Pharmacoepidemiological assessment of drug-cancer associations

Anton Pottegård
Pharmacoepidemiological assessment of drug-cancer associations

Anton Pottegård

Clinical Pharmacology and Pharmacy
Department of Public Health
University of Southern Denmark
"We are chemical apes: having discovered the capacity to extract, purify, and react molecules to produce new and wondrous molecules, we have begun to spin a new chemical universe around ourselves. Our bodies, our cells, our genes are thus being immersed and reimmersed in a changing flux of molecules — pesticides, pharmaceutical drugs, plastics, cosmetics, estrogens, food products, hormones, even novel forms of physical impulses, such as radiation and magnetism. Some of these, inevitably, will be carcinogenic. We cannot wish this world away; our task, then, is to sift through it vigilantly to discriminate bona fide carcinogens from innocent and useful bystanders."

Dr. Siddhartha Mukherjee in “The Emperor of All Maladies”
PREFACE

The text in front of you was drafted during the spring and summer of 2017 and finalized in the summer of 2018 to summarize my work concerning the assessment of carcinogenic or chemopreventive effects of prescription drugs. The 10 papers included in the thesis are selected to represent my applied work, thus leaving out my more methodological contributions. The latter are, however, cited throughout the text. Further, a preliminary version of the thesis was redrafted as a methodological review published in Basic and Clinical Pharmacology and Toxicology in 2018. Nevertheless, the thesis focuses on my work with specific drug-cancer associations, with 10 papers that I believe tell a coherent story in their own right.

Many people deserve recognition for their contribution to the work reported in this thesis. This includes both the 25 co-authors that have contributed to the 10 specific papers as well as the more than 300 different collaborators worldwide that I have had the privilege to work with. While I cannot give due credit to all, I need to thank specifically Søren Friis, that has been a co-author on all 10 papers in the thesis and that has grown to be a close friend over the years. Furthermore, I would like to thank Laurel Habel, Sigrún Alba Johannesdottir Schmidt, Til Stürmer, and David Gaist that have all pushed me to do better drug-cancer research, as well as my wonderful colleagues at Clinical Pharmacology and Pharmacy at the University of Southern Denmark, that make me look forward to going to work each and every day. Finally, while many people deserve mention, Jesper Hallas, in all his quiet supremacy, stands out. Having introduced me to the field of pharmacoepidemiology and many of my current collaborators, supervised my PhD thesis, and in all regards shaped me as the researcher that I am today, he is now my most important partner in crime.

Around the time where the idea of writing up a thesis was hatched my daughter Esther was born. While it may seem forced to thank a 3-year-old for her contribution to a scientific dissertation, it is, nevertheless, what I must do. As a young ambitious researcher, it is easy to get caught up in strategies to improve your Hirsch-index or how to get your research published in high-ranking journals. However, seeing my daughter discover the world anew has served as a due reminder that the process of getting somewhere is, as it should be, usually more fun than arriving. The continued support and forbearance of my family, in particular my unbelievably patient wife, is the foundation that allows me to pursue my passion and also the only thing that keeps me sane. It is much more than I deserve.

Anton Pottegård (July 2018)
LIST OF PAPERS

Papers on single drug-cancer associations

I  Use of benzodiazepines or benzodiazepine related drugs and the risk of cancer: a population-based case-control study
Pottegård A, Friis S, Andersen M, Hallas J.

II  Long-term use of statins and risk of renal cell carcinoma: A population-based case-control study
Pottegård A, Clark P, Friis S, Hallas J, Lund L.
Eur Urol. 2016 May;69(5):877-82

III  Long-term lithium use and risk of renal and upper urinary tract cancers
Pottegård A, Hallas J, Jensen BL, Madsen K, Friis S.

IV  Long-term use of lithium and risk of colorectal adenocarcinoma: A nationwide case-control study
Pottegård A, Ennis Z, Hallas J, Jensen BL, Madsen K, Friis S.
Br J Cancer. 2016 Mar 1;114(5):571-5

V  Use of tricyclic antidepressants and risk of glioma: A nationwide case-control study
Pottegård A, García Rodríguez LA, Rasmussen L, Damkier P, Friis S, Gaist D.
Br J Cancer. 2016 May 24;114(11):1265-8

VI  Use of sildenafil and other phosphodiesterase inhibitors and risk of melanoma
Pottegård A, Schmidt SAJ, Olesen AB, Achacoso N, Van Den Eden SK, Hallas J, Sørensen HT, Friis S, Habel LA.
Br J Cancer. 2016 Sep 27;115(7):895-900

Screening paper

VII  Identification of associations between prescribed medications and cancer: A nationwide screening study
Pottegård A, Friis S, Christensen Rd, Habel LA, Gagne JJ, Hallas J.
EBioMedicine. 2016 May; 7:73-9

Papers on hydrochlorothiazide and skin cancer

VIII  Hydrochlorothiazide use is strongly associated with risk of lip cancer
Pottegård A, Hallas J, Olesen M, Svendsen MT, Habel LA, Friedman GD, Friis S.

