Drug repositioning in chemoprevention of colorectal cancer

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LUND UNIVERSITY

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on 7th of June at 13:30 in 37:an CRC, Department of Clinical Science

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Title and subtitle: Drug repositioning in chemoprevention of colorectal cancer

Abstract:

Background

Colorectal cancer (CRC) ranks as the third most frequently occurring cancer and the second leading cause of cancer-related deaths worldwide. In response to the high demand for an effective prevention strategy, chemoprevention, using medications to block the pathogenetic pathways of disease, might be an attractive strategy to offer a more effective option at a low cost. Therefore, we aimed to assess the potential beneficial effects of already-approved drugs on the chemoprevention of CRC, especially in high-risk groups.

Methods

Papers I, II, and III were population-based cohort studies. By accessing several nationwide Swedish registers, we identified individuals who had ever been previously prescribed melatonin (Paper I), proguanil/atovaquone (Paper II), and selective serotonin reuptake inhibitor (SSRI, Paper III), respectively, and matched them with comparisons who did not use the drugs based on age and sex. The Cox regression model was used to calculate hazard ratios (HRs) and 95% CI confidence intervals (CIs). Paper IV was a nested case-control study exploring the combined effect of SSRIs and aspirin against CRC. We identified CRC cases and randomly matched them to controls conditional on birth year and sex using incidence-density sampling. The conditional logistic regression model was used to calculate odds ratios (ORs) and 95% CIs.

Results

We found that uses of melatonin (Paper I), proguanil/atovaquone (Paper II), and SSRIs (Paper III) were all associated with a reduced CRC risk, with adjusted HRs and 95% CIs, 0.82 (0.72-0.92), 0.76 (0.62-0.93) and 0.77 (0.70-0.85), respectively. Tests for trends showed significant dose-response correlations (P <0.001). The decrease in CRC risk was independent of tumor location and stage at diagnosis. In Paper IV, both aspirin and SSRIs monotherapy were negatively associated with CRC risk, but the combined use of aspirin and SSRIs was associated with an even lower CRC risk (adjusted OR, 0.77, 95% CI, 0.67-0.89) than aspirin monotherapy (adjusted OR, 0.91, 95% CI, 0.87-0.97) or SSR monotherapy (adjusted OR, 0.93, 95% CI, 0.86-1.00). A significant interaction was observed at the additive scale (P <0.001).

Conclusion

We identified several potential chemopreventive agents against CRC. Our findings call for further studies to confirm the underlying mechanisms and the plausibility of clinical recommendations.

Keywords: Drug repositioning; Colorectal cancer; Pharmacoepidemiology; Chemoprevention; Melatonin; Proguanil; Atovaquone; Aspirin; Selective serotonin reuptake inhibitor.

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To my dear grandpa

Table of Contents

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List of papers included in the thesis

This thesis is based on the following four articles that are appended at the end of this thesis. Reprinted with permission from the respective publishers.

Paper I

Zhang N, Sundquist J, Sundquist K, Ji J. Use of Melatonin Is Associated With Lower Risk of Colorectal Cancer in Older Adults. Clin Transl Gastroenterology. 2021 Aug 3;12(8):e00396.

Paper II

Zhang N, Sundquist J, Sundquist K, Ji J. Proguanil and atovaquone use is associated with lower colorectal cancer risk: a nationwide cohort study. BMC Medicine. 2022 Nov 10;20(1):439.

Paper III

Zhang N, Sundquist J, Sundquist K, Ji J.Use of Selective Serotonin Reuptake Inhibitors Is Associated with a Lower Risk of Colorectal Cancer among People with Family History. Cancers (Basel). 2022 Nov 29;14(23):5905.

Paper IV

Zhang N, Sundquist J, Sundquist K, Zhang ZG, Ji J. Combined Use of Aspirin and Selective Serotonin Reuptake Inhibitors Is Associated With Lower Risk of Colorectal Cancer: A Nested Case-Control Study. Am J Gastroenterol. 2021 Jun 1;116(6):1313-1321.

List of papers not included in the thesis

1. **Zhang N**, Sundquist J, Sundquist K, Ji J. An Increasing Trend in the Prevalence of Polypharmacy in Sweden: A Nationwide Register-Based Study. Front Pharmacol. 2020 Mar 18;11:326.

2. Huang S[#], **Zhang NQ**[#], Xu CJ[#], Huang WQ[#], Li DX, Li J, Yao LL, Sundquist K, Sundquist J, Jiang SH, Xing X, Hu LP, Zhang ZG, Ji J, Zhang XL. Dipyridamole enhances the anti-cancer ability of aspirin against colorectal cancer by inducing apoptosis in an unfolded protein response-dependent manner. Cell Oncol (Dordr). 2023 Mar 20. doi: 10.1007/s13402-023-00789-7.

3. Xiao J, Ji J, **Zhang N**, Yang X, Chen K, Chen L, Huang W. Association of genetically-predicted lipid traits and lipid-modifying targets with heart failure. Eur J Prev Cardiol. 2022 Dec 15:zwac290.

4. Li Y, Sundquist K, **Zhang N**, Wang X, Sundquist J, Memon AA. Mitochondrial related genome-wide Mendelian randomization identifies putatively causal genes for multiple cancer types. EBioMedicine. 2023 Jan 10;88:104432.

5. Li Y, Sundquist K, Wang X, **Zhang N**, Hedelius A, Sundquist J, Memon AA. Association of Mitochondrial DNA Copy Number and Telomere Length with Prevalent and Incident Cancer and Cancer Mortality in Women: A Prospective Swedish Population-Based Study. Cancers (Basel). 2021 Jul 30;13(15):3842.

[#] Contributed equally.

Abbreviations

AIC	Akaike information criterion
ATC	Anatomical therapeutic chemical classification
CCI	Charlson comorbidity index score
CI	Confidence interval
COPD	Chronic obstructive pulmonary disease
COX-2	Cyclooxygenase-2
CRC	Colorectal cancer
DDD	Defined daily dose
GWAS	Genome-wide association studies
HR	Hazard ratio
IBD	Inflammatory bowel disease
ICD	International classification of diseases
PDE5	Phosphodiesterase-5
RERI	Relative excess risk for interaction
RR	Risk ratio
RWD	Real world data
RCT	Randomized controlled trials
SSRI	Selective serotonin reuptake inhibitor
USPSTF	US Preventive Services Task Force

Introduction

Colorectal cancer

Epidemiology

Colorectal cancer (CRC) is ranked as the third most common cancer and the second leading cause of cancer-related mortality worldwide. In 2020 it was estimated that there were over 1.9 million new cases of CRC, which resulted in approximately 935,000 deaths[1]. The incidence and prevalence of CRC have been posited to economic and social development [2]. The CRC incidence in developed countries was approximately four-fold greater than that in developing countries, whereas the mortality rate caused by CRC was roughly the same due to a higher fatality rate in developing countries. The incidence rates vary across different areas. The highest rate was in European regions, Australia/New Zealand, and Northern America. The global burden caused by CRC has been steadily rising, with a more than 150% increase in incident cases (842,098 vs. 2.17 million) and a more than 100% increase in mortality rate (518,126 vs. 1.09 million) from 2009 to 2019 [3,4]. In spite of the fact that aging and population growth can account for the majority of this rise, changes in age-specific incidence rates contribute to 20% of the escalation [3]. On the other hand, the high incidence rate among the elderly masks the worrying rise of early-onset CRC (diagnosed before the age of 50), especially rectal cancer and distal colon cancer [5,6]. It was estimated that the burden of CRC is expected to continue to increase to 3.2 million new cases and 1.6 million deaths by the year 2040, with developed countries contributing to the majority of the incident cases [7].

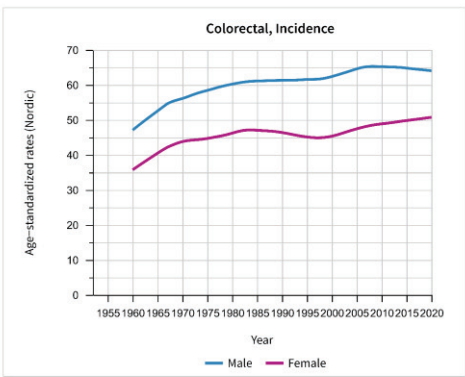
Sweden is one of the countries that have the highest CRC rate in the world. There has been a continuous increase in CRC incidence over the past few decades, while the mortality rate has slightly decreased (**Figure 1**) [8]. The improvement in survival rate in Sweden might be attributed to upgraded registration of diagnosis and treatment [9], nationwide audits [10], initiatives to centralize treatment, and reduction in waiting times [11].

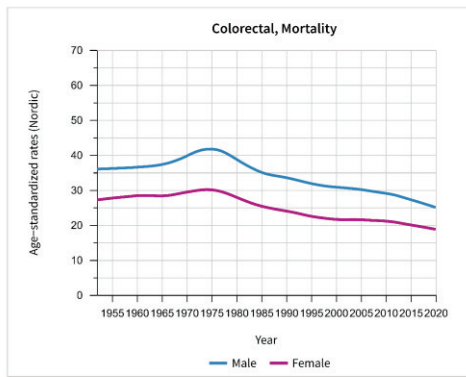
International Agency for Research on Cancer
World Health Organization

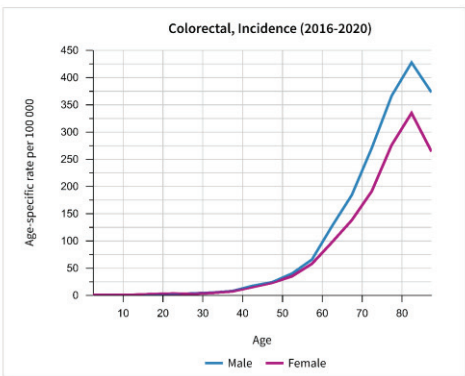
NORDCAN
Association of the Nordic Cancer Registries

Sweden
Colorectal

	Male	Female
Number of new cases per year (incidence 2016-2020)	3 582	3 187
Proportion of all cancers (%)	10.8	11.1
Proportion of all cancers except non-melanoma skin (%)	11.9	12.3
Risk of getting the disease before age 75 (%)	3.6	2.8
Age-standardized incidence rate (Nordic)	64.5	50.7
– Estimated annual change latest 10 years (%)	-0.2	0.4
Number of deaths per year (2016-2020)	1 390	1 297
Proportion of all cancer deaths (%)	11.5	11.8
Risk of dying from the disease before age 75 (%)	1.1	0.8
Age-standardized death rate (Nordic)	25.6	19.2
– Estimated annual change latest 10 years (%)	-1.9	-1.5
Persons living with the diagnosis at the end of 2020 (prevalence)	28 542	28 791
Number of persons living with the diagnosis per 100 000	546.5	558.3
Relative survival (%) with [95% CI] (2016-2020)		
1-year	87.1 [86.5-87.7]	86.8 [86.2-87.4]
5-year	69.1 [68.2-70.0]	70.9 [70.0-71.8]







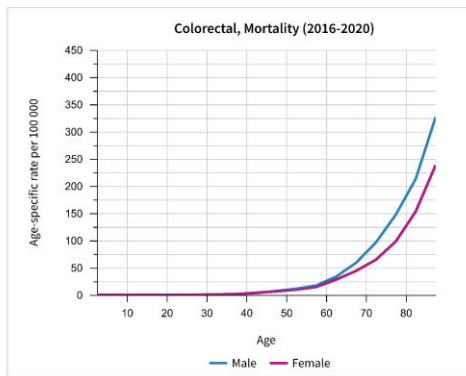


Figure 1. Colorectal cancer incidence and mortality rates in Sweden. Data source: NORDCAN. https://nordcan.iarc.fr/en/factsheets

Risk factors

Environmental and hereditary factors are both involved in the pathogenesis of CRC (**Figure 2**) [5].

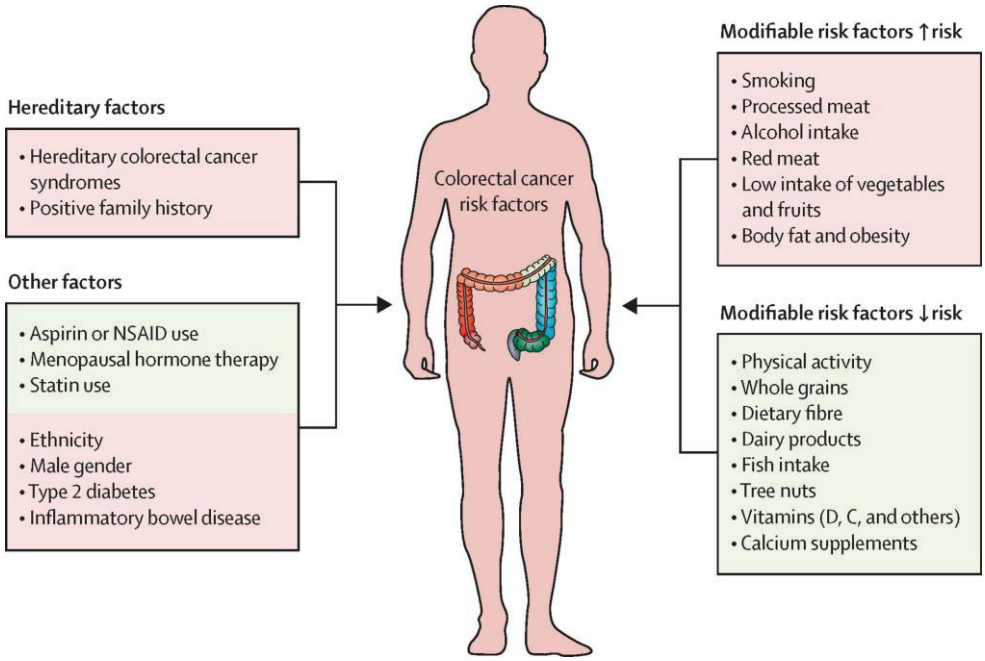


Figure 2. List of risk factors for colorectal cancer. Reprinted from Lancet. 2019. Vol.19 Issue 394. Pages 1467-1480. Dekker E, Tanis PJ, Vleugels JLA, et al. Colorectal cancer. © 2019 Elsevier Ltd.

Age, sex, and ethnicity

Age is one of the risk factors for CRC due to that cancer is an aging-related disease. In Sweden, the incidence and mortality rates of CRC increase dramatically after the age of 50. Male sex is also a risk factor. The age-standardized rates are higher in males than in females both in incidence and mortality, and the difference becomes more remarkable after 50 years of age (**Figure 1**). One of the plausible explanations for the vulnerability in males might be due to the difference in hormone levels. Men do not benefit from the same protective effect of endogenous estrogen as women [12]. In addition, men are more likely to be affected by environmental factors than by genetic factors in colorectal carcinogenesis. Migration epidemiology has suggested that male immigrants, who came to Sweden before the age of 30, had a CRC incidence rate similar to that of native residents after residing in Sweden for five decades. However, the transition in CRC risk toward the host country was observed to be less prominent in women [13]. The incidence and mortality rates vary by ethnicity. According to statistics from the American Cancer Society in 2023, CRC

incidence and mortality are highest in American Indian and Alaska Native individuals, followed closely by non-Hispanic blacks (hereafter, blacks), and lowest in Asians/Pacific Islanders [14]. However, Swedish nationwide registers do not include data on ethnicity but rather country or region of birth.

