRIOR TO THE INDEX STOP FROM THE EXPOSURE CALCULATION

E.G. CAN BE USED TO CALCULATE EXPOSURE UP TO XXX

DAYS PRIOR TO END OF FOLLOW UP

EXPSTART1 = FIXED NUMBER OF DAYS PRIOR/POST INDEX FROM WHICH TO START CALCULATING EXPOSURE

SPECIFY -VE FOR PRE INDEX AND +VE FOR POST INDEX (0 =

INDEX)

EXPSTOP1 = FIXED NUMBER OF DAYS PRIOR/POST INDEX TO STOP CALCULATING EXPOSURE AT

SPECIFY -VE FOR PRE INDEX AND +VE FOR POST INDEX (0 =

INDEX)

EXPSTART(N) AND EXPSTOP(N) REPRESENT A SINGLE WINDOW OF EXPOSURE WITH REFERENCE TO THE INDEX DATE

I.E. SPECIFYING EXPSTART1 = -365 AND EXPSTOP = 1 WILL CALCULATE EXPOSURE FOR THE 365 DAYS IMMEDIATELY PRIOR TO THE INDEX DATE

UP TO 10 SEPARATE EXPOSURE WINDOWS CAN BE SPECIFIED WITH THIS MACRO

```
LIB1 = LIBRARY NAME FOR FILES
LIB2 = LIBRARY NAME FOR FILES (THIS CAN BE THE SAME AS LIB1 IF
NECESSARY)
```

```
*/
      (drugname=      ,
        startdate=   ,
        enddate=     ,
        index=       ,
        indexfile=   ,
        indexstart=  ,
        indexstartfile= ,
        indexstop=   ,
        indexstopfile= ,
        offset=      0,
        expstart1=   ,
        expstop1=    ,
        expstart2=   ,
        expstop2=    ,
        expstart3=   ,
        expstop3=    ,
        expstart4=   ,
        expstop4=    ,
        expstart5=   ,
        expstop5=    ,
        expstart6=   ,
        expstop6=    ,
        expstart7=   ,
        expstop7=    ,
        expstart8=   ,
        expstop8=    ,
        expstart9=   ,
        expstop9=    ,
        expstart10=  ,
        expstop10=   ,
        lib1=        ,
        lib2=        );
```

```
/*ADD INDEXSTART DATE*/
```

```
PROC SORT DATA= &lib2..&drugname;
BY patient_id;
RUN;
PROC SORT DATA= &lib1..&indexfile;
BY patient_id;
RUN;
DATA &lib2..&drugname._exposure;
MERGE &lib1..&indexfile (IN=AA KEEP=patient_id &index) &lib2..&drugname (IN=BB);
BY patient_id;
IF AA=1 AND BB=1;
RUN;
```

```
/*ADD INDEXSTART DATE*/
```

```
%IF &indexstartfile ^= %STR()
%THEN %DO;
    PROC SORT DATA= &lib2..&drugname._exposure;
    BY patient_id;
    RUN;
    PROC SORT DATA= &lib1..&indexstartfile;
```

```

        BY patient_id;
        RUN;
        DATA &lib2..&drugname._exposure;
        MERGE      &lib1..&indexstartfile      (IN=AA      KEEP=patient_id      &indexstart)
&lib2..&drugname._exposure (IN=BB);
        BY patient_id;
        IF BB=1;
        RUN;
%END;
/*ADD INDEXSTOP DATE*/
%IF &indexstopfile ^= %STR()
%THEN %DO;
        PROC SORT DATA= &lib2..&drugname._exposure;
        BY patient_id;
        RUN;
        PROC SORT DATA= &lib1..&indexstopfile;
        BY patient_id;
        RUN;
        DATA &lib2..&drugname._exposure;
        MERGE      &lib1..&indexstopfile      (IN=AA      KEEP=patient_id      &indexstop)
&lib2..&drugname._exposure (IN=BB);
        BY patient_id;
        IF BB=1;
        RUN;
%END;
/*CREATE MACRO VARIABLES FOR START AND END AS SASDATES*/
DATA _NULL_;
start="&startdate"d;
end="&enddate"d;
CALL SYMPUT('startnum',TRIM(LEFT(start)));
CALL SYMPUT('endnum',TRIM(LEFT(end)));
RUN;
/*CALCULATE EXPOSURE IN SPECIFIED INTERVAL WITH REFERENCE TO INDEX DATE*/
DATA &lib2..&drugname._exposure;
SET &lib2..&drugname._exposure;

ARRAY  &drugname._days (&startnum:&endnum)  &drugname._days&startnum      -
&drugname._days&endnum;
ARRAY  &drugname._dose (&startnum:&endnum)  &drugname._dose&startnum      -
&drugname._dose&endnum;

%IF &indexstart ^= %STR()
%THEN %DO;
        &drugname._expdays_indexstart=0;
        &drugname._expdose_indexstart=0;
        DO i = &startnum TO &endnum;
                IF i >= (&indexstart) AND i <= (&index)
                THEN DO;
                        IF &drugname._days(i) = 1 THEN &drugname._expdays_indexstart =
&drugname._expdays_indexstart + 1;
                        IF &drugname._dose(i) ^= . THEN &drugname._expdose_indexstart =
&drugname._expdose_indexstart + &drugname._dose(i);
                END;
        END;
        &drugname._expdaysx_indexstart = &drugname._expdays_indexstart / ((&index)-
(&indexstart)+1);