IX  Hydrochlorothiazide use and risk of non-melanoma skin cancer: A nationwide case-control study from Denmark
Pedersen SA, Gaist D, Schmidt SAJ, Hölmich LR, Friis S, Pottegård A.

X  Association of hydrochlorothiazide use and risk of malignant melanoma
Pottegård A, Arnspang S, Schmidt SAJ, Hölmich LR, Friis S, Gaist D.
JAMA Intern Med. 2018 May 29 (Epub).
TABLE OF CONTENTS

PREFACE.................................................................................................................................... 1
LIST OF PAPERS...................................................................................................................... 2
TABLE OF CONTENTS......................................................................................................... 3
SUMMARY.................................................................................................................................. 4
DANSK RESUME ..................................................................................................................... 6
INTRODUCTION .................................................................................................................... 8
METHODS ............................................................................................................................... 10
   Data sources .......................................................................................................................... 10
   Cancer outcomes .................................................................................................................. 11
   Sampling of cases and controls ........................................................................................... 12
   Exposure ascertainment ...................................................................................................... 13
   Analysis .................................................................................................................................. 15
   Screening algorithm ............................................................................................................ 16
RECAP OF STUDIES ............................................................................................................. 18
   Benzodiazepines and (all) cancer [I] ................................................................................... 18
   Statins and renal cell cancer [II].......................................................................................... 19
   Lithium and upper urinary tract cancers [III] ...................................................................... 19
   Lithium and colorectal adenocarcinoma [IV] .................................................................... 20
   Tricyclic antidepressants and glioma [V] ........................................................................... 20
   Phosphodiesterase inhibitors and melanoma [VI] ............................................................ 21
   Screening for drug-cancer associations [VII] .................................................................... 22
   Hydrochlorothiazide and skin cancer [VIII-X] ................................................................. 22
CONCLUSIONS AND PERSPECTIVES ........................................................................... 24
   Scientific dissemination ........................................................................................................ 24
   Methodological developments ............................................................................................ 26
   Screening studies ................................................................................................................ 29
   Concluding remarks ........................................................................................................... 30
REFERENCES ......................................................................................................................... 31
APPENDIX: PAPERS I-X ..................................................................................................... 37
ENGLISH SUMMARY

Use of prescription drugs represents an exogenous exposure that may infer an increased, or even decreased, risk of cancer. While a few such effects have been established, the typically long induction period before an increased risk of cancer becomes evident, coupled with the low incidence of most individual cancers, makes drug-cancer association studies particularly challenging. Consequently, the list of established carcinogenic or chemopreventive pharmaceuticals is very short, while a large number of drugs are currently suspected of possessing carcinogenic properties, with more studies being required to confirm these associations.

This thesis includes 10 original studies. Six studies pertain to single drug-cancer associations, one study presents a screening algorithm designed to identify new drug-cancer associations, and three studies looks further into the association between hydrochlorothiazide and skin cancer, a signal derived from the screening study.

Within the nationwide Danish health registries, we conducted case-control studies, with cases obtained via the Danish Cancer Registry, sex- and age-matched controls sampled via riskset sampling, and drug exposure estimated using data from the Danish Prescription Registry. The odds ratio (OR) associating a given drug and cancer was then estimated using conditional logistic regression, while adjusting for relevant confounders. The screening algorithm was based on largely similar methodology, dividing all cancer cases into 99 distinct outcomes based on topography and histology and performing analyses for all drugs or drug classes with 10 or more users among the cancer cases (observed or expected). All signals, i.e. drug-cancer associations, that were found to have an association meeting a pre-specified requirement for strength, while also meeting subsequent tests for specificity and dose-response patterns, were reported in full.