Hereditary factors

Approximately 35-40% of CRC cases are linked to inherited susceptibility to CRC (**Figure 3**) [15]. Roughly 25% of CRC cases showed a familial clustering of the disease, without any apparent genetic cancer syndrome [16]. Previous meta-analyses revealed that the risk of CRC among individuals, with at least one affected first-degree relative (parents, siblings, or children), is increased by about 2-times compared with the general population, and the observed associations were more pronounced when the first-degree relatives were diagnosed with CRC before the age of 50 years [17,18]. Heritability estimates for colon and rectum cancer were, respectively, 16% and 15% according to a previous Nordic twin study [19]. Around 5% of the cases are attributed to hereditary cancer syndromes, which can be subdivided as hereditary nonpolyposis colorectal cancer (or Lynch syndrome) and familial adenomatous polyposis [16]. The rest of CRC heritability is contributed by low-penetrance genetic variations identified from genome-wide association studies (GWAS), and other inherited aberrations yet to be discovered.

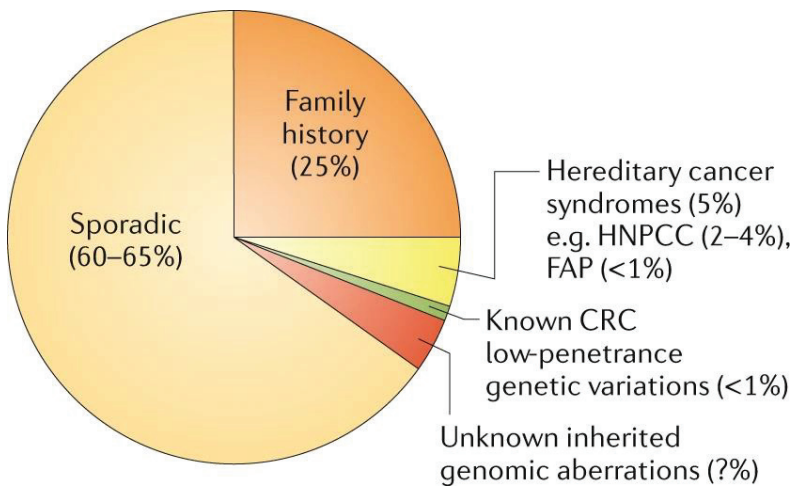


Figure 3. Proportion of colorectal cancer cases associated with sporadic and hereditary factors. Reprinted from Nat Rev Gastroenterol Hepatol. 2019. Vol.16 Pages 713-732. Keum, N., GiovannuCCI, E. Global burden of colorectal cancer: emerging trends, risk factors and prevention strategies. © 2019, Springer Nature Ltd.

Environmental factors

Around 60-65% of CRC cases develop sporadically, indicating that CRC incidence in a population is largely affected by modifiable diet and lifestyle factors.

Cigarette smoking considerably elevates the risk of many types of cancers including CRC; this is consistently reinforced by epidemiological studies [20,21]. A recent meta-analysis summarizing 188 original studies showed that cigarette smoking increased 15-20% of the CRC risk in duration- and intensity-dependent patterns, both in men and women. Quitting smoking could reduce CRC risk [22].

Alcohol consumption has been well recognized as a risk factor for CRC [23-25]. Results from the latest meta-analysis, which pooled 32 cohort studies, indicated that moderate and heavy drinking both contributed to the increased risk of CRC, with risk ratios (RRs) and 95% confidence intervals (CIs) of 1.24 (1.17-1.32), and 1.54 (1.15-2.06) respectively, but did not increase CRC mortality [25].

Obesity, defined as individuals with a body mass index (BMI, weight/[height in m]^2) greater than or equal to 25, is a growing global public health issue, leading to an increase in the risk of several debilitating and deadly diseases including cancers [26-29]. In Europe, obesity contributed to 11% of CRC cases [30]. Obesity was reported to be associated with a 30-70% increased CRC risk in men, while the association was less strong in women [29]. Obesity may also increase the chances of cancer recurrence or mortality and may have an impact on initial treatment decisions [31-36].

Physical activity is a protective factor that can significantly reduce CRC incidence as well as mortality [37,38]. The biological pathways are incompletely understood but generally center on maintaining healthy body weight, enhancing insulin sensitivity and digestive tract immune function, and reducing chronic low-grade inflammation [27].

Diet can be the most modifiable risk factor for CRC. The gut microbiota's structure and function can be altered by diet through modulating metabolites product [39-41]. It has been suggested that high consumption of red meat and ultra-processed meat was associated with an increased risk of CRC [42]. In contrast, a diet rich in fiber, fruits, vegetables, whole grains, nuts, dairy products, vitamin D, and calcium, can help to reduce the risk of developing CRC [43-48].

Inflammatory bowel disease (IBD), including ulcerative colitis and Crohn's disease, is a risk factor for CRC. It shares the same factors that also trigger CRC, thus individuals with a diagnosis of IBD are 2-6 fold more likely to develop CRC than the general population [49,50]. IBD was also reported to be associated with a higher rate of recurrence and mortality rate [51,52].

CRC screening and diagnosis

The majority of CRC cases develop slowly from precursor lesions like adenomatous polyps or sessile serrated lesions, providing a timeframe for early detection of both cancer and precursor lesions through screening [53]. Several screening options for CRC are accessible, such as stool-based tests (e.g., fecal immunochemical testing, shorted as FIT, and multitarget stool DNA test), blood-based tests (e.g., septin 9), and imaging-based tests (e.g., CT colonography, colon capsule, flexible sigmoidoscopy, and colonoscopy) [54,55]. Assuming 100% adherence to CRC screening for individuals aged 50 to 75, the strategies of colonoscopy every 10 years, annual FIT, sigmoidoscopy every 10 years with annual FIT, and CT colonography every five years result in median reductions in the lifetime risk of dying from CRC by 87%, 81%, 82%, and 85%, respectively. Although most guidelines recommend that individuals at average risk of CRC begin screening at the age of 50 years, initiating screening at 45 years of age was found to be more efficient and resulted in a better trade-off between the benefits and the burden of screening than starting at 50 years of age [56]. However, CRC screening recommendations vary across countries due to differences in population risk, economic and healthcare resources, and patient and societal values [57]. In Sweden, organized CRC screening is only available in Stockholm and Gotland counties at the regional level, with a compliance rate of approximately 60% [58]. Individuals between the ages of 60 and 69 are invited to undergo biennial screening for FIT. If blood is detected, a follow-up colonoscopy is performed [59].

Patients with CRC typically show symptoms such as rectal bleeding, chronic abdominal pain, microcytic anemia, and altered bowel habits [60]. The median age of diagnosis is 67 years. The gold standard for diagnosing CRC is a complete colonoscopy up to the cecum, coupled with a biopsy for histopathological examination, due to its high diagnostic performance [61]. This procedure serves as both a diagnostic and therapeutic opportunity by enabling tumor localization and potential endoscopic excision of polyps [62].

Drug repositioning

Drug repositioning breaks the drug shortage bottleneck

Considering the high incidence and mortality of CRC, there is an increasing need for oncology drugs, both for prevention and treatment. Despite the rapid development in technology and enhanced knowledge of carcinogenesis, the translation of "*de novo*" drugs into clinical practice has been slower than anticipated [63]. On average, drug development takes around 12 to 13 years and costs approximately 2 to 3 billion USD to bring a *de novo* drug from the bench to the

bedside [64]. It is estimated that it takes 4-8 years for the pre-clinical period including basic study, drug design, *in vitro* and *in vivo* experiments, and 6-8 years for three phases of clinical trials and FDA approval (**Figure 4**). However, the rate of success is low, with only one out of every 5000-10,000 prospective antitumor agents being approved by the FDA, and only 5% of oncology drugs that enter Phase I clinical trials eventually receive approval [65].

The cost of cancer treatment is continuously increasing. However, national healthcare services worldwide and in Sweden cannot support the current explosion in the cost of new oncology drugs, and a significant decrease in the cost of new oncology drugs is warranted. Drug repositioning (also called drug repurposing, reprofiling, or re-tasking), the application of known drugs and compounds with a purpose outside the scope of the original medical indication, might be a promising strategy for breaking the current drug shortage bottleneck. Reduced time, cost, and risk are the key advantages of drug repurposing. Since the safety profiles including pharmacokinetic, pharmacodynamic, and toxicity profiles of the old drugs have already been approved in the original preclinical and Phase I studies, there is a drastic reduction in development cost and time [66,67].

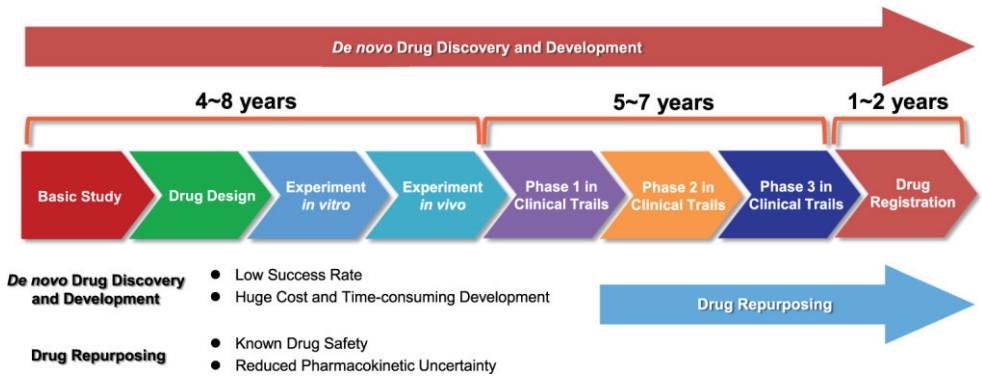


Figure 4. The estimated time and main steps in de novo drug discovery and development and drug repurposing for cancer therapy. Reprinted from Sig Transduct Target Ther. 2020. Vol. 5, Issue 1, Pages 113. Zhang, Z., Zhou, L., Xie, N., et al. Overcoming cancer therapeutic bottleneck by drug repurposing. ©The authors.

Approaches for drug repositioning

A mechanistic evaluation of an old drug's anticarcinogenic potential requires systematic approaches, including computational and experimental methods, to analyze its effectiveness and therapeutic potential (**Figure 5**) [68-70]. Computational approaches based on Real World Data (RWD) are attracting more and more attention recently. As its name implies, RWD primarily comprises a systematized collection of electronic health records of patients in the real world, which are

characterized by extensive and intricate datasets on patient outcomes [71-74]. Electronic health records contain extensive data on patient medical records, including their medical history, laboratory test results, medication use, and adverse events [75]. Furthermore, the abundance of electronic health records data provides ample statistical power for achieving significant results [76]. Randomized controlled trials (RCTs) are considered the gold standard for establishing causal inferences and testing the efficacy of interventions in clinical care [77]. However, RCTs may not be appropriate or feasible for addressing all research questions. For instance, RCTs might face ethical issues in identifying harmful risk factors, and are not practical for assessing long-term outcomes or rare adverse events, determining trends in management, or identifying disparities in access to treatments. These aspects of knowledge can only be obtained using RWD [78]. Studies using RWD can address a wider range of questions and include a broader cross-section of the population compared to RCTs, which usually have strict inclusion and exclusion criteria. This makes RWD studies more generalizable to the community and provides more timely evidence [79]. RWD offers an efficient approach to conducting post-approval surveillance studies of medical interventions, as they allow for longer follow-up periods and larger sample sizes to identify rare outcomes [80].

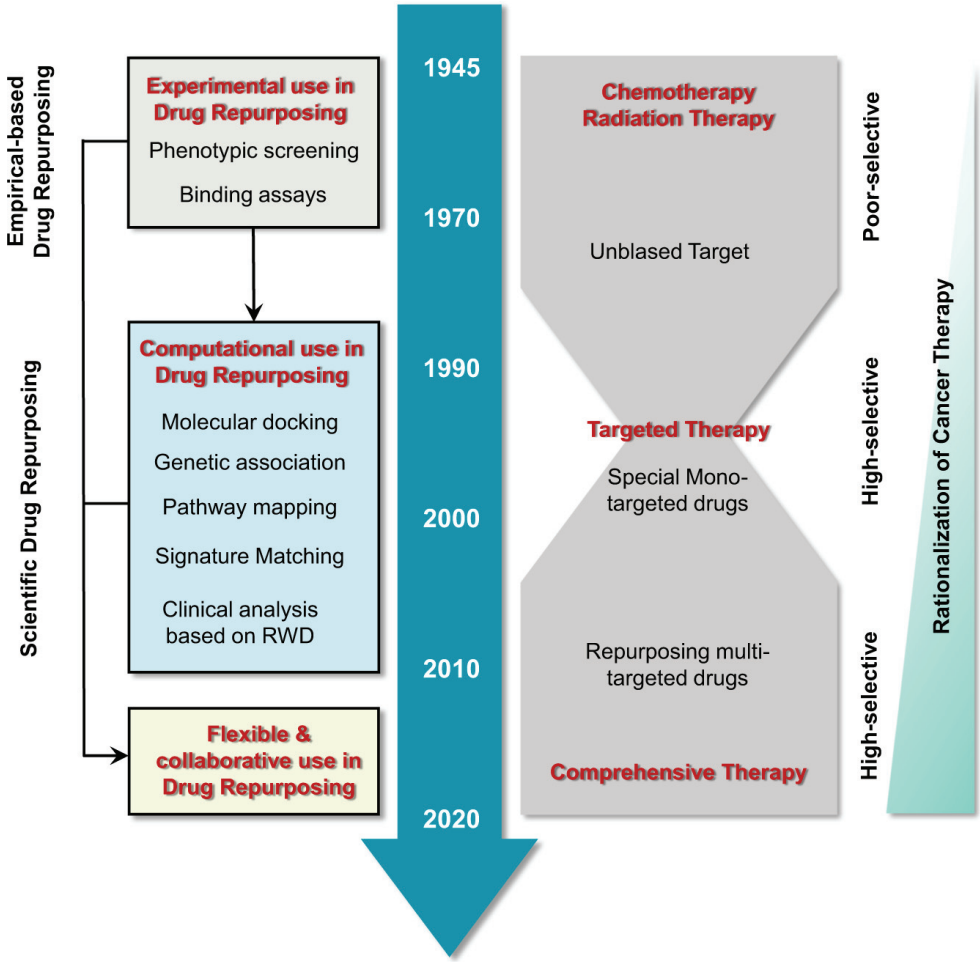


Figure 5. Technological approaches to drug repurposing for cancer therapy. Reprinted from Sig Transduct Target Ther. 2020. Vol. 5, Issue 1, Pages 113. Zhang, Z., Zhou, L., Xie, N., et al. Overcoming cancer therapeutic bottleneck by drug repurposing. ©The authors.