```

```

        &drugname._expdosex_indexstart = &drugname._expdose_indexstart / ((&index)-
(&indexstart)+1);
%END;

%IF &indexstop ^= %STR()
%THEN %DO;
    &drugname._expdays_indexstop=0;
    &drugname._expdose_indexstop=0;
    DO i = &startnum TO &endnum;
        IF i >= (&index) AND i <= (&indexstop-&offset)
            THEN DO;
                IF &drugname._days(i) = 1 THEN &drugname._expdays_indexstop =
&drugname._expdays_indexstop + 1;
                IF &drugname._dose(i) ^= . THEN &drugname._expdose_indexstop =
&drugname._expdose_indexstop + &drugname._dose(i);
            END;
        END;
        &drugname._expdaysx_indexstop = &drugname._expdays_indexstop / ((&indexstop-&offset)-
(&index)+1);
        &drugname._expdosex_indexstop = &drugname._expdose_indexstop / ((&indexstop-&offset)-
(&index)+1);
    %END;

%IF &expstart1 ^= %STR()
%THEN %DO;
    &drugname._expdays1=0;
    &drugname._expdose1=0;
    DO i = &startnum TO &endnum;
        IF i >= (&index+&expstart1) AND i <= (&index+&expstop1)
            THEN DO;
                IF &drugname._days(i) = 1 THEN &drugname._expdays1 =
&drugname._expdays1 + 1;
                IF &drugname._dose(i) ^= . THEN &drugname._expdose1 =
&drugname._expdose1 + &drugname._dose(i);
            END;
        END;
        &drugname._expdaysx1 = &drugname._expdays1 / ((&index+&expstop1)-
(&index+&expstart1)+1);
        &drugname._expdosex1 = &drugname._expdose1 / ((&index+&expstop1)-
(&index+&expstart1)+1);
    %END;

%IF &expstart2 ^= %STR()
%THEN %DO;
    &drugname._expdays2=0;
    &drugname._expdose2=0;
    DO i = &startnum TO &endnum;
        IF i >= (&index+&expstart2) AND i <= (&index+&expstop2)
            THEN DO;
                IF &drugname._days(i) = 1 THEN &drugname._expdays2 =
&drugname._expdays2 + 1;
                IF &drugname._dose(i) ^= . THEN &drugname._expdose2 =
&drugname._expdose2 + &drugname._dose(i);
            END;
        END;
        &drugname._expdaysx2 = &drugname._expdays2 / ((&index+&expstop2)-
(&index+&expstart2)+1);

```

```

        &drugname._expdosex2      =      &drugname._expdose2      /      ((&index+&expstop2)-
(&index+&expstart2)+1);
%END;