The first six studies were performed based on previous studies reporting either an increased or decreased risk of cancer associated with use of specific drugs. The first study concerned use of benzodiazepines and risk of all cancer, finding no overall association, except for what could be attributed to residual confounding from smoking and alcohol use (OR 1.09, 95%CI 1.04-1.14). The second study investigated the potential chemopreventive effects of statins towards renal cell carcinoma, finding little evidence of such an effect (OR 1.06, 95%CI 0.91-1.23). The third study investigated the risk of upper urinary tract cancers associated with lithium use, returning a reassuring null finding (OR 1.3, 95%CI 0.8-2.2). In a follow-up study, the association between lithium use and colorectal adenocarcinoma was investigated. While no overall association was seen (OR 1.13, 95%CI 0.89-1.43) a slightly increased risk was seen specifically for the distal part of the colon (OR 1.52, 95%CI 1.05-2.20). The fifth study investigated the putative chemopreventive effect of tricyclic antidepressants towards glioma. While
interpretation was hindered by limited statistical power, a tendency towards a protective effect was seen (OR 0.72, 95%CI 0.41-1.25). The sixth study investigated the association between use of phosphodiesterase inhibitors and melanoma using both US and Danish data, which generally found little evidence of such an association (Denmark: OR 1.22, 95%CI 0.99-1.49; US: OR 0.95, 95%CI 0.78-1.14).

The seventh study was the screening algorithm. A total of 22,125 drug-cancer pairs underwent evaluation. Initially, 4561 signals met the requirement for strength of association, of which 1020 also met the requirements for specificity and dose-response pattern. These associations were published in full, as a repository of potential associations to be evaluated in future studies. The eighth, ninth and tenth study followed up on a signal that was obtained in the screening study, namely the apparent association between use of hydrochlorothiazide and risk of skin cancer. The three studies investigated hydrochlorothiazide’s association to lip cancer, non-melanoma skin cancer, and melanoma, respectively. For squamous cell carcinoma of the lip, an OR of 7.7 (95% CI 5.7-10.5) was found, with clear evidence of a dose-response pattern. For basal cell carcinoma of the skin, a weaker but dose-dependent association was seen increasing to an OR of 1.54 (95% CI 1.38-1.71). For squamous cell carcinoma of the skin, a stronger dose-response relationship was seen, reaching an OR of 7.38 (95% CI 6.32-8.60). Lastly, for melanoma, a marginally increased risk was seen (OR 1.22; 95% CI 1.06-1.36) with little evidence of dose-response pattern. Stronger associations were, however, seen specifically for nodular (OR 2.05; 95% CI 1.54-2.72) and lentigo melanoma (OR 1.61; 95% CI 1.03-2.50).

Finally, major challenges for the conduct of pharmacoepidemiological drug-cancer studies are discussed. This includes the pursuit of false hypotheses, stemming from less sound analyses or ill-advised interpretations of drug-cancer studies. Further, future methodological developments are discussed, in particular the emergence of more useful data sources, the challenges in conducting studies of long-term outcomes, and the lack of data on core risk factors for cancer, such as alcohol consumption, obesity, and smoking. Lastly, future challenges of hypothesis-free screening are discussed, including the need for basic development of screening decision rules and post-processing of large number of signals and issues related to re-use of data for confirmatory studies that was also used in the screening study generating the hypothesis.
Brug af receptpligtige lægemidler udgør en udefrankommende påvirkning der kan medføre en øget, eller endda nedsat, risiko for kræft. Mens enkelte sådanne sammenhænge er blevet vist, så medfører den meget lange induktionsperiode før en øget kræfrisiko bliver tydelig, sammen med den lave forekomst af de fleste individuelle kræftformer, at studier af sammenhænge mellem lægemidler og kræft er særligt udfordrende. Som en konsekvens er listen over etablerede kræftfremkaldende eller -beskyttende lægemidler meget kort, mens et meget stort antal lægemidler er mistænkt for at have sådanne egenskaber, hvor flere studier er påkrævet for at kunne bekræfte sammenhængen.


Baseret på data fra de nationale danske sundhedsregistre, gennemførte vi case-kontrollstudier, med cases fundet vi Cancerregisteret, køns- og aldersmatchede kontroller identificeret via riskset sampling og lægemiddeleksponering opgjort ud fra data fra Lægemiddelstatistikregisteret. Odds ratioen (OR) der forbinder et givet lægemiddel og kræft blev estimeret ved hjælp af betinget logistisk regression, med justering for relevante confoundere. Screenings-algoritmen var baseret på tilsvarende metodologi, med opdeling af kræft-cases i 99 forskellige udfald, baseret på topografi og histologi, og med analyse af alle lægemidler og lægemiddelgrupper hvor der var mere end 10 brugere blandt cases (observeret eller forventet). Alle signaler, dvs. lægemiddel-kræft-sammenhænge, som udviste en sammenhæng som opfyldte et prædefineret krav for styrke, og som også opfyldte efterfølgende tests for specificitet og dosis-respons-mønstre, blev afrapporteret.