Potential candidates for CRC chemoprevention

Several promising agents have been repurposed for the prevention of CRC based on numerous basic, clinical, and RWD evidence during the past several decades.

Aspirin

One of the most successful drugs used in chemoprevention against CRC is aspirin (acetylsalicylic acid). Aspirin is a non-steroidal anti-inflammatory drug and is

primarily used to reduce pain, fever, and inflammation. Aspirin was also recommended as prophylaxis for cardiovascular disease [81]. Over recent decades, numerous pre-clinical, RCTs, and observational evidence indicated a chemopreventive effect of low-dose aspirin against cancer, especially CRC [82-94]. Aspirin's chemopreventive effect in CRC depends on its inhibitory effect on cyclooxygenase-2 (COX-2), and involves multiple mechanisms. The combined effect of antiplatelet and anti-inflammatory of aspirin may prevent inflammation-associated tumorigenesis in CRC [95]. Based on the evidence mentioned above, the US Preventive Services Task Force (USPSTF) recommended the use of low-dose aspirin for primary prevention of CRC in individuals aged 50 to 59 in 2007 [96]. However, the overall advantage of aspirin was comparatively reduced in the revised evaluation in 2022 [97]. Furthermore, aspirin use may elevate the risk of gastrointestinal bleeding and intracranial hemorrhage, emphasizing the persistent ambiguity regarding the impacts of aspirin when utilized for primary prevention since chemopreventive agent selection must weigh the risk, as well as its related benefits, at the same time for each patient.

Metformin

Metformin, a biguanide antihyperglycemic agent, is widely recognized as the first-line oral medication for treating type 2 diabetes mellitus by global recommendations, as it functions as an insulin sensitizer. Metformin was regarded as “the aspirin of the 21st century” for its role in the prevention of cancers similar to aspirin [98]. Results from a meta-analysis pooling 12 studies reported a 20% reduced risk of colon cancer [99]. Metformin has been found to possess an antitumor effect, which can be largely attributed to its capability to modulate a range of upstream and downstream molecular targets that are involved in key cellular processes such as apoptosis, autophagy, cell cycle regulation, oxidative stress, inflammation, metabolic homeostasis, and epigenetic regulation [100-120]. However, achieving the therapeutic concentration of metformin that induces cell growth arrest is challenging with conventional administration routes and may be difficult to apply in clinical settings [121].

Statin

Statins are a class of drugs that are widely used to lower cholesterol levels. They are commonly prescribed for their lipid-lowering properties, which have been shown to reduce the risk of cardiovascular disease and stroke in patients with hyperlipidemia [122]. The effects of statin go beyond its primary indication of inhibiting cholesterol biosynthesis, including the modulation of cell growth, apoptosis, and inflammation, which can potentially influence the development of cancer [123-130]. Evidence from clinical and epidemiological studies is, however, inconclusive. Several meta-analyses using different inclusion criteria and methodologies drew differing

conclusions, both for CRC incidence and mortality [131-138]. The antitumor effect of statin needs to be validated in well-designed RCTs with sufficient power.

Phosphodiesterase-5 inhibitor

The phosphodiesterase-5 (PDE5) inhibitor, which was originally indicated for systemic hypertension and angina, has been successfully repurposed as a treatment for erectile dysfunction. Now, it is being repurposed once again as a potential preventive agent against CRC [139]. Results from a recent meta-analysis, including four retrospective studies, indicated that the use of PDE5 inhibitor was associated with a significant 15% reduced CRC risk [140]. Due to its low side-effect profile, PDE5 inhibitor is a suitable option for chemoprevention and is frequently used for the daily, long-term management of pulmonary arterial hypertension and benign prostate hyperplasia [141-149]. However, there is not enough available evidence for females because of a limited amount of female users. Thus, the current findings are not yet applicable for practical use unless validated in prospective cohort studies and well-designed RCTs for long-term use and explore the effects on women.

Melatonin

Melatonin, which regulates the night and day cycles or sleep-wake cycles, is a naturally indolic hormone primarily secreted by the pineal gland in humans and mammals [150]. Melatonin is commonly used in clinical practice as a short-term treatment for insomnia, including conditions like jet lag or sleep disturbances related to shifting work patterns, and it is usually administered orally [151]. Due to its potential in preventing cancer development and aiding cancer treatment, melatonin has received significant attention, as circadian rhythm disruption has been identified as a contributing factor in cancer [152]. Besides the pineal gland, the digestive tract could also produce melatonin, which is not dependent on circadian rhythms but on specific food consumption [153,154]. By estimates, the concentration of melatonin in the digestive tract is 10 to 100 folds of that in the blood which is released from the pineal gland. This high level of melatonin in the gut contributes to the regulation of regeneration and function of gastrointestinal epithelium and enhances the immune activity of the gut [154,155]. In addition to its crucial function in the gastrointestinal tract, melatonin is recognized for its anti-oxidant, anti-inflammatory, immune-modulating, and oncostatic properties [156-158]. Melatonin has been suggested to have a protective effect in breast [159-163], prostate [164,165], and ovarian cancer [166] as evidenced by several epidemiological studies, yet conflicting results have been reported by other studies [167-170]. Nevertheless, there is a dearth of population-based evidence regarding the correlation between melatonin and CRC. Additionally, previous epidemiological investigations have primarily concentrated on melatonin levels in the body, including urinary melatonin excretion or serum melatonin concentrations, rather than investigating its chemopreventive properties as an oral medication.

Proguanil

Proguanil is a member of the biguanide family and has a structural analog of metformin. Proguanil is usually used to treat or prevent malaria. Lea et al. reported that proguanil exhibits potent anti-proliferative effects compared to other biguanides such as buformin, phenformin, and phenyl biguanide, particularly against bladder and colon cancer cells [171]. Furthermore, chemical alterations to proguanil have been demonstrated to exert a more potent impact on the proliferation and migration of human cancer cell lines [172] In clinical settings, proguanil is commonly administered alongside another antimalarial drug to enhance its therapeutic efficacy. In Sweden, the combination of proguanil and atovaquone in fixed doses is frequently used to attain superior prevention outcomes against drug-resistant malaria strains. Atovaquone has been shown to possess antitumor properties by inhibiting mitochondrial oxidative phosphorylation; a major generator of oxygen radicals [173]. However, evidence from population-based studies examining the association between the use of proguanil/atovaquone and CRC is lacking.

Selective serotonin reuptake inhibitors (SSRIs)

SSRIs are a first-line antidepressant in clinical practice, and can also be prescribed to treat anxiety, insomnia, and chronic pain. SSRIs function by inhibiting neural reuptake, which leads to an increase in the level of serotonin in the brain. This results in improved information transmission between neuron cells. All marketed SSRIs function in this manner. Several population-based epidemiological studies suggest that the use of SSRIs might contribute to a reduction in the risk for cancer, including colorectal, kidney, ovarian, and liver cancer [174-177]. Several studies conducted in vitro and in vivo have highlighted their anti-tumor effects. These findings suggest that SSRIs could be a viable option for the chemoprevention of CRC [178,179]. However, a meta-analysis of six observational studies found that the overall risk was only marginally insignificant (adjusted RR, 0.89; 95% CI, 0.79-1.01) [180]. As the evidence is still limited, it is thus imperative to investigate the antineoplastic potential of SSRIs on CRC.

Aims

The overall aim of this thesis was to explore the potential drug repositioning opportunities for the chemoprevention of CRC, with a main focus on individuals at high risk.

The specific aims of each study were as follows:

I. To explore the association between the use of melatonin and the risk in older adults.

II. To investigate the association between exposure to proguanil and atovaquone and the risk of CRC among people with a family history of CRC.

III. To examine the link between exposure to SSRIs and CRC risk in individuals with a family history of CRC.

IV. To explore whether the use of aspirin and SSRIs - either as monotherapy or combined - can have a clinical benefit against CRC.

Methods

Data source

The projects included in this thesis were based on data derived from Swedish nationwide registers. **Table 1** lists the detailed description of the registers used in the thesis.

Table 1. Summary of the Swedish registers used in this thesis.

Register	Period	Brief description
Swedish Cancer Register	1958-2018	Nationwide data with close to 100% histological verification of most cancers. Cancer data includes site of tumor, histological type, stage, and date of diagnosis[181,182].
Cause of Death Register	1961-2018	Date, cause, and contributory cause(s) of death. Missing 0.9%[183].
National Patient Register	1964-2018	The National Patient Register was established in 1964. Since 1987, it includes all in-patient care, including somatic and psychiatric diseases and co-morbidities, diagnoses, and lengths of stay with a high level of completeness (97%). Since 2001, it covers outpatient visits, day surgery, and psychiatric care from both private and public caregivers [184].
Multi-Generation Register	1932-2018	Data includes all individuals who had a residence permit in Sweden from 1961 and onwards and who were born from 1932 onwards (index persons). It includes data on index persons and their first-degree relatives (e.g. parents and siblings) [185].
Total Population Register	1960-2018	Data includes individually linked data on 11.8 million people and 3.7 million nuclear families. Variables include age, sex, marital status, mobility, individual income, family income, pension, social welfare, wealth, housing tenure, education, employment, occupation, country of birth, and urban/rural status. Missing < 0.5% [186].
Prescription Register	2005-2018	Data includes information regarding drug utilization and expenditures for all prescribed drugs in the entire Swedish population with missing individual identity data of less than 0.3%. All drugs are classified according to the Anatomical Therapeutic Chemical (ATC) classification system [187,188].

Ethical statement

The projects included in this thesis were approved by the Regional Ethical Review Board in Lund, Sweden, on February 6th, 2013 (Dnr 2012/795 and later amendments). The Swedish personal identification number was replaced with serial numbers to ensure people's integrity. Implicit consent instead of written informed consent was used in register-based projects. Individuals are informed through advertisements in the newspapers about the use of their data, and they have the right to opt out of being included in the research datasets. The use of implicit consent has been deemed appropriate by Swedish law and ethical guidelines.

Assessment of exposure

We retrieved information on drug use from the Swedish Prescribed Drug Register, which was established on July 1st, 2005. The Swedish Prescribed Drug Register contains information regarding drug utilization and expenditures for all prescribed drugs in the entire Swedish population with missing individual identity data of less than 0.3% [187,188]. Dispensation records contain the information on the date of prescription, ATC codes, defined daily dose (DDD), DDD per package, and the number of packages prescribed. We used ATC codes to identify the drug exposures: melatonin (Paper I), proguanil and atovaquone (Paper II), SSRIs (Paper III), aspirin and SSRIs (Paper IV). The summary information on the drugs is listed in **Table 2**.

In order to explore the dose-response relationships for drug exposures, we first calculated how many days a prescription lasted by multiplying DDD per package and the number of packages prescribed, and then sum all the prescriptions for each individual during follow-up time into cumulative defined daily dose (cDDD).

Table 2. Summary of the drug exposures included in this thesis.

Drug name	Drug class (level 1)	Drug subclass (level 2)	Drug subclass (level 3)	Drug subclass (level 4)	ATC code
Melatonin	Nervous system	Psycholeptics	Hypnotics and sedatives	Melatonin receptor agonists	N05CH
Proguanil/atovaquone	Antiparasitic products, insecticides and repellents	Antiprotozoals	Antimalarials	Biguanides	P01BB
Proguanil					P01BB01
proguanil and atovaquone					P01BB51
SSRI	Nervous system	Psychoanaleptics	Antidepressants	Selective serotonin reuptake inhibitors	N06AB
citalopram					N06AB04
escitalopram					N06AB10
fluoxetine					N06AB03
paroxetine					N06AB05
sertraline					N06AB06
Aspirin	Blood and blood forming organs	Antithrombotic agents	Antithrombotic agents	Platelet aggregation inhibitors	B01AC06

Assessment of outcome

The outcome of the four projects is the diagnosis of CRC. We identified information on CRC diagnoses using the 10th International Classification of Diseases (ICD) codes C18, C19, and C20 from the Swedish Cancer Register.

The Swedish Cancer Register provides information on the TNM staging system since 2002, including tumor size (T), nodal status (N), and metastatic disease (M). The stage at diagnosis of CRC can be determined by combining the T, N, and M categories, ranging from stage I to stage IV (the most advanced). The stages at diagnosis include stage I (T1 or T2), stage II (T3 or T4), stage III (any T, N1, or N2), and stage IV (any M1).

The Cause of Death Register provides information on the date, cause, and contributory cause(s) of death, thus we could identify individuals who had died during the study period.

Study population

In Paper I, we used a retrospective cohort study design. The source population consists of 9,147,428 cancer-free Swedish-born residents registered in the Swedish Total Population Register from July 2005 through December 2015. By linking to the Swedish Prescription Register, we identified individuals who were ever previously prescribed melatonin from July 2005 to December 2015. In order to adopt a new user design, we applied a washout period of one year and a half, which meant that individuals who had been prescribed melatonin before January 2007 were regarded as prevalent melatonin users (n = 3,156) and thus were excluded from the study.

A total of 58,557 melatonin users aged 50 and older were recruited in Paper I. Matched comparisons without prescription of melatonin were randomly selected based on birth year, sex, education, and first-degree family history of CRC on a ratio of 1:3.

The index date for the start time point of follow-up was the date of the first prescription of melatonin users and their matched comparisons. We followed the study population from the index date until i) the first date of CRC diagnosis; ii) the date of death from any cause; iii) the end of the study period (31st December 2015), whichever came first. Individuals with a follow-up time of fewer than three months were excluded (**Figure 6**).

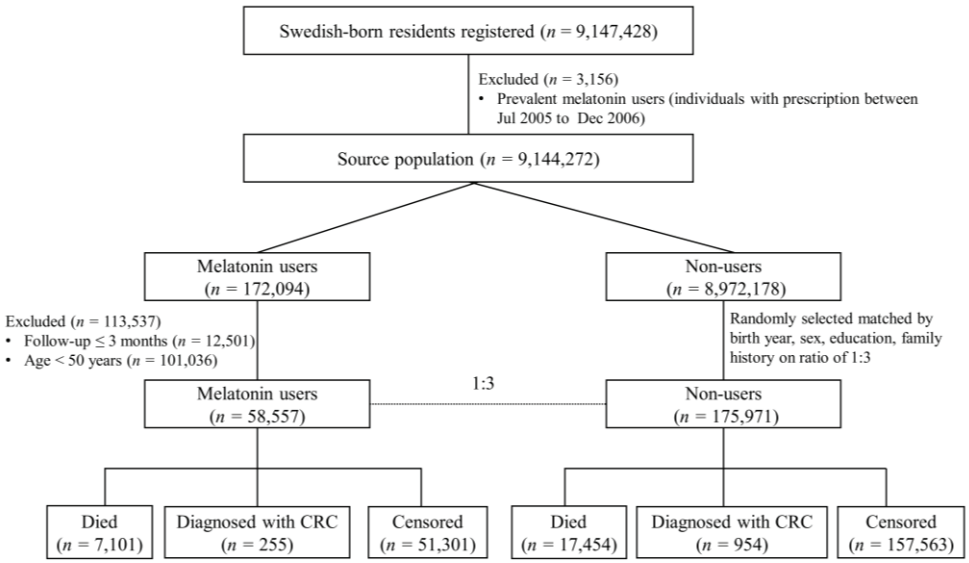


Figure 6. Study flow chart of Paper I.