```

```

%IF &expstart3 ^= %STR()
%THEN %DO;
    &drugname._expdays3=0;
    &drugname._expdose3=0;
    DO i = &startnum TO &endnum;
        IF i >= (&index+&expstart3) AND i <= (&index+&expstop3)
            THEN DO;
                IF &drugname._days(i) = 1 THEN &drugname._expdays3 =
&drugname._expdays3 + 1;
                IF &drugname._dose(i) ^= . THEN &drugname._expdose3 =
&drugname._expdose3 + &drugname._dose(i);
                    END;
            END;
        &drugname._expdaysx3      =      &drugname._expdays3      /      ((&index+&expstop3)-
(&index+&expstart3)+1);
        &drugname._expdosex3      =      &drugname._expdose3      /      ((&index+&expstop3)-
(&index+&expstart3)+1);
%END;

```

```

%IF &expstart4 ^= %STR()
%THEN %DO;
    &drugname._expdays4=0;
    &drugname._expdose4=0;
    DO i = &startnum TO &endnum;
        IF i >= (&index+&expstart4) AND i <= (&index+&expstop4)
            THEN DO;
                IF &drugname._days(i) = 1 THEN &drugname._expdays4 =
&drugname._expdays4 + 1;
                IF &drugname._dose(i) ^= . THEN &drugname._expdose4 =
&drugname._expdose4 + &drugname._dose(i);
                    END;
            END;
        &drugname._expdaysx4      =      &drugname._expdays4      /      ((&index+&expstop4)-
(&index+&expstart4)+1);
        &drugname._expdosex4      =      &drugname._expdose4      /      ((&index+&expstop4)-
(&index+&expstart4)+1);
%END;

```

```

%IF &expstart5 ^= %STR()
%THEN %DO;
    &drugname._expdays5=0;
    &drugname._expdose5=0;
    DO i = &startnum TO &endnum;
        IF i >= (&index+&expstart5) AND i <= (&index+&expstop5)
            THEN DO;
                IF &drugname._days(i) = 1 THEN &drugname._expdays5 =
&drugname._expdays5 + 1;
                IF &drugname._dose(i) ^= . THEN &drugname._expdose5 =
&drugname._expdose5 + &drugname._dose(i);
                    END;
            END;
        &drugname._expdaysx5      =      &drugname._expdays5      /      ((&index+&expstop5)-
(&index+&expstart5)+1);

```

```

        &drugname._expdosex5      =      &drugname._expdose5      /      ((&index+&expstop5)-
(&index+&expstart5)+1);
%END;

%IF &expstart6 ^= %STR()
%THEN %DO;
    &drugname._expdays6=0;
    &drugname._expdose6=0;
    DO i = &startnum TO &endnum;
        IF i >= (&index+&expstart6) AND i <= (&index+&expstop6)
        THEN DO;
            IF &drugname._days(i) = 1 THEN &drugname._expdays6 =
&drugname._expdays6 + 1;
            IF &drugname._dose(i) ^= . THEN &drugname._expdose6 =
&drugname._expdose6 + &drugname._dose(i);
                END;
            END;
            &drugname._expdaysx6      =      &drugname._expdays6      /      ((&index+&expstop6)-
(&index+&expstart6)+1);
            &drugname._expdosex6      =      &drugname._expdose6      /      ((&index+&expstop6)-
(&index+&expstart6)+1);
%END;

%IF &expstart7 ^= %STR()
%THEN %DO;
    &drugname._expdays7=0;
    &drugname._expdose7=0;
    DO i = &startnum TO &endnum;
        IF i >= (&index+&expstart7) AND i <= (&index+&expstop7)
        THEN DO;
            IF &drugname._days(i) = 1 THEN &drugname._expdays7 =
&drugname._expdays7 + 1;
            IF &drugname._dose(i) ^= . THEN &drugname._expdose7 =
&drugname._expdose7 + &drugname._dose(i);
                END;
            END;
            &drugname._expdaysx7      =      &drugname._expdays7      /      ((&index+&expstop7)-
(&index+&expstart7)+1);
            &drugname._expdosex7      =      &drugname._expdose7      /      ((&index+&expstop7)-
(&index+&expstart7)+1);
%END;

%IF &expstart8 ^= %STR()
%THEN %DO;
    &drugname._expdays8=0;
    &drugname._expdose8=0;
    DO i = &startnum TO &endnum;
        IF i >= (&index+&expstart8) AND i <= (&index+&expstop8)
        THEN DO;
            IF &drugname._days(i) = 1 THEN &drugname._expdays8 =
&drugname._expdays8 + 1;
            IF &drugname._dose(i) ^= . THEN &drugname._expdose8 =
&drugname._expdose8 + &drugname._dose(i);
                END;
            END;
            &drugname._expdaysx8      =      &drugname._expdays8      /      ((&index+&expstop8)-
(&index+&expstart8)+1);