De første seks studier blev gennemført på baggrund af tidligere studier der angav enten en øget eller nedsat risiko for kræft associeret med brug af specifikke lægemidler. Det første studie angik brug af benzodiazepiner og risiko for alle kræftformer og fandt ingen overordnede sammenhæng, ud over hvad der kunne tilskrives residualconfounding fra rygning og alkoholindtag (OR 1.09, 95%CI 1.04-1.14). Det andet studie undersøgte den potentielle kræftforebyggende effekt af statiner mod renalcellecarcinomer og fandt ingen tegn på en sådan effekt (OR 1.06, 95%CI 0.91-1.23). Det tredje studie undersøgte risikoen for kræft i de øvre urinveje, inklusiv nyrerne, associeret med brug af lithium, hvilket returnerede et betydeligt nulever resultat (OR 1.3, 95%CI 0.8-2.2). I et opfølgende studie blev sammenhængen mellem lithium-brug og colorektale adenocarcinomer
undersøgt. Mens der ikke blev set nogen overordnet sammenhæng (OR 1.13, 95%CI 0.89-1.43) sås der en let øget risiko specifikt for den distale del af colon (OR 1.52, 95%CI 1.05-2.20). Det femte studie undersøgte den påståede kræftbeskyttende effekt af tricykliske antidepressiva i forhold til gliomer. Mens fortolkningen blev besværliggjort af begrænset statistisk power, så sås en tendens mod en beskyttende effekt (OR 0.72, 95%CI 0.41-1.25). Det sjette studie undersøgte sammenhængen mellem brug af phosphodiesterasehæmmere og melanomer ved brug af både amerikanske og danske data og fandt generelt ingen tegn på en sådan sammenhæng (Danmark: OR 1.22, 95%CI 0.99-1.49; Amerika: OR 0.95, 95%CI 0.78-1.14).


De tre studier undersøgte hydrochlorothiazids sammenhæng med henholdsvis læbekræft, non-melanom hudkræft og melanomer. For pladecellekræft på læben blev der fundet en OR på 7.7 (95% CI 5.7-10.5) med klare tegn på en dosis-respons-sammenhæng. For basalcellkræft i huden sås en svagere men dosis-afhængig sammenhæng, stigende op til en OR på 1.54 (95% CI 1.38-1.71). For pladecellekræft i huden sås en stærkere dosis-respons-sammenhæng, de nåede en OR på 7.38 (95% CI 6.32-8.60). Slutteligt sås for melanomer en margintalt øget risiko (OR 1.22; 95% CI 1.06-1.36) uden tegn på dosis-respons-sammenhæng. Stærkere sammenhænge blev dog set specifikt for nodulære melanomer (OR 2.05; 95% CI 1.54-2.72) og lentigo melanomer (OR 1.61; 95% CI 1.03-2.50).

INTRODUCTION

Everyone, that is laymen, clinicians, and researchers alike, are familiar with the “war on cancer”. During the 19th and most of the 20th century, the army fighting on our behalf in this war consisted almost solely of surgeons. Gradually, a wider array of soldiers has been enrolled, including oncologists, hematologists, chemists, pharmacists, nurses, and bioinformatics just to name a few, each employing a different arsenal of weapons, targeting different parts of the enemy. One such soldier, most often fighting his battle far away from the day-to-day skirmish in the cancer wards, is the epidemiologist.

The contributions of epidemiologists to the main battle were, for many decades, far and few between. Important exceptions include John Hill’s observations in 1761 that use of snuff infers an increased risk of lip, mouth, and throat cancers [1] and the observation made by Pott in 1775 that chimney-sweeps had a markedly higher risk of scrotal cancer than other people [2], likely attributable to their exposure to soot. The turning point, however, came with the realization that the epidemic of lung cancers observed during the 50s and 60s was caused by smoking [3]. This was the first time that an outside exposure had been established as the primary cause of a highly prevalent cancer – at a time where the primary focus for most cancer researchers were on either genetics and viruses as the primary driver of oncogenesis.