In Paper II, we used a retrospective cohort study design with a focus on individuals with a family history of CRC who are in a high-risk group for developing CRC. Using data from the Swedish Multi-generation Register identifies the first-degree relatives (e.g. parents and siblings) for all individuals who had a residence permit in Sweden from 1961 and onwards and who were born from 1932 onwards (index persons). By further linking to the Swedish Cancer Register, we identified all adults who have one or more first-degree relatives diagnosed with CRC (n = 477,582).

By linking to the Swedish Prescription Register, we identified individuals who were ever previously prescribed proguanil/atovaquone from July 2005 to December 2018. In order to adopt a new user design, we applied a washout period of a half year, which means that individuals who were prescribed proguanil/atovaquone before January 2006 were regarded as prevalent proguanil/atovaquone users (n = 591) and thus were excluded from the study. A total of 16,817 proguanil/atovaquone users were recruited in Paper II. Matched comparisons without a prescription of proguanil/atovaquone were randomly selected based on birth year and sex on a ratio of 1:10.

The index date for the start time point of follow-up was the date of the first prescription of proguanil/atovaquone users and their matched comparisons. We followed the study population from the index date until i) the first date of CRC diagnosis; ii) the date of death from any cause; iii) the end of the study period (31st

December 2018), whichever came first. Individuals with a follow-up time of fewer than three months were excluded (**Figure 7**).

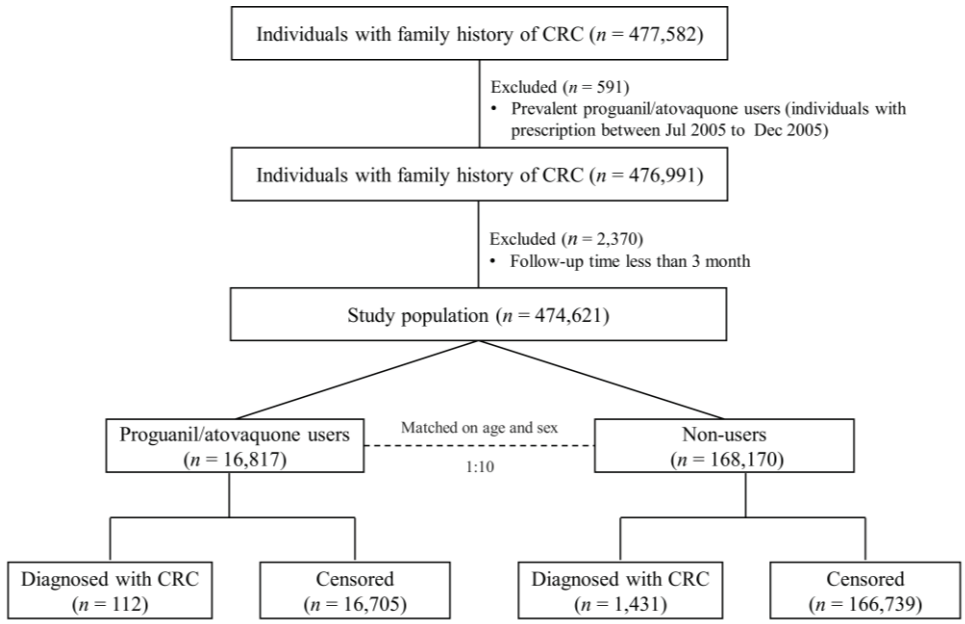


Figure 7. Study flow chart of Paper II.

In Paper III, we used a retrospective cohort study design, the source population was also the individuals with a family history of CRC (n = 477,582). We then excluded prevalent SSRIs users who received a prescription for SSRIs between July 2005 to December 2005 (n = 23,118). Furthermore, we then excluded individuals with a follow-up time of fewer than three months and those younger than 50 years of age.

By linking to the Swedish Prescription Register, we identified individuals who were ever previously prescribed SSRIs from July 2005 to December 2018. To adopt a new user design, we applied a washout period of a half year, which means that individuals who were prescribed SSRIs before January 2006 were regarded as prevalent SSRIs users (n = 23,118) and thus were excluded from the study. A total of 38,617 SSRIs users were recruited in Paper III. Matched comparisons without a prescription of SSRIs were randomly selected based on birth year and sex on a ratio of 1:3.

The index date for the start time point of follow-up was the date of the first prescription of SSRI users and their matched comparisons. We followed the study population from the index date until i) the first date of CRC diagnosis; ii) the date

of death from any cause; iii) the end of the study period (31st December 2018), whichever came first (**Figure 8**).

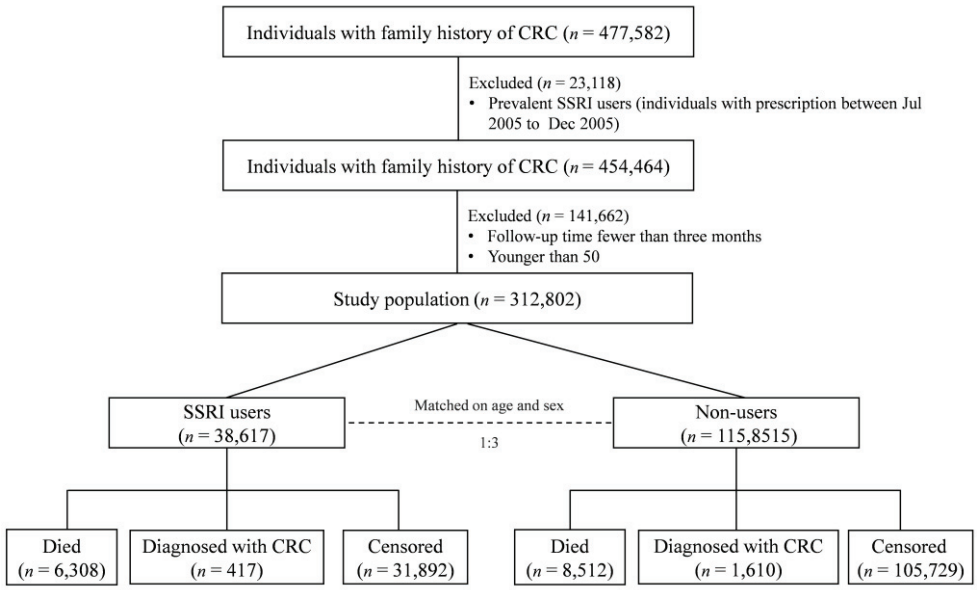


Figure 8. Study flow chart of Paper III.

In Paper IV, we used a nested case-control study design. The source population consists of 9,147,428 Swedish-born residents registered from July 2005 to March 2014 in the Swedish Total Population Register. To adopt a new user design, a half-year wash-out window was applied, thus the cohort entry date was set as January 2006. Individuals with a prescription for aspirin or SSRIs or individuals who had a diagnosis of any cancer before the entry date were excluded. Using data from the Swedish Cancer Register, we identified a total of 24,786 CRC patients between January 2007 and March 2014. We used incidence-density sampling to randomly select nested controls from the source cohort, matched by age and sex on a ratio of 1:3. The index date was the date of the CRC diagnosis for CRC cases and their matched controls (Figure 9). Individuals with a first prescription within one year before the index date were not regarded as SSRI users in order to minimize reverse causality by protopathic bias, which is, for example, that the symptoms that prompted the patients to use the medication were early symptoms of cancer, such as abdominal pain, feeling unwell or tiredness [189], thus they were likely to consult a doctor before the diagnosis of CRC and hence be prescribed aspirin to relieve these symptoms.

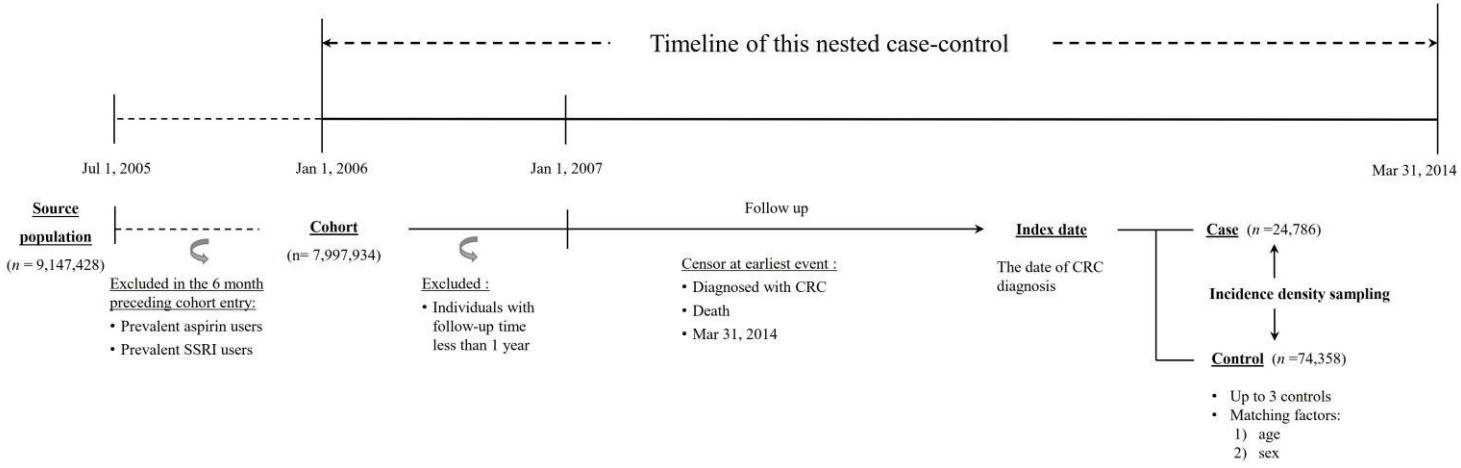


Figure 9. Study flow chart of Paper IV.

Assessment of Covariates

Information on potential confounding was retrieved from several Swedish nationwide registers, including birth year (continuous variable), sex (male or female), birth country (Sweden or others), the highest education (1-9, 10-11, ≥12 years), income (lowest, middle-low, middle-high, and highest), IBD (identified from the National Patient Register using ICD-10 codes "K50" for Crohn's disease and "K51" for ulcerative colitis, yes/no), obesity (identified from the National Patient Register using ICD-10 code "E66", yes/no), chronic obstructive pulmonary disease (COPD, identified from the National Patient Register using ICD-10 codes "J41-J44", yes/no) as a proxy for smoking, prescription of other medication (metformin and statin, identified from the Swedish Prescription Register using ATC codes "A10BA02" and "C10AA" respectively, yes/no), history of colonoscopy within last 10 years (identified from the National Patient Register using operation codes "UJF32" and "UJF42", yes/no), outpatient visits per year (from the National Patient Register, 0, 1, 2, ≥3) and Charlson Comorbidity Index score (CCI, 0, 1, 2, ≥3).CCI is a sum of weighted scores assigned to 17 comorbidities [190]. CCI has been established as a useful tool to measure comorbid disease status [191].

Statistical analysis

Cox regressions were used in Papers I, II and III to calculate the hazards ratios (HRs) and 95% CIs. Considering that the study population of Paper I was elderly individuals with a high mortality rate [192], and in Paper III, the suicide rate in SSRI users was higher than the general population [193], we applied competing risk cox regression models to control the competing risk of death. Dose-response analyses were conducted by categorizing the use of melatonin (Paper I) and proguanil/atovaquone (Paper II) into three groups based on cDDD and then tested for the trend by entering the cDDD as a continuous variable in the regression model. In Paper III, we evaluated the non-linear dose-response relationship between SSRIs doses and CRC incidence using restricted cubic spline (RCS) curves. Our best-fit curve was determined by testing three-to-seven cutoff points and selecting the model with the lowest Akaike information criterion (AIC). Five knots (0.05, 0.275, 0.5, 0.725, and 0.95) were selected in the final model.

We used a conditional logistic regression model to calculate ORs and 95% CIs of CRC associated with aspirin and SSRIs use for individually matched case-control studies in Paper IV. The interactive effect of aspirin and SSRIs was assessed on multiplicative and additive scales. Multiplicative interaction was evaluated by the ratio of OR ($OR_{combined}/(OR_{aspirin}*OR_{SSRIs})$), which was obtained by adding the

multiplicative interaction term to the logistic regression model as indicator variables. We calculated additive interactions using the relative excess risk for interactions (RERI). Dose-response analysis was conducted by assigning the combined users into four groups: low aspirin & low SSRIs, low aspirin & high SSRIs, high aspirin & low SSRIs, and high aspirin & high SSRIs, while individuals without the use of aspirin and SSRIs were set as the reference group.

Stratified analyses were conducted for all the four projects based on several important factors, including cancer sites, stage at cancer diagnosis, age, and sex (**Table 3**).

Table 3. Overview of statistical analyses for the projects included in this thesis.

Study	Study population	Study design	Exposure	Main analysis	Multivariable model	Stratified analysis
Paper I	Older people	Cohort	Melatonin	Cox regression	Age at index, sex, education, family history of crc, personal history of inflammatory bowel disease, use of colonoscopy, obesity, copd, CCI, use of aspirin, use of statin, use of metformin.	Cancer sites Stage at cancer diagnosis Age Sex Family history of crc
Paper II	Individuals with family history	Cohort	Proguanil/ atovaquone	Cox regression	Age at index, sex, education, birth country, income, history of inflammatory bowel disease, copd, obesity, outpatient visits, history of colonoscopy, use of aspirin, use of statin, CCI.	Cancer sites Stage at cancer diagnosis Age Sex
Paper III	Individuals with family history	Cohort	SSRI	Cox regression	Age at index, sex, education, birth country, income, history of inflammatory bowel disease, copd, obesity, outpatient visits, history of colonoscopy, use of aspirin, use of statin, use of metformin, CCI.	Cancer sites Stage at cancer diagnosis Age Sex
Paper IV	Adults	Nested case-control	Aspirin and SSRI	Conditional logistic regression	Age at index, sex, education, family history of crc, history of inflammatory bowel disease, use of colonoscopy, outpatient visits, obesity, chronic obstructive pulmonary disease, charlson comorbidity index, use of statin, use of metformin.	Cancer sites Stage at cancer diagnosis

Results

Paper I

As shown in **Table 4**, a total of 58,657 melatonin users and 175,971 non-users were recruited in this study. The incidence rate in the melatonin users was 10.40 per 10,000 person-years, which was lower than that in the comparisons (12.82 per 10,000 person-years). Melatonin use was associated with an 18% reduced risk of CRC (95% CI, 0.72-0.92) after adjustment for several confounding factors. Subgroup analysis by cancer site showed that the protective effect of melatonin was slightly stronger in rectal cancer (adjusted HR, 0.73; 95% CI, 0.58-0.93) than in distal colon cancer (adjusted HR, 0.89; 95% CI, 0.74-1.08) and proximal colon cancer (adjusted HR, 0.80; 95% CI, 0.63-1.01). We also observed that the inverse association was stronger in earlier-stage cancers (adjusted HR, 0.78; 95% CI, 0.65-0.94) than advanced-stage cancers (adjusted HR, 0.82; 95% CI, 0.68-0.99).