```

```

        &drugname._expdosex8      =      &drugname._expdose8      /      ((&index+&expstop8)-
(&index+&expstart8)+1);
%END;

```

```

%IF &expstart9 ^= %STR()
%THEN %DO;
    &drugname._expdays9=0;
    &drugname._expdose9=0;
    DO i = &startnum TO &endnum;
        IF i >= (&index+&expstart9) AND i <= (&index+&expstop9)
            THEN DO;
                IF &drugname._days(i) = 1 THEN &drugname._expdays9 =
&drugname._expdays9 + 1;
                IF &drugname._dose(i) ^= . THEN &drugname._expdose9 =
&drugname._expdose9 + &drugname._dose(i);
            END;
        END;
        &drugname._expdaysx9      =      &drugname._expdays9      /      ((&index+&expstop9)-
(&index+&expstart9)+1);
        &drugname._expdosex9      =      &drugname._expdose9      /      ((&index+&expstop9)-
(&index+&expstart9)+1);
%END;

```

```

%IF &expstart10 ^= %STR()
%THEN %DO;
    &drugname._expdays10=0;
    &drugname._expdose10=0;
    DO i = &startnum TO &endnum;
        IF i >= (&index+&expstart10) AND i <= (&index+&expstop10)
            THEN DO;
                IF &drugname._days(i) = 1 THEN &drugname._expdays10 =
&drugname._expdays10 + 1;
                IF &drugname._dose(i) ^= . THEN &drugname._expdose10 =
&drugname._expdose10 + &drugname._dose(i);
            END;
        END;
        &drugname._expdaysx10     =      &drugname._expdays10     /      ((&index+&expstop10)-
(&index+&expstart10)+1);
        &drugname._expdosex10     =      &drugname._expdose10     /      ((&index+&expstop10)-
(&index+&expstart10)+1);
%END;

```

```

KEEP
patient_id
&drugname._expdays1 - &drugname._expdays10
&drugname._expdose1 - &drugname._expdose10
&drugname._expdaysx1 - &drugname._expdaysx10
&drugname._expdosex1 - &drugname._expdosex10
&drugname._expdays_indexstart
&drugname._expdaysx_indexstart
&drugname._expdose_indexstart
&drugname._expdosex_indexstart
&drugname._expdays_indexstop
&drugname._expdaysx_indexstop
&drugname._expdose_indexstop
&drugname._expdosex_indexstop;

```


RUN;

%MEND DRUGEXPOSURE2;

Appendix Three

WHO ATC Drug Codes

Anti-diabetic:	A10
Insulin:	A10A
Metformin:	A10BA, A10BD02, A10BD03, A10BD05, A10BD07, A10BD08
Sulfonylureas:	A10BB, A10BD02, A10BD04, A10BD06
Other anti-diabetic drugs:	A10BF, A10BG, A10BH, A10BX, A10BD03, A10BD04, A10BD05, A10BD06, A10BD07, A10BD08, A10BD09
Aspirin:	B01AC06, M01BA03, N02BA01, N02BA51, N02BA71
Non-aspirin NSAID:	M01A
Statins	C10AA

ICD-O-2 Tumour Site Codes

Chapter 6 and Chapter 7

Colon:	C18.0 – C18.9
Rectal:	C19.9, C20.9

Chapter 8

Proximal colon:	C18.0, C18.1, C18.2, C18.3
Distal colon:	C18.4, C18.5, C18.6, C18.7
Rectal:	C19.9, C20.9
Other:	C18.8, C18.9

ICD-O-2 Tumour Morphology Codes

Adenocarcinoma:	8140/3, 8210/3, 8211/3, 8246/3, 8261/3, 8263/3
Other:	8000/3, 8070/3, 8480/3, 8481/3, 8490/3, 8550/3