One such outside exposure that might increase – or even decrease – an individual’s risk of developing cancer, is the use of prescription drugs. A few such associations have been established, such as the carcinogenic properties of phenacetin [4,5] and the chemopreventive effects of aspirin towards colorectal cancer [6,7]. Cancer effects of drug use are, however, typically only evident after considerable induction periods. As an example, the increased risk of breast cancer associated with use of female hormones becomes apparent only after many years of continued use [7]. This, together with the low incidence of most individual cancers, severely limits the ability of traditional pharmacovigilance systems based on spontaneous reporting of adverse effects to detect these effects. Consequently, the assessment of drug-cancer associations is largely left to observational research. For many reasons, however, the field of cancer pharmacoepidemiology is still in its infancy. One important reason is the fact that the challenges discussed above, e.g. the long induction time and the low prevalence of individual cancers, infer very high demands for the data sources used in such studies, especially regarding duration of follow-up and the size of the population covered. Thus, only a handful drug-cancer associations are currently recognized as fully established by the International Agency for Research on Cancer (IARC) [8,9], while numerous drugs are suspected of possessing carcinogenic properties although more data is required to confirm these associations [8].
The public health importance of identifying carcinogenic effects of drugs is readily apparent, since even small carcinogenic effects of widely used drugs will translate into many drug-induced cancer cases. Identification of such associations allows for appropriate handling of patients, e.g. by introducing periodic screening of patients using the drug in question, changing guidelines to recommend other and safer treatment alternatives or, in some cases, withdrawing the drug from the market all together. However, identifying causal carcinogenic effects is not the only objective for studies on cancer risk and use of drugs. Often, a safety signal, i.e., a putative drug-cancer association, arise during clinical development, e.g. from animal models or as an unexpected high number of a specific cancer during phase III trials. Another source of such safety signals is single observational studies reporting a new potential association. In these cases, providing solid evidence for the lack of an association also holds considerable value by reassuring prescribers and patients of the safety of drugs, which in turn promotes their appropriate use. Lastly, the identification of chemopreventive drug effects also hold value. While it will rarely be feasible to simply give the drug as prophylactic treatment to patients, such associations might provide clues that can lead to better understanding of tumor development and prevention.

The work presented in this thesis includes six studies of single drug-cancer associations [I-VI]. Four of these was prompted by previous studies suggesting an increased risk of cancer associated with use of benzodiazepines (all cancer [I]), lithium (renal and colon cancer [III-IV]), and sildenafil (melanoma [VI]), while another two studies investigated putative chemopreventive properties of statins towards renal cancer [II] and tricyclic antidepressants towards glioma [V]. Further, the establishment of a screening algorithm, designed to identify associations between use of prescription drugs and cancer risk [VII] is presented. Lastly, the screening study identified a signal regarding use of hydrochlorothiazide and skin cancer, which was investigated further in three studies on use of hydrochlorothiazide and risk of lip cancer [VIII], non-melanoma skin cancer [IX], and melanoma [X].
METHODS

Using the nationwide Danish health registries, we have conducted a series of case-control studies and a screening study based on similar methodology. In brief, to conduct a case-control study, we identify individuals with a cancer diagnosis (cases) and a group of cancer-free individuals (controls) with a similar sex- and age-composition. We then estimate each individual’s use of drugs based on prescription fills. By comparing the use of drugs among cases to that among controls, using conditional logistic regression and adjusting for relevant confounders, we obtain an estimate of the association between use of the given drug or drug class and the cancer outcome in question.

Data sources

Virtually all medical care in Denmark is furnished by the national health authorities. Coupled with the wealth of registries documenting the use of the Danish health care system, this allows true population-based register linkage studies that covers all inhabitants of Denmark. Data from the different data sources can be unambiguously linked using the personal identification number (“CPR-nummer”), a unique identifier assigned to all Danish residents since 1968 [10]. For the work presented in this thesis, all linkages were performed within Statistics Denmark, a governmental institution that collects and processes information for a variety of statistical and scientific purposes.

We used five Danish nationwide registries. The primary data sources were the Danish Cancer Registry [11,12] and the National Prescription Registry [13], while supporting information was obtained from the National Patient Register [14], Registers in Statistics Denmark on educational level and income [15,16], and the Civil Registration System [17].

The Danish Cancer Registry [11,12] has recorded cases of cancer on a nationwide basis since 1943. Only incident cancer cases are recorded, and the registry thus contains no data on relapses or secondary cancers within the same organ. Data has historically been collected manually, with some input from the Registry of Causes of Deaths [18] and, since 1987, the Danish National Patient Register [14]. In 2004, a modernization process was completed, and the Cancer Registry is now based on data from the Danish National Patient Register [14] as well as the National Pathology Registry [19]. The Cancer Registry has been shown to have accurate and almost complete ascertainment of cancer cases. Cancer diagnoses in the Cancer Registry are recorded according to the International Classification of Diseases, version 10 (ICD-10), and the ICD for Oncology (ICD-O-1-3) for topography and morphology codes.
The Danish National Prescription Registry [13] contains data on all prescription drugs dispensed to Danish citizens since 1995. Only prescriptions filled at community pharmacies