Stratified analysis by age group suggested that the inverse associations were significant in people aged 60 and above (adjusted HR and 95% CI for age group 60-69 was 0.78 and 0.63-0.98; adjusted HR and 95% CI for the age group above 70 was 0.81 and 0.68-0.95 for age group), but not significant in the age group 50-59 (adjusted HR, 0.94; 95% CI, 0.70-1.26). The use of melatonin was significantly associated with reduced risk in women (adjusted HR, 0.79; 95% CI, 0.68-0.93), but not in men (adjusted HR, 0.86; 95% CI, 0.71-1.05). We tested the dose-response relationship between melatonin use and CRC by modeling the cDDD of melatonin in three groups: <30 cDDD, 30-89 cDDD, and >= 90 cDDD. We observed an increased protective effect of melatonin as the doses increased, with adjusted HRs and 95% CIs of 0.91 (0.76-1.09), (0.67-1.01), and 0.66 (0.50-0.86), respectively. Test for the trend of the dose-response relationship showed significant results (*P* <0.001) (**in Paper I**).

Table 4. HRs and 95% CIs of CRC risk associated with melatonin use among the elderly.

	Individuals	Person-years	CRC diagnoses	IR, per 10,000 person-year	Crude			Adjusted[a]		
					HR	95% CI	*P* value	HR	95% CI	*P* value
Ever use of melatonin										
No	175971	744141	954	12.82	1			1		
Yes	58657	245304	255	10.40	0.80	0.71-0.91	<0.001	0.82	0.72-0.92	0.001
Cancer site										
Proximal colon										
Non-users	175971	744141	380	5.11	1			1		
Melatonin users	58657	245304	112	4.57	0.88	0.74-1.06	0.184	0.89	0.74-1.08	0.237
Distal colon										
Non-users	175971	744141	272	3.66	1			1		
Melatonin users	58657	245304	71	2.89	0.78	0.62-0.99	0.037	0.80	0.63-1.01	0.058
Rectum										
Non-users	175971	744141	283	3.80	1			1		
Melatonin users	58657	245304	66	2.69	0.70	0.55-0.89	0.004	0.73	0.58-0.93	0.011
Stage at cancer diagnosis										
Stage I or II										
Non-users	175971	744141	418	5.62	1			1		
Melatonin users	58657	245304	110	4.48	0.79	0.66-0.95	0.013	0.78	0.65-0.94	0.010
Stage III or IV										
Non-users	175971	744141	420	5.64	1			1		
Melatonin users	58657	245304	110	4.48	0.78	0.65-0.94	0.010	0.82	0.68-0.99	0.041

[a] Adjusted for age at index, sex, education, family history of CRC, personal history of inflammatory bowel disease, use of colonoscopy, obesity, COPD, CCI, use of aspirin, use of statin, use of metformin.

Paper II

As shown in **Table 5**, we recruited 16,817 proguanil/atovaquone users and 168,170 non-users. Following an average of 7.1 years, the CRC incidence rate in the proguanil/atovaquone users was 9.32 per 10,000 person-years, while the incidence rate was 12.07 in the non-users. Proguanil/atovaquone use was associated with a reduced risk of CRC with an adjusted HR and 95% CI of 0.76 (0.63-0.92). We found that the protective effect of proguanil/atovaquone was significant in colon cancer (adjusted HR, 0.78; 95% CI, 0.61-0.99), but marginally insignificant in rectal cancer (adjusted HR, 0.72; 95% CI, 0.51-1.02). When stratified by cancer stages at diagnosis, the association was significant in cancer diagnosed in advanced stages (adjusted HR, 0.69; 95% CI, 0.51-0.92), but not in cancer diagnosed in early stages (adjusted HR, 0.88; 95% CI, 0.65-1.20).

The effect of proguanil/atovaquone was in a dose-dependent pattern. The HR of CRC risk was 0.82 (95% CI, 0.64-1.05) among proguanil/atovaquone users with the lowest cumulative dose (<6 cDDD), decreased to 0.67 (95% CI, 0.44-1.03) among users with medium dose (7-12 cDDD), and 0.62 (95% CI, 0.37-1.04) among individuals with the highest cumulative dose (>12 cDDD). We also observed a duration-dependent association. The adjusted HR of CRC was 0.84 (95% CI, 0.66-1.09) among users with the lowest duration (<1 week), decreased to 0.78 (95% CI, 0.51-1.19) among users with medium duration (1 week to 1 year), and 0.57 (95% CI, 0.36-0.90) among users with the highest duration (>1 year). Both trends for the dose- and duration-response correlation were significant (P <0.001). The associations between proguanil/atovaquone use and CRC risk were significant among individuals older than 50 (adjusted HR and 95% CI, 0.75 and 0.61-0.93), but not significant in the younger adults (adjusted HR, 0.81; 95% CI, 0.47-1.40). When stratified by sex, women benefitted more from the use of proguanil/atovaquone use (adjusted HR, 0.69; 95% CI, 0.59-0.81) compared with men (adjusted HR, 0.83; 95% CI, 0.71-0.98) (in Paper II).

Table 5. HRs and 95% CIs of CRC risk associated with proguanil/atovaquone use.

	Individuals	Person-years	CRC diagnoses	IR, per 10,000 person-year	Crude			Adjusted		
					HR	95% CI	*P* value	HR	95% CI	*P* value
Ever use of proguanil/atovaquone										
No	168170	1185525	1431	12.07	1			1		
Yes	16817	120203	112	9.32	0.76	0.63-0.92	0.004	0.76	0.62-0.93	0.006
Cancer site										
Colon cancer										
Non-users	168170	1185525	933	7.87	1			1		
Users	16817	120203	75	6.24	0.78	0.62-0.98	0.003	0.78	0.61-0.99	0.042
Rectal cancer										
Non-users	168170	1185525	498	4.2	1			1		
Users	16817	120203	37	3.08	0.72	0.52-1.00	0.049	0.72	0.51-1.02	0.072
Cancer stage										
Stage I and II										
Non-users	168170	1185525	525	4.43	1			1		
Users	16817	120203	48	3.99	0.89	0.67-1.17	0.395	0.88	0.65-1.20	0.424
Stage III and IV										
Non-users	168170	1185525	745	6.28	1			1		
Users	16817	120203	52	4.33	0.68	0.52-0.89	0.005	0.69	0.51-0.92	0.011

[a] Adjusted for age at index, sex, education, birth country, income, history of inflammatory bowel disease, COPD, obesity, outpatient visits, history of colonoscopy, use of aspirin, use of statin, CCI.

Paper III

As shown in **Table 6**, after an average of 6.8 years of follow-up time, the incidence of CRC among 38,617 SSRI users was 1.65/1000 person-years, which was significantly lower than that among 115,851 non-users (2.00/1000 person-years). We observed an inverse association between SSRIs use and CRC risk among elderly individuals with a family history, with an adjusted HR of 0.77 (95% CI, 0.70-0.85). We further investigated the effect of subtypes of SSRIs and found that reduced CRC risk was significantly associated with the use of citalopram (adjusted HRs, 0.77; 95% CIs, 0.67-0.88) and sertraline (adjusted HRs, 0.69; 95% CIs, 0.57-0.83), but not with fluoxetine, paroxetine, and escitalopram probably due to the limited number of cases. We observed a slightly stronger inverse relationship in rectal cancer (0.73; 95% CI, 0.63-0.91) compared with colon cancer (0.79; 95% CI, 0.70-0.89). When stratified by cancer stages at diagnosis, the association was slightly stronger for cancers diagnosed in the advanced stages (adjusted HR, 0.73; 95% CI, 0.63-0.85) in comparison with those diagnosed in early stages (adjusted HR, 0.80; 95% CI, 0.68-0.93).

The significant inverse association between SSRIs use and CRC risk was observed among the age group 60 to 69 years (adjusted HR, 0.61, 95% CI, 0.51-73) but not among age groups 50 to 59 years (adjusted HR, 0.91; 95% CI, 0.76-1.09) and 70 years or older (adjusted HR, 0.86; 95% CI, 0.73-1.01). When stratified by sex, the benefit from using SSRIs was more pronounced in women (adjusted HR, 0.74; 95% CI, 0.64-0.84) compared with men (adjusted HR, 0.84; 95% CI, 0.72-0.97) (in Paper III).

The result of the RCS curve is shown in **Figure 10**. We observed a non-linear dose-response relationship between SSRIs use and CRC risk.

Table 6. HRs and 95% CIs of CRC risk associated with SSRIs use among individuals with family history of CRC.

	Individuals	Person-years	CRC diagnoses	IR, per 1000 person-year	Crude			Adjusted [a]		
					HR	95% CI	*P* value	HR	95% CI	*P* value
SSRIs use										
Non-users	115851	804265	1610	2.00	1			1		
SSRIs users	38617	253019	417	1.65	0.77	0.70-0.85	<0.001	0.77	0.70-0.85	<0.001
Subtype of SSRIs										
Fluoxetine	1515	14224	21	1.47	0.74	0.49-1.13	0.164	0.75	0.47-1.18	0.210
Citalopram	15587	129042	227	1.76	0.77	0.68-0.88	<0.001	0.77	0.67-0.88	<0.001
Paroxetine	1116	10759	13	1.21	0.64	0.38-1.09	0.103	0.66	0.36-1.20	0.172
Sertraline	9761	81764	117	1.43	0.71	0.60-0.85	<0.001	0.69	0.57-0.83	<0.001
Escitalopram	3995	34586	51	1.47	0.77	0.58-1.01	0.055	0.84	0.64-1.11	0.219
Cancer site										
Colon cancer										
Non-users	115851	804265	1125	1.40	1			1		
SSRIs users	38617	253019	298	1.18	0.79	0.71-0.89	<0.001	0.79	0.70-0.89	<0.001
Rectal cancer										
Non-users	115851	804265	485	0.6	1			1		
SSRIs users	38617	253019	119	0.47	0.73	0.61-0.87	<0.001	0.73	0.63-0.91	0.003
Cancer stage										
Stage I and II										
Non-users	115851	804265	616	0.77	1			1		
SSRIs users	38617	253019	169	0.67	0.82	0.72-0.95	0.010	0.80	0.68-0.93	0.004
Stage III and IV										
Non-users	115851	804265	785	0.98	1			1		
SSRIs users	38617	253019	186	0.74	0.71	0.61-0.82	<0.001	0.73	0.63-0.85	<0.001

[a] Adjusted for age at index, sex, education, birth country, family history of CRC, history of inflammatory bowel disease, COPD, obesity, history of colonoscopy, use of aspirin, use of statin, use of metformin, CCI.

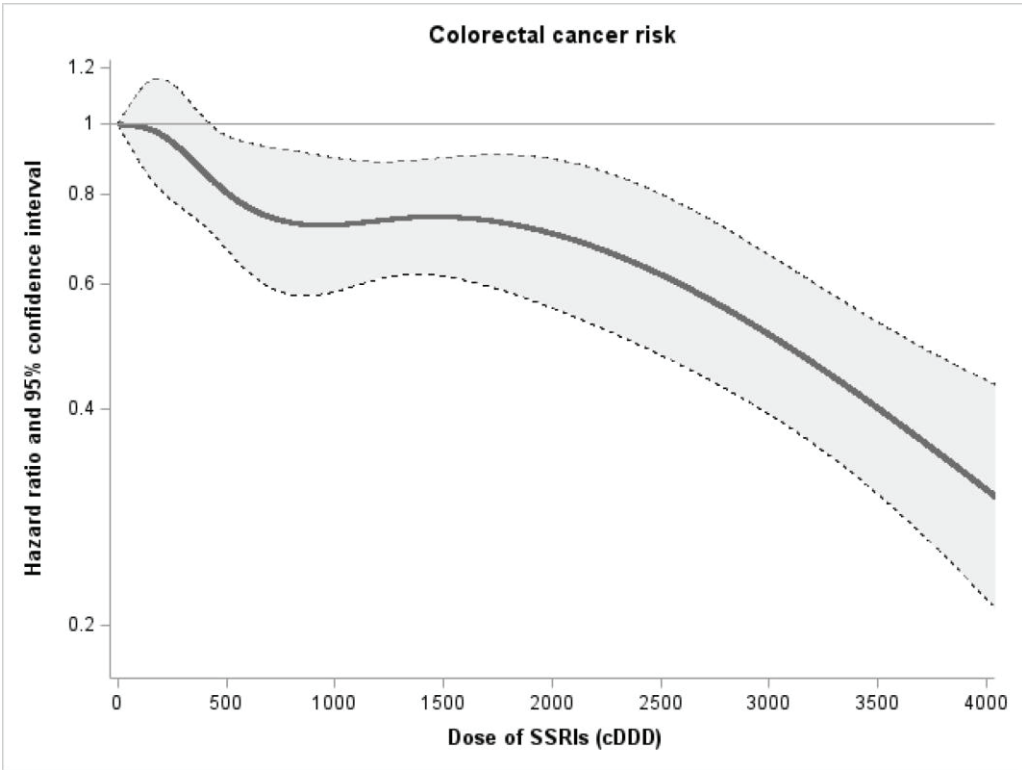


Figure 10. Association between different doses of SSRIs use and the risk of CRC using restricted cubic spline, with 95% CIs (the grey area). Adjusted for age at index, sex, education, birth country, income, history of inflammatory bowel disease, COPD, obesity, outpatient visits, history of colonoscopy, use of aspirin, use of statin, use of metformin, CCI.

Paper IV

Table 8 lists the frequencies and ORs for use of aspirin and SSRIs in 24,786 cases and 74,358 controls. We observed a negative association between overall aspirin use and CRC risk, with an OR of 0.91 (95% CI, 0.87-0.96) after adjustment for several confounding factors. The overall use of SSRIs was also inversely associated with CRC risk, with an OR of 0.91 (95% CI, 0.85-0.97). We further explored the effect of subtypes of SSRIs and found that reduced CRC risk was significantly associated with the use of citalopram (adjusted OR, 0.91; 95% CI, 0.84-0.99), and sertraline (adjusted OR, 0.85; 95% CI, 0.75-0.97), but not with fluoxetine, paroxetine, and escitalopram. Furthermore, we divided the users of aspirin and SSRIs into three groups: those taking aspirin alone, those taking SSRIs alone, and those taking both. The adjusted ORs for CRC between aspirin monotherapy, SSRIs monotherapy, and combined use of both aspirin and SSRIs were 0.91(0.87-0.97), 0.93 (0.86-1.00), and 0.77 (0.67-0.89), respectively, compared with non-users. SSRIs and aspirin interaction was significant at the additive scale with a RERI of -0.07 (95% CI, -0.11 to -0.03), but not at the multiplicative scale ($P = 0.241$).