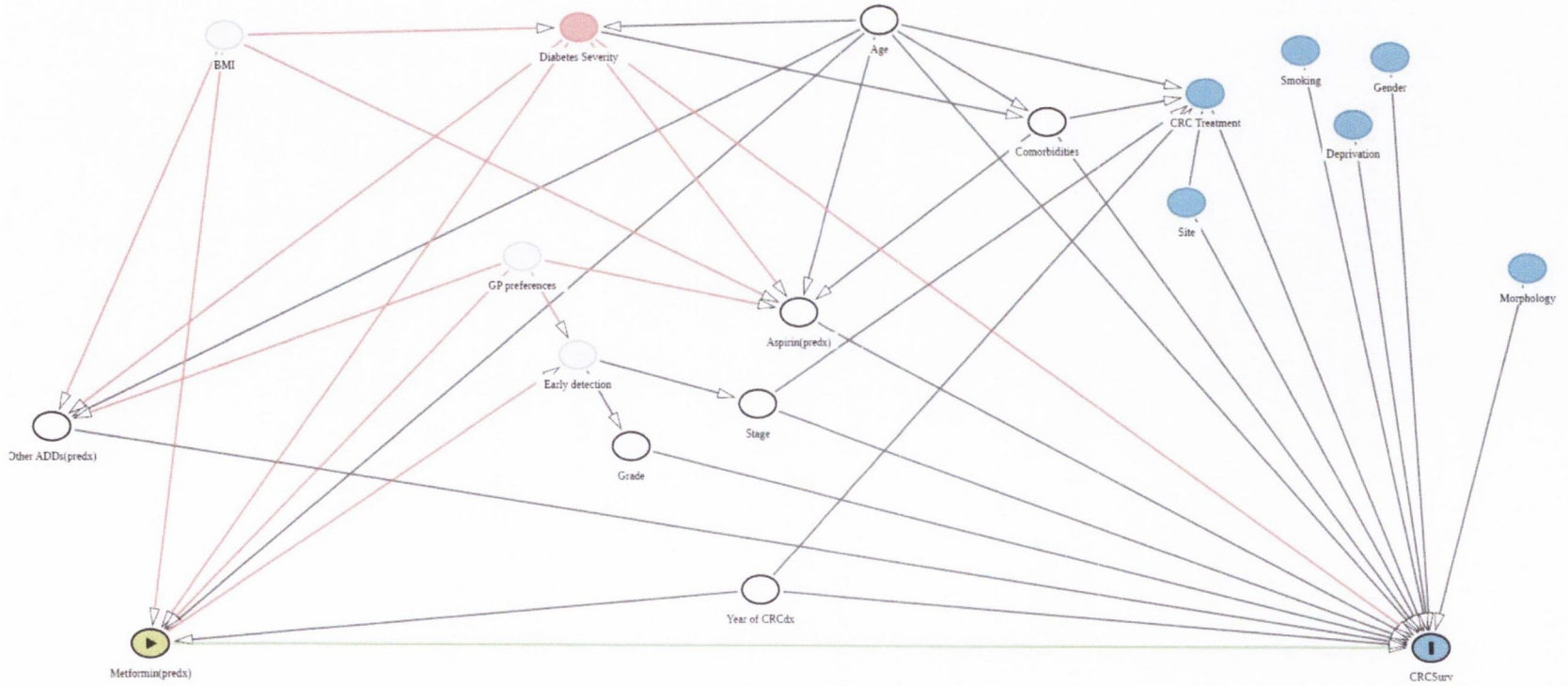


Figure: Directed Acyclic Graph showing putative relationships between pre-diagnostic metformin exposure (Metformin(predx)) and colorectal cancer-specific survival (CRCSurv)

(drawn using <http://www.dagitty.net/>. Minimal adjustment set indicated by white oval nodes)

dx: diagnosis

ADDs: anti-diabetic drugs

- exposure
- outcome
- ancestor of exposure
- ancestor of outcome
- ancestor of exposure and outcome
- adjusted variable
- unobserved (latent)
- other variable
- causal path
- biasing path