We observed significant inverse associations between aspirin monotherapy and the risk of proximal colon cancer (adjusted OR, 0.89; 95% CI, 0.81-0.97) and rectal cancer (adjusted OR, 0.88; 95% CI, 0.80-0.97). SSRIs monotherapy was only significantly associated with reduced rectal cancer risk. The combined use was negatively associated with distal colon cancer risk, with an adjusted OR of 0.71 (95% CI, 0.55-0.93). When stratified by cancer stages of diagnosis, the results were largely consistent in both early-stage cancers and advanced-stage cancers (in Paper IV).

The results of dose-dependent analyses of aspirin and SSRIs associated with CRC are listed in **Figure 11**. The risk of CRC was significantly decreased by an increased dose of aspirin and SSRIs use (adjusted ORs and 95% CIs for four quartiles were 0.94 (0.86-1.04), 0.94 (0.86-1.02), 0.88 (0.80-0.97) and 0.86 (0.79-0.95) for aspirin use, P for trend <0.001; 0.95 (0.85-1.06), 0.95 (0.83-1.08), 0.81 (0.71-0.92) and 0.92 (0.81-1.05) for SSRIs use, P for trend = 0.003). For combined users, the lowest CRC risk was observed in the high aspirin + low SSRIs group, but the observed associations were largely consistent for the four groups.

Table 8. ORs and 95% CIs of CRC associated with SSRIs or aspirin use.

	Cases (*n* = 24786)		Controls (*n* = 74358)		Crude			Adjusted [a]		
	No.	%	No.	%	OR	95% CI	*P* value	OR	95% CI	*P* value
Aspirin										
Non-users	22098	89.2	66222	89.1	1			1		
Aspirin users	2688	10.8	8136	10.9	0.99	0.94-1.04	0.662	0.91	0.87-0.96	<0.001
SSRIs										
Non-users	23487	94.8	70141	94.3	1			1		
Any type	1299	5.2	4217	5.7	0.92	0.86-0.98	0.010	0.91	0.85-0.97	0.003
Citalopram	863	3.5	2792	3.8	0.92	0.85-1.00	0.046	0.91	0.84-0.99	0.021
Escitalopram	155	0.6	440	0.6	1.06	0.88-1.27	0.547	1.06	0.88-1.28	0.539
Fluoxetine	65	0.3	189	0.3	1.03	0.78-1.37	0.826	1.01	0.76-1.34	0.949
Paroxetine	47	0.2	160	0.2	0.88	0.64-1.22	0.446	0.87	0.63-1.21	0.414
Sertraline	327	1.3	1130	1.5	0.87	0.77-0.98	0.023	0.85	0.75-0.97	0.018
Aspirin or SSRIs										
Non-users	21069	85.0	62962	84.7	1			1		
Aspirin monotherapy	2418	9.8	7179	9.7	1.01	0.96-1.06	0.857	0.91	0.87,0.97	0.001
SSRIs monotherapy	1029	4.2	3260	4.4	0.94	0.94-1.01	0.105	0.93	0.86-1.00	0.047
Combined users	270	1.1	957	1.3	0.84	0.84-0.96	0.013	0.77	0.67-0.89	<0.001
Multiplicative interaction								0.91	0.77-1.07	0.241
Additive interaction								-0.07	-0.11 to -0.03	0.001

[a] Adjusted for age at index, sex, education, family history of CRC, history of inflammatory bowel disease, use of colonoscopy, outpatient visits, obesity, chronic obstructive pulmonary disease, CCI, use of statin, use of metformin.

	Case	Control	Adjusted OR (95% CI) [a]	*P* for trend
Dose of overall aspirin use				<0.001
Quartile 1	625 (2.5)	1860 (2.5)	0.94 (0.86-1.04)	
Quartile2	723 (2.9)	2130 (2.9)	0.94 (0.86-1.02)	
Quartile3	603 (2.4)	1865 (2.5)	0.88 (0.80-0.97)	
Quartile4	737 (3)	2281 (3.1)	0.86 (0.79-0.95)	
Dose of overall SSRI use				0.003
Quartile 1	287 (1.2)	928 (1.2)	0.95 (0.85-1.06)	
Quartile2	381 (1.5)	1161 (1.6)	0.95 (0.83-1.08)	
Quartile3	299 (1.2)	1075 (1.4)	0.81 (0.71-0.92)	
Quartile4	332 (1.3)	1053 (1.4)	0.92 (0.81-1.05)	
Dose of combined use				
Low aspirin + low SSRI	60 (0.2)	217 (0.3)	0.72 (0.53-0.97)	
Low aspirin + high SSRI	60 (0.2)	197 (0.3)	0.78 (0.58-1.06)	
High aspirin + low SSRI	68 (0.3)	250 (0.3)	0.69 (0.52-0.92)	
High aspirin + high SSRI	82 (0.3)	293 (0.4)	0.71 (0.55-0.92)	

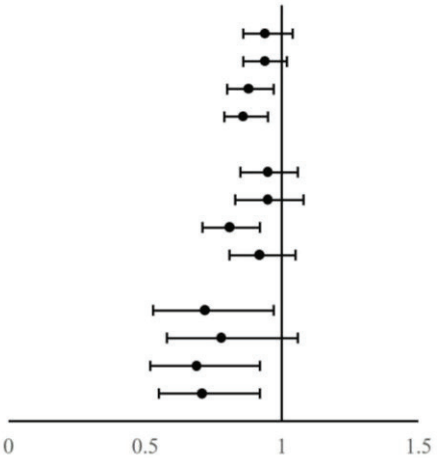


Figure 11. ORs and 95% CIs of CRC associated with different doses of SSRIs or aspirin use. SSRIs, selective serotonin reuptake inhibitors; OR, odds ratios; CI, confidence interval. [a] Adjusted for age at index, sex, education, family history of CRC, history of inflammatory bowel disease, use of colonoscopy, outpatient visits, obesity, chronic obstructive pulmonary disease, CCI, use of statin, use of metformin.

Discussion

Main findings

This thesis identified several chemopreventive agents against CRC, especially among individuals in the high-risk group. We found that the use of melatonin was associated with an 18% reduced risk of CRC among elderly individuals, the use of proguanil/atovaquone with a 24% reduced CRC risk among individuals with a family history of CRC, while the use of SSRIs was associated with a 23% reduced CRC risk among elderly individuals with a family history of CRC. We also found that the combined use of aspirin and SSRIs could achieve a better prevention effect than aspirin monotherapy or SSRIs monotherapy, which suggests that combining several agents that target different pathways could give better prevention against the development of CRC.

Our results in context

Drug repositioning is a growing endeavor from both academia and the life sciences industry to find effective drugs for cancer treatment and chemoprevention. Chemopreventive agents are expected to be popularized for widespread and long-term use, thus they need to be low toxic with fewer side effects, easy to take and administer (ideally available for oral use), and be cost-effective [194].

Melatonin

Results from Paper I indicate that melatonin could be an effective chemopreventive agent in older adults. Although numerous *in vivo* and *in vitro* studies back the biological mechanism of melatonin against CRC [195-207], population-based studies have not yet investigated the association between oral use of melatonin and CRC risk. Previous epidemiological studies investigating the association between melatonin and other types of cancer mainly focused on the body's circadian melatonin levels. Results from two meta-analyses observed an inverse correlation between urinary melatonin excretion and breast cancer risk, with a linear dose-

response trend [160,161]. Another two case-control studies suggested that increased urinary melatonin level was associated with a lower risk of prostate cancer in males[164,165]. A retrospective study found that the increased serum level of melatonin was significantly associated with reduced ovarian cancer risk [166]. However, another study found no association between urinary melatonin levels and the risk of ovarian cancer [170]. Several clinical trials have evaluated this drug as adjuvant therapy in cancers and found that the concurrent use of melatonin could improve the efficacy of cytotoxic drugs in chemotherapy treatment of metastatic CRC patients. Besides CRC, studies proved the same effect of melatonin in various solid cancers, including enhancing the therapeutic efficacy, improving tolerance to chemotherapy, relieving the side effect caused by chemotherapies and radiation, and significantly improving survival rate [208,209].

Unfortunately, we are not able to disentangle whether the observed association in Paper I was attributed to the antitumor effect of melatonin itself or the benefit from good quality sleep. Previous epidemiological studies have found that individuals who worked rotating night shifts had an increased risk of CRC, indicating that a regular circadian melatonin pattern would seem to be preventive for humans experiencing chronic light pollution [210,211].

Melatonin stands out as a promising chemopreventive agent for its excellent safety profile. As an endogenously produced hormone melatonin is well tolerated with rare side effects reported. Results from a systematic review suggested that most of the side effects were mild and short-lived (fatigue, headache, and sleepiness), while severe side effects (abnormal increase in blood pressure and heart rate) were reported at an extremely high dose or in specific populations. Dosing according to natural circadian rhythms can easily manage most untoward effects [212].

Proguanil/atovaquone

In Paper II, we found the use of proguanil/atovaquone could reduce CRC risk in people with a family history, and the effect is even stronger for advanced CRC. To the best of our knowledge, this is the first epidemiological study to explore the role of proguanil/atovaquone in the prevention of cancer. Several pre-clinical studies support the antitumor effect of proguanil and atovaquone. Mitochondrial dysfunction is one of the hallmarks of cancer development; inhibiting mitochondrial metabolism is an effective target for cancer therapy [213]. Unlike metformin functioning as an inhibitor of complex 1 of the mitochondrial electron transport chain, proguanil exerts an antitumor effect outside the mitochondria due to its poor uptake into mitochondria [214]. Even so, proguanil exhibited stronger growth inhibition against colon and bladder cancer cells than other biguanides including phenformin, buformin, and phenyl biguanide. This suggests that proguanil may inhibit cancer cell proliferation at extra-mitochondrial sites.

Atovaquone also inhibits mitochondrial respiration in solid tumors. Atovaquone is capable of reducing oxygen consumption and reducing hypoxia by targeting mitochondrial complex III, which in turn down-regulates mitochondrial respiration, thereby inhibiting cell growth, survival, and migration in hypopharyngeal, colon and lung cancer cell lines [215]. As solid tumors typically have hypoxic microenvironments, reducing oxygen consumption could enhance the sensitivity to radiotherapy, thus subsequently improving the clinical outcomes [216]. A similar effect was also observed in other cancers, including breast [173], retinoblastoma [215], cervical [217], thyroid [218], and kidney cancers [219].

Proguanil and atovaquone are known to have a favorable safety profile, with the infrequent occurrence of adverse events [220]. Several reported side effects were mild and short-term, including anorexia, nausea, vomiting, abdominal pain, diarrhea, headache, dizziness, and coughing [221]. Only two serious cases of vanishing bile duct syndrome and anaphylaxis have been reported [222,223].

SSRIs

The findings of Paper II indicate that SSRIs may offer protection against CRC development, and the effect is particularly pronounced in advanced-stage cancer. Although the biological mechanisms underlying the anti-tumor effect of SSRIs are not entirely clear, various hypotheses have been suggested and validated *in vivo* and *in vitro*, indicating that SSRIs can exert anti-cancer effects through multiple pathways, including activation of serotonin receptors [224] and downstream MAPK/ERK pathway [225], inhibition of proliferation of colon cancer cells by activating the JNK-c-Jun pathway [226,227], suppression of NF-κB signaling pathway [228,229], autophagic cell death [230-233], and modulation of serotonin metabolism activity [234]. Nevertheless, population studies have not been able to draw conclusive results concerning the role of SSRIs in protecting against CRC up till now. A case-control study from the US reported a 45% reduced risk of developing CRC in those who regularly used SSRIs [174]. A Canadian registry-based nested case-control study found that individuals with high cumulative doses of SSRIs before CRC diagnosis (0-5 years) had a 30% reduced risk of developing CRC [235]. However, studies conducted in Denmark, Finland, and the US provided scant evidence to support a protective association between the use of SSRIs and the risk of CRC [236-240]. Besides, it is common for cancer patients to be prescribed SSRIs to manage depression, which can be triggered by a cancer diagnosis and its associated treatment [241]. The utilization of SSRIs as an adjuvant therapy could significantly enhance the overall quality of life in patients with advanced cancer [242], and improve cancer-specific survival in patients with kidney cancer [243]. Thereafter, SSRIs may also serve as a promising adjunctive therapy for cancer treatment.

All types of SSRIs are generally well-tolerated compared with other antidepressants, but they may cause some side effects including nausea, headache, diarrhea, sexual

dysfunction, weight gain, and insomnia. Rare but serious side effects may include serotonin syndrome, suicidal thoughts, and bleeding disorders [244].

Aspirin and SSRIs

Recent studies have highlighted the complexity of CRC carcinogenesis, emphasizing the importance of addressing both intra-tumor and inter-tumor heterogeneity. This complexity implies that combining therapies that target multiple dysregulated cellular pathways may be more effective than using single agents or sequential drug combinations [245,246]. Aspirin has a well-recognized role in inhibiting cyclooxygenases (COX), especially COX-2 [247]. SSRIs have been also reported to affect *COX-2* expression, which might work synergistically with aspirin in the prevention of CRC [234,248]. Besides the COX-2-dependent mechanism, both aspirin and SSRIs have been shown to activate the AMPK/mTOR pathway [230,231,249,250], inhibit NF-κB pathway [228,229,251], and inhibit DNA repair of tumor cells [235,241]. In addition, the concomitant use of SSRIs with prior anti-platelet therapy may potentially enhance the anti-cancer effect of aspirin by yielding supplementary anti-platelet effects [252]. Given the mechanisms discussed, combining SSRIs and aspirin may lead to a synergistic effect on CRC prevention, by working synergistically in the same pathways, and targeting other pathways involved in CRC [253], thus can achieve an enhanced effect than monotherapies.

There might be a concern that both aspirin and SSRIs are associated with an increased risk of gastrointestinal bleeding since they both have anti-platelet properties [254]. Therefore, the safety profile for a combination of aspirin and SSRIs is highly needed before application in clinical practice.