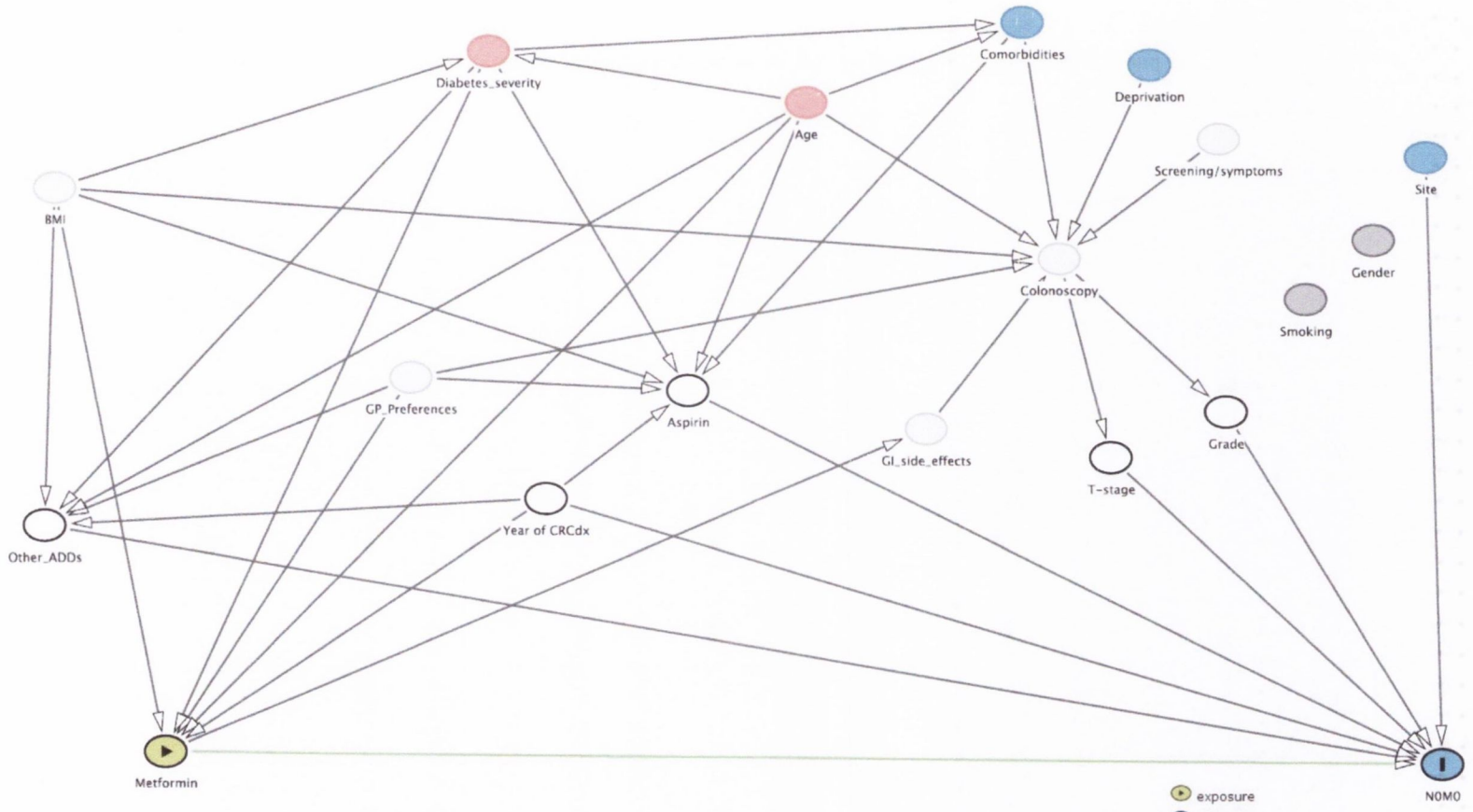
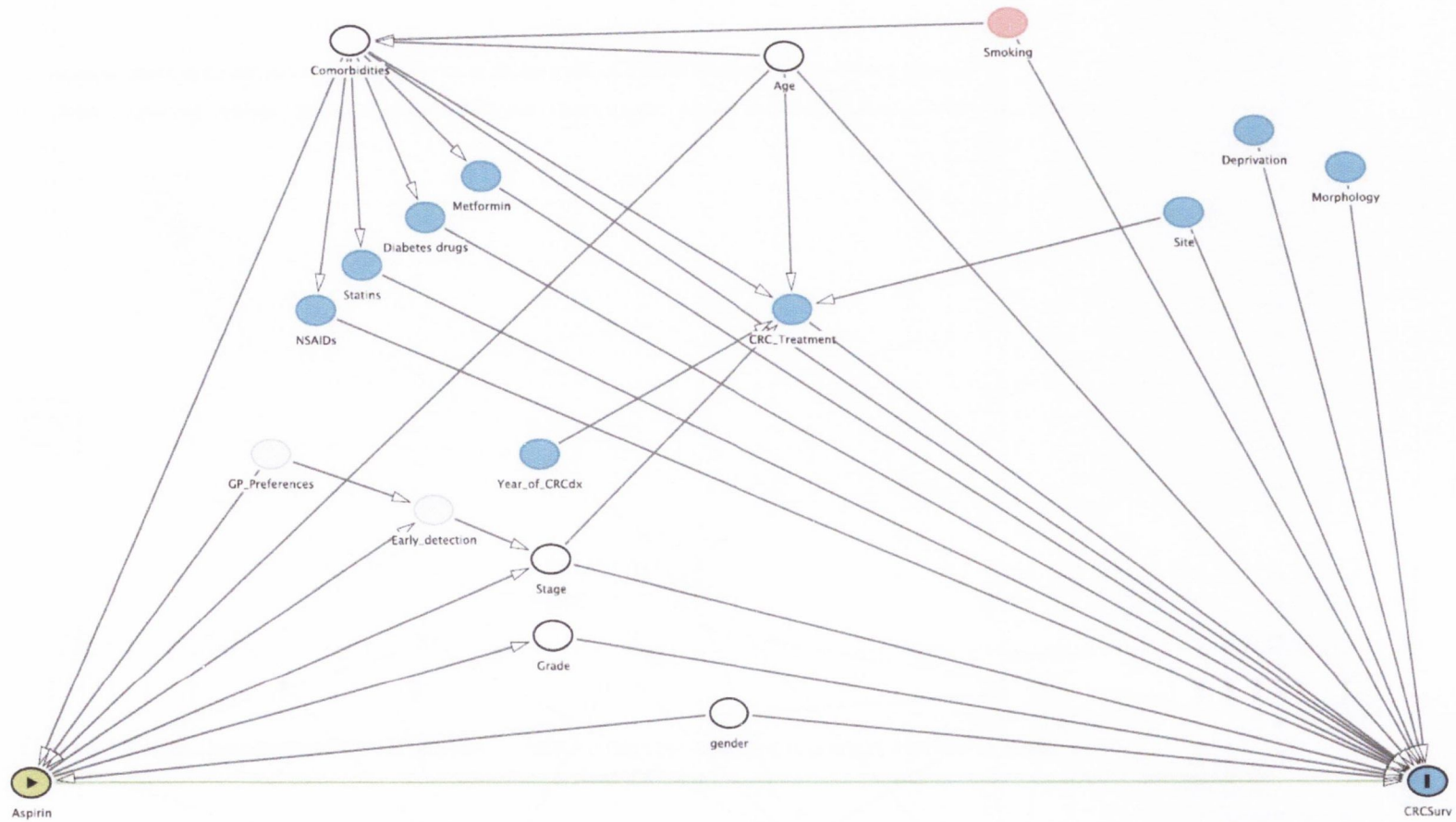


Figure: Directed Acyclic Graph showing putative relationships between pre-diagnostic metformin exposure (Metformin(predx)) and the presence/absence of lymph node or distant metastases at diagnosis (NOM0).

(drawn using <http://www.dagitty.net/>. Minimal adjustment set indicated by white oval nodes)

dx: diagnosis

ADDs: anti-diabetic drugs



- exposure
- outcome
- ancestor of exposure
- ancestor of outcome
- ancestor of exposure and outcome
- adjusted variable
- unobserved (latent)
- other variable
- causal path
- biasing path

Figure: Directed Acyclic Graph showing putative relationships between pre-diagnostic aspirin exposure and colorectal cancer-specific survival (CRCSurv)

(drawn using <http://www.dagitty.net/>. Minimal adjustment set indicated by white oval nodes)

dx: diagnosis