Methodological considerations

Study design

RCTs are the best way to prove causal associations, however, it is not always feasible to conduct an RCT. Compared with RCTs, RWD provides valuable information with larger sample sizes on the practical usage of drugs and real-world outcomes related to both efficacy and toxicity. Such information serves as a valuable supplement and complement provided by RCTs. Cohort design and case-control design are widely used in register-based pharmacoepidemiology. Paper I to Paper III used a cohort study design with the advantage of estimating the real incidence rates and attributable proportions in the population. However, in some situations, it is better to use a case-control design rather than a cohort design. As for Paper IV,

we aimed to explore the combined use of aspirin and SSRIs - it is difficult to estimate the exposure period for those who ever previously used two drugs if we use a cohort design. If we start to follow the combined users since the initiation of the first drug, for example, aspirin, it will result in an immortal time period from the initiation of aspirin till the initiation of SSRI, which will lead to an overestimation of the protective effect of combined use. But if we start to follow the combined users since the initiation of the second drug, it will lead to an underestimation because the first drug has already taken effect before the follow-up starts. In this case, we chose a nested case-control design to avoid the issue of assessing the drug exposure period. A case-control study well nested in a nationwide source cohort could also evaluate the incidence rates and attributable proportions in the population.

We adopted a new user design for the four projects in this thesis. In Sweden, drugs are dispensed for up to 90 days, thus by applying a wash-out period of six months we can exclude the prevalent regular users of the medications. By using a new user design, we were able to follow the exposure groups from the first prescription of the drugs, thus it could emulate the intervention part of an RCT (intervention vs. placebo) [255]. The new-user design ensures the proper sequence of measuring confounders, treatment, and outcomes, preventing inadvertent adjustments for variables influenced by treatment and reducing the likelihood of finding associations based on reversed causation, thereby safeguarding the integrity of the study's findings [256].

Drug exposure assessment

Although the quality of the Swedish Prescription Register is very good overall and covers almost the whole population in Sweden, we lacked information on the use of over-the-counter drugs and medication used during hospitalizations. The Swedish healthcare system provides a ‘pharmaceutical benefit scheme’ that all the residents in Sweden only pay a maximum of SEK 2400 a year (Högkostnadsskydd) for prescribed drugs; the county council pays for costs exceeding that amount. Over-the-counter medications are generally not subsidized. Therefore, although melatonin is available without a prescription in Sweden, melatonin users tend to prefer obtaining their medication through prescriptions. When assessing the dose of drug use, we used the quantities of drug dispensation. However, we could not know whether the prescribed medications were finished thus the accurate dosages were difficult to estimate.

Potential bias

Selection bias

The most common selection bias in pharmacoepidemiology is healthy user bias. Individuals who were prescribed drugs have a higher propensity to care more about their health status and thus adhere to other healthy behaviors and preventative care. The healthy user effect will distort the drug-outcome association and overstate the preventive effect of the medications being studied.

Indication bias is also a type of selection bias that occurs when the indication of drug exposure can modify the outcome of interest. In drug repositioning studies, there will always be an indication that is not the outcome of our interest. It is important to critically interpret the results after ruling out the confounding by the primary indication of the drugs.

Information bias

Misclassification bias is a common issue in register-based pharmacoepidemiology. It occurs when there is an error in the classification of exposure or outcome status. As we discussed above, we lacked information on over-the-counter drugs and medication used during hospitalizations, which can lead to misclassifications of exposure.

Immortal time bias

Immortal time bias occurs when the study design creates a span of time in the observation or follow-up period of a cohort during which the outcome of interest cannot occur, thus leading to a distorted estimation of the treatment effect. This is often seen in retrospective cohort studies, where the period of time between the start of follow-up and the initiation of drugs is considered as "immortal time". In this thesis, we defined the drug exposure period from the drug initiation till the outcome event or end of the study, thus avoiding the bias by immortal time.

Protopathic bias

Protopathic bias occurs when the symptoms that prompted the patients to use the medication were early symptoms of the outcome of interest that has not yet been diagnostically detected. Therefore, protopathic bias can be considered a specific instance of bias of "reverse causality". This bias is identified by an inversion of the causal relationship between the outcome of interest (disease onset) and the suspected cause (use of medications). In this thesis, we applied a lag time in exposure assessment to mitigate protopathic bias.

Strengths and limitations

This thesis has several strengths across the four projects. The nationwide coverage of registers ensures external validity while the high quality of register-based data

and verified disease diagnoses reduces recall bias and minimizes misclassifications of the outcome. In addition, the matched cohort design used in all four projects helps to control for the confounding effects of important factors.

Several limitations need to be acknowledged. Firstly, the results might be affected by healthy user bias. To account for health behavior differences, the regressions model was adjusted by incorporating colonoscopy as a proxy, since it is offered opportunistically in Sweden, and people with better health awareness and knowledge are more likely to undergo colonoscopies. Additionally, the frequency of outpatient visits was also considered, as it is strongly linked to adherence to CRC screenings. Using colonoscopy and outpatient visits as proxies for healthcare engagement could potentially limit the impact of divergent health behaviors between users and non-users. Furthermore, the results of stratification analyses indicated strong correlations between drug exposure and advanced-stage CRC diagnosis compared to early-stage diagnosis, which suggests that detection bias may have only had a minor impact on our findings. Secondly, our results were inevitably affected by indication bias. To eliminate the possibility of potential confounding by indication bias, we performed several sensitivity analyses, such as investigating the association between the use of active comparator (drugs with the same indications) and CRC risk. In Paper IV, we additionally performed a Mendelian Randomization analysis to further rule out the indication bias by depression. Thirdly, as we discussed above, the lack of information on over-the-counter medications might lead to a misclassification of melatonin use in Paper I. We conducted a sensitivity analysis to account for the misclassification of melatonin exposure, and the results were consistent with those of the main analysis. This suggests that the findings are robust and reliable. Fourthly, while modifiable lifestyle factors greatly impact CRC, our nationwide databases do not provide complete information on certain potential confounding factors, such as smoking, alcohol consumption, and dietary habits. Nevertheless, we attempted to mitigate the impact of these factors by adjusting for COPD in regression models, which serves as a crude proxy for smoking. Furthermore, we accounted for education status, which has a strong association with lifestyle factors and can partially exclude their confounding effects.

Clinical perspectives

This thesis identified several promising chemoprevention agents. The potential mechanism, efficacy, and safety profile require to be validated by pro-clinical studies and well-designed RCTs in the future.

Conclusion

In conclusion, the overall aim of this thesis was to explore the potential drug repositioning opportunities for the chemoprevention of CRC. Below is the specific conclusion for each project.

Paper I

Our study revealed a negative correlation between the utilization of melatonin and the incidence of CRC in older individuals. This association was found to be consistent regardless of the location of CRC or the stage of cancer at the time of diagnosis.

Paper II

This cohort study conducted on a population suggests that individuals with a family history of CRC who use proguanil/atovaquone have a lower risk of developing CRC, and the magnitude of the risk reduction is directly proportional to the dosage used.

Paper III

Our population-based cohort study indicates that regular use of SSRIs is linked to a reduced risk of CRC in individuals with a family history of CRC, and this risk reduction appears to follow a dose-dependent pattern.

Paper IV

This nested case-control study indicates that using aspirin and SSRIs, either alone or in combination, is linked to a decreased risk of CRC, and the decrease follows a dose-response relationship. The observed significant interaction on an additive scale indicates that the combination of aspirin and SSRIs may have a synergistic effect on reducing the risk of CRC development.

Future perspectives

The findings of this thesis provide promising insights into the potential drug repositioning opportunities for the chemoprevention of CRC. However, there is still much to be explored in this field, and future research should focus on the following aspects:

1. Investigating the underlying mechanisms: The exact mechanisms by which these drugs act on CRC prevention are not fully understood. Further research should be conducted to investigate the molecular and cellular mechanisms underlying the protective effects of these drugs.
2. Long-term follow-up studies: Although the current studies have shown positive results, long-term follow-up studies are needed to confirm the chemopreventive effects of these drugs. Future studies should focus on assessing the long-term benefits and risks associated with these drugs.
3. Identification of high-risk groups: As the chemopreventive effects of these drugs appear to vary among different population groups, future research should focus on identifying the high-risk groups who may benefit the most from these drugs. This could include individuals with specific genetic or lifestyle risk factors for CRC.
4. Combination therapies: The observed synergistic effect of aspirin and SSRIs on CRC prevention highlights the potential for combination therapies in CRC chemoprevention. Future research should explore the use of other drug combinations that may have similar or complementary effects.

In summary, the findings of this thesis provide a basis for future research in the field of CRC chemoprevention. Further investigation into the mechanisms of action, long-term effects, identification of high-risk groups, and combination therapies may provide valuable insights into the development of more effective strategies for CRC prevention.

Popular science summary

Colorectal cancer (CRC) is one of the most common types of cancer, and it can be fatal if not detected and treated early. Researchers have been exploring ways to prevent CRC, and this thesis explores the possibility of using drugs that are already on the market for other conditions.

The first paper in the thesis looked at the use of melatonin, a hormone that regulates sleep, in preventing CRC. The study found that people who used melatonin had a lower risk of developing CRC, regardless of the stage or location of the cancer.

The second paper investigated the use of proguanil/atovaquone, a medication used to treat and prevent malaria, in preventing CRC. The study found that people with a family history of CRC who used higher doses of the medication had a lower risk of developing CRC.

The third paper looked at the use of selective serotonin reuptake inhibitors (SSRIs), a type of antidepressant, in preventing CRC. The study found that regular use of SSRIs was linked to a lower risk of developing CRC, and this effect was more significant in people with a family history of the disease.

Finally, the fourth paper investigated the use of aspirin and SSRIs, either alone or in combination, in preventing CRC. The study found that using these drugs was associated with a lower risk of developing CRC, and the effect was greater with higher doses. When aspirin and SSRIs were used together, there was a synergistic effect in reducing the risk of CRC.

Overall, this thesis provides evidence that existing drugs may have potential in preventing CRC, and further research could lead to new and effective strategies for preventing this deadly disease.

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Additional analyses

Supplemental material to the thesis "Drug repositioning in chemoprevention of colorectal cancer" by Naiqi Zhang, Lund University.

In order to test the robustness of our results, we conducted several additional analyses to account for the impact of time-dependent variables, immortal time bias, competing risks, and dose-response.

We have also added additional discussion on the dose-response, biological plausibility, and competing risks.

Paper I: Melatonin and colorectal cancer

The source population comprises 2,640,805 Swedish-born residents aged 50 and older as of the index date, which was 1st January, 2006. To implement a new user design, we instituted a six-month washout period (Jul 2005- Dec 2005), where prevalent users were excluded from the present analysis (n = 245). Individuals were followed from index date until i) the first date of colorectal cancer (CRC) diagnosis; ii) the date of death from any cause; iii) the end of the study period (31st December 2018), whichever came first. We used a competing risk Cox regression model in order to control for the competing risk of death. Use of melatonin was modeled as a time-dependent variable, where individuals were initially considered nonusers until they received the first melatonin dispensation.

We found a marginally insignificant association between melatonin and CRC risk, with an adjusted hazard ratio (HR) of 0.93, 95% confidence interval (CI) of 0.84-1.02, and *P* value of 0.122 (Supplementary Table 1).

Supplementary Table 1. Melatonin use and CRC risk among individuals aged 50 and older.

	Individuals, n	Person-years	CRC diagnoses, n	IR, per 1000 person-year	Crude			Adjusted*		
					HR	95% CI	*P* value	HR	95% CI	*P* value
Melatonin										
Non-users	257637	29371958	48684	1.66	1			1		
Users	64923	807979	435	0.54	0.92	0.83-1.01	0.069	0.93	0.84-1.02	0.122

*Adjusted for age at index, sex, education, income, region of residence, family history, history of inflammatory bowel disease, Chronic obstructive pulmonary disease, obesity, history of colonoscopy, use of aspirin, use of statin, Charlson comorbidity index score.

We additionally conducted an analysis using propensity score (PS) matching. A total of 64,918 melatonin users aged 50 and older were included. For each melatonin user, five matched comparisons without prescription of melatonin were randomly selected based on PS calculated by age at index, sex, education, income, region of residence, family history, history of inflammatory bowel disease, chronic obstructive pulmonary disease, obesity, history of colonoscopy, use of aspirin, use of statin, Charlson comorbidity index score.. All individuals were followed from 1st Jan 2006 until i) the first date of CRC diagnosis; ii) the date of death from any cause; iii) the end of the study period (31st December 2018), whichever came

first. We used a competing risk Cox regression model in order to control for the competing risk of death. Use of melatonin was modeled as a time-dependent variable.

We found that the use of melatonin was associated with 11% reduced CRC risk, with an adjusted HR of 0.89, 95% CI of 0.81-0.98, and *P* value of 0.018 (Supplementary Table 2).

Supplementary Table 2. Melatonin use and CRC risk among individuals aged 50 and older using propensity score matching.

	Individuals, n	Person-years	CRC diagnoses, n	IR, per 1000 person-year	Crude			Adjusted*		
					HR	95% CI	*P* value	HR	95% CI	*P* value
Melatonin										
Non-users	324526	3789190	6156	1.62	1			1		
Users	64918	807915	435	0.54	0.89	0.81-0.98	0.017	0.89	0.81-0.98	0.018

*Adjusted for PS calculated by age at index, sex, education, income, region of residence, family history, history of inflammatory bowel disease, Chronic obstructive pulmonary disease, obesity, history of colonoscopy, use of aspirin, use of statin, Charlson comorbidity index score.

Paper II: Proguanil/atovaquone and colorectal cancer

The source population consisted of 444,684 cancer-free Swedish-born individuals who had at least one first-degree relative diagnosed with CRC, and aged 18 and older at the index date (1st Jan 2006). To implement a new user design, we instituted a six-month washout period (Jul 2005- Dec 2005), where prevalent users were excluded from the present analysis (n = 591). Individuals were followed from index date until i) the first date of CRC diagnosis; ii) the date of death from any cause; iii) the end of the study period (31st December 2018), whichever came first. Use of proguanil/atovaquone was modeled as a time-dependent variable, where individuals were initially considered nonusers until they received the first proguanil/atovaquone dispensation.

We found that the use of proguanil/atovaquone was associated with 30% reduced CRC risk (crude HR: 0.70, 95% CI: 0.58-0.84, $P < 0.001$). However, the association lost significance after adjusting for confounders (adjusted HR: 0.86, 95% CI: 0.71-1.04, $P = 0.115$) (Supplementary Table 3).

Supplementary Table 3. Proguanil/atovaquone use and CRC risk among individuals with family history.

	Individuals, n	Person-years	CRC diagnoses, n	IR, per 1000 person-year	Crude			Adjusted*		
					HR	95% CI	*P* value	HR	95% CI	*P* value
Proguanil										
Non-users	427429	5303522	6869	1.30	1			1		
Users	16403	210397	113	0.54	0.70	0.58-0.84	<0.001	0.86	0.71-1.04	0.115

*Adjusted for age at index, sex, education, income, region of residence, history of inflammatory bowel disease, Chronic obstructive pulmonary disease, obesity, history of colonoscopy, use of aspirin, use of statin, Charlson comorbidity index score.

Considering that the confounders played a major role in the association, we additionally conducted an analysis using PS matching. A total of 16,394 proguanil/atovaquone users with family history were included. For each proguanil/atovaquone user, five matched comparisons without prescription of melatonin were randomly selected based on PS calculated by age at index, sex, education, income, region of residence, family history, history of inflammatory bowel disease, chronic obstructive pulmonary disease, obesity,

history of colonoscopy, use of aspirin, use of statin, Charlson comorbidity index score. All individuals were followed from 1st Jan 2006 until i) the first date of CRC diagnosis; ii) the date of death from any cause; iii) the end of the study period (31st December 2018), whichever came first. Use of proguanil/atovaquone was modeled as a time-dependent variable.

We found that use of proguanil/atovaquone was associated with 26% reduced CRC risk (adjusted HR: 0.76, 95% CI: 0.62-0.90, $P = 0.002$) (Supplementary Table 4).

Supplementary Table 4. Proguanil/atovaquone use and CRC risk among individuals with family history using propensity score matching.

	Individuals, n	Person-years	CRC diagnoses, n	IR, per 1000 person-year	Crude			Adjusted*		
					HR	95% CI	*P* value	HR	95% CI	*P* value
Proguanil										
Non-users	81795	1034015	1043	1.01	1			1		
Users	16394	210281	113	0.54	0.75	0.62-0.90	0.003	0.74	0.62-0.90	0.002

*Adjusted for PS calculated by age at index, sex, education, income, region of residence, history of inflammatory bowel disease, Chronic obstructive pulmonary disease, obesity, history of colonoscopy, use of aspirin, use of statin, Charlson comorbidity index score.

Paper III: SSRI and colorectal cancer

The source population consisted of 444,684 cancer-free Swedish-born individuals who had at least one first-degree relative diagnosed with CRC, and aged 18 and older at the index date (1st Jan 2006). To implement a new user design, we instituted a six-month washout period (Jul 2005- Dec 2005), wher prevalent users were excluded from the present analysis ($n = 21,056$). Individuals were followed from index date till i) the first date of CRC diagnosis; ii) the date of death from any cause; iii) the end of the study period (31st December 2018), whichever came first. We used a competing risk Cox regression model in order to control for the competing risk of death. Use of SSRI was modeled as a time-dependent variable, where individuals were initially considered nonusers until they received the first SSRI dispensation.

As shown in Supplementary Table 5, we found that the use of SSRI was associated with 19% reduced CRC risk (adjusted HR: 0.81, 95% CI: 0.74-0.89, $P < 0.001$).

Supplementary Table 5. SSRI use and CRC risk among individuals with family history.

	Individuals, n	Person-years	CRC diagnoses, n	IR, per 1000 person-year	Crude			Adjusted*		
					HR	95% CI	*P* value	HR	95% CI	*P* value
SSRI										
Non-users	362954	4516479	6206	1.37	1			1		
Users	60674	754108	496	0.66	0.81	0.74-0.89	<0.001	0.81	0.74-0.89	<0.001

*Adjusted for age at index, sex, education, income, region of residence, history of inflammatory bowel disease, Chronic obstructive pulmonary disease, obesity, history of colonoscopy, use of aspirin, use of statin, Charlson comorbidity index score.

We additionally conducted an analysis using PS matching. A total of 60,543 SSRI users with family history were included. For each SSRI user, three matched comparisons without prescription of melatonin were randomly selected based on PS calculated by age at index, sex, education, income, region of residence, family history, history of inflammatory bowel disease, chronic obstructive pulmonary disease, obesity,

history of colonoscopy, use of aspirin, use of statin, Charlson comorbidity index score. All individuals were followed from 1st Jan 2006 until i) the first date of CRC diagnosis; ii) the date of death from any cause; iii) the end of the study period (31st December 2018), whichever came first. We used a competing risk Cox regression model in order to control for the competing risk of death. Use of SSRI was modeled as a time-dependent variable.

We observed an inverse association between SSRI use and CRC risk, with an adjusted HR of 0.79, 95% CI of 0.73-0.87, and *P* value < 0.001 (Supplementary Table 6).

Supplementary Table 6. SSRI use and CRC risk among individuals with family history using propensity score matching.

	Individuals, n	Person-years	CRC diagnoses, n	IR, per 1000 person-year	Crude			Adjusted*		
					HR	95% CI	*P* value	HR	95% CI	*P* value
SSRI										
Non-users	178584	2213185	3011	1.36	1			1		
Users	60543	752527	494	0.65	0.79	0.72-0.86	<0.001	0.79	0.73-0.87	<0.001

*Adjusted for PS calculated by age at index, sex, education, income, region of residence, history of inflammatory bowel disease, Chronic obstructive pulmonary disease, obesity, history of colonoscopy, use of aspirin, use of statin, Charlson comorbidity index score.

In order to explore the dose-response relationship between SSRI use and CRC risk, we conducted a nested case-control study. The source population consisted of 444,684 Swedish-born individuals who had at least one first-degree relative diagnosed with CRC. Prevalent SSRI users were excluded from the study population using a time window from Jul 2005 to Dec 2005 (n = 21,056). We identified 2916 CRC patients diagnosed between Jan 2007 and Dec 2018. For each CRC case, five nested controls were randomly selected using incidence-density sampling among cohort members who had no cancer diagnosis before selection, matched by propensity score.

We found that the use of SSRI was associated with lower CRC risk, with adjusted OR of 0.84, and 95%CI of 0.76-0.94. The OR of CRC risk was 0.93 (95% CI, 0.76-1.05) among SSRI users with the lowest cumulative dose, decreased to 0.91 (95% CI, 0.75-1.11) among users with medium-low dose, and 0.66 (95% CI, 0.53-0.82) among individuals with the medium-high dose, and then increased to 0.88 (95% CI, 0.72-1.08) among individuals with the highest dose (Supplementary Table 7).

Supplementary table 7. Dose-response analysis of SSRI use and CRC risk among individuals with family history using nested case-control study design.

	Case		Control		Crude			Adjusted*		
	No.	%	No.	%	HR	95% CI	*P* value	HR	95% CI	*P* value
SSRI										
Non-users	2458	84.3	11871	82.0	1					
Users	458	15.7	2599	18.0	0.84	0.76-0.94	0.002	0.84	0.76-0.94	0.002
Dose										
Quartile 1	119	4.1	612	4.2	0.93	0.76-1.14	0.472	0.93	0.76-1.14	0.471
Quartile 2	128	4.3	651	4.5	0.91	0.75-1.11	0.373	0.91	0.75-1.11	0.373
Quartile 3	93	3.2	679	4.7	0.66	0.53-0.82	<0.001	0.66	0.53-0.82	<0.001
Quartile 4	121	4.1	657	4.6	0.88	0.72-1.08	0.213	0.88	0.72-1.08	0.213

*Adjusted for PS score calculated by age at index, sex, education, income, region of residence, history of inflammatory bowel disease, Chronic obstructive pulmonary disease, obesity, history of colonoscopy, use of aspirin, use of statin, Charlson comorbidity index score.

Discussion

Definition of exposure status

In our main analysis, we aimed to explore the association between different kinds of medications (melatonin, proguanil/atovaquone, SSRIs) with CRC risk by adopting a study approach similar to 'per protocol analyses'. The individuals who did not use these medications during the whole follow-up period were defined as the control population and matched with the population who have ever used these medications. However, it should be noted that such a definition of the control population considered future events, i.e., we needed to follow all these individuals until the end of the study to make sure that they never had used these medications. The use of future exposure information to define baseline cohort membership is a practice that comes with the potential of introducing bias into research findings. This bias can be particularly challenging to predict, as both its direction and magnitude may vary based on the specific study context and the nature of the exposures being considered [1]. The incorporation of information about exposures that occur after the initial cohort selection may complicate the assessment of causal relationships and the interpretation of study results, introducing complexities that researchers must carefully consider in their interpretations.

To exclude one potential bias in our main analyses, we additionally carried out a couple of analyses by using a time-varying exposure study design to define the status of exposure, i.e., whether the individuals had ever used the medications or not. Results from the additional analyses using time-varying exposure showed similar results to the main analyses, and few of them showed borderline significance. It should be noted that immortal time bias can most often be ruled out when using a time-varying exposure study design. However, the status of covariates, such as region of residence, history of inflammatory bowel disease, COPD, obesity, history of colonoscopy, use of aspirin, use of statin as well as CCI, which were defined at baseline might change during the follow-up periods, especially for those who used the medications a couple of years after the index date. For example, one can use the SSRIs in the year 2016, which means that this person has not used SSRIs for ten years, in which periods it should be defined as non-exposure before the year 2016. However, these covariates could have changed during the past ten years between 2006 and 2015; thus, our results from the time-varying exposure study design might not fully adjust for their confounding effect. Even though time-varying covariates were considered in the Cox analyses, such analyses might not be possible to carry out as including too many covariates in the analyses would need much more computing power.

Time-related biases in pharmacoepidemiology

Time-related biases, such as immortal time bias, latency time bias, and time-window bias, frequently occur in pharmacoepidemiologic research [2].

Immortal time bias

Immortal time bias refers to a period of follow-up during which death or an outcome of interest can not occur because of the exposure definition. It is common in observational studies that there is a discrepancy

between the date of cohort entry (or disease diagnosis) and the date of first drug prescription. When follow-up begins from the date of cohort entry, it leads to "immortal" person-time in patients who start treatment vs. those who do not as individuals must survive long enough to be classified as treated. Misclassified immortal time would bias the results in favor of a treatment effect. In the main analyses of this thesis, individuals were followed from the date of the first dispensation of the drugs, thus avoiding bias due to immortal time. Results from additional analyses using time-varying exposure showed similar results to the main analyses, suggesting that immortal time bias plays a minor role in the present study.

However, the dose-response relationship of drug exposure in a cohort study is particularly susceptible to the influence of immortal time bias. This is because individuals receiving higher doses must survive for a certain period to accumulate a sufficient amount of drug use. Other methods, such as employing time-varying exposure to define cumulative doses [3], were suggested to have more advantages to account for immortal time bias regarding the dose-response relationship analyses. In the time-varying exposure analyses, the cumulative dose was modeled as a time-varying variable, updated at every risk set, calculated as the sum of all prescription doses until the time of the event, and classified according to the predefined categories. In our study regarding the association between melatonin and proguanil/atovaquone with CRC risk, we had some reservations about employing time-varying exposures to define cumulative doses. Firstly, the administration of melatonin and proguanil/atovaquone differs somewhat from other medications, such as antihypertensive or antihyperlipidemic drugs, which are typically taken daily. Melatonin, for instance, may only be used sporadically, such as for insomnia or jet lag. If an individual has two prescriptions (each for 30 tablets) with a two-year interval, it's challenging to ascertain whether they used it within the first 30 days or throughout the entire two years. This makes defining the date of reaching cumulative dosages difficult. Secondly, our study focused on CRC, which has a development time of at least 10 years. Consequently, determining the latency period poses great challenges [4]—should the follow-up cease when they reach the defined cumulative level, or should we continue monitoring them for a few more years? If we continue the follow-up, those in the highest cumulative dosages group will have much less follow-up time.

To address this concern, we further conducted a dose-response analysis of SSRI usage using a nested case-control design. The results of this analysis indicated that the relationship between the dose of SSRI use and the risk of colorectal cancer followed a U-shaped dose-response pattern. While a nested case-control study design could be reasonable for studying dose-response relationships, we believe it warrants further discussion.

Time-window bias

Another time-related bias in case-control studies is time-window bias, which resulted from the use of a time-independent approach in control selection. Controls were defined as individuals who did not experience the study outcome during the observation period, leading to exposure assessment over a shorter time span for cases compared to controls. In Paper IV, we adopted time-dependent sampling, following the principle of incidence density sampling thus effectively mitigating this bias [5]. This approach ensures that cases and their matched controls are observed for an equal length of time, thereby eliminating discrepancies in the time windows for exposure measurement.

Latency time bias

Carcinogenesis is broadly acknowledged as a multi-stage biological process. Cancer latency periods differ across cancer types and are predominantly uncertain; however, it is generally believed that they span at least several years. When exploring the associations between drugs and cancer in pharmacoepidemiology, it is crucial to take into account the pertinent latency periods of cancer and the potential challenges related

to reverse causality and detection bias [4]. We applied a one-year lagging time in Paper IV to account for this issue.

Observational studies serve as a valuable means of drug repositioning. Nevertheless, they may be prone to time-related biases, which can be mitigated through the use of appropriate design and analysis approaches. It is essential to interpret pharmacoepidemiological results with caution, and any findings should undergo rigorous evaluation in well-designed randomized trials before considering their clinical applications.

Biological plausibility

The plausibility of biological mechanisms for the potential antitumor effects of proguanil and atovaquone is not well understood but some hypotheses are worth attention. Metformin, a member of the biguanide family with a similar structure and function as proguanil, was observed to elicit an antitumor effect by modulation of the gut microbiota and rescues Fusobacterium nucleatum-induced colorectal tumourigenesis [6]. Similarly, another *in vivo* study suggested that oral use of metformin is associated with changes in the gut microbiome and reductions in MC38 tumor cell growth in mice [7]. The changes induced by the gut microbiome alteration seem to adhere to a non-dose-dependent pattern, implying that even low doses may potentially exhibit anti-tumour effects, although the specific biological mechanisms need to be examined further.

Competing risk of death

It should be noted that the proportion of deaths in the cohort of SSRI users is higher as compared to non-SSRI users, which means that the cohort of SSRI users might die before they get cancer, and this would in turn not protect against CRC. To account for the higher mortality in the cohort of SSRI users, a competing risk Cox regression model was used in the main analyses as well as the additional analyses. Such analyses can partly exclude bias due to the different mortality between the study population and the control population. However, we should note that such discrepancies regarding the different mortality are mainly due to the indication of using SSRIs, i.e., that the cohort of SSRI users had ever been diagnosed with mental disorders, including depression, anxiety, etc.. Thus, the best study design to account for such bias is to use an active comparison study design, i.e., the control population should use other medications to treat depression, anxiety, etc. instead of non-SSRI users in our analyses. Unfortunately, SSRIs are the most common medications to treat depression and anxiety in Sweden, whereas other medications are used less frequently, which makes an active comparison study design difficult. Thus, we adopted a sensitivity analysis to explore the risk of CRC among individuals who had ever used tricyclic antidepressants (TCAs) to treat depression and anxiety, and we did not find a negative association between TCA use and CRC risk, suggesting that the protective effect of SSRI use on CRC risk might not be biased by a higher mortality in the cohort of SSRI users. Additionally, some statistical methods could be considered to account for the bias, such as the use of combined endpoints (combining all-cause mortality with selected non-fatal events), which have been used in cardiovascular research [8]. In future studies, a combined study approach regarding the antitumor effect of the studied medicines on cancer risk should be employed, such as animal models and CRISPR techniques to understand the underlying mechanism of, for example, SSRIs against tumor development.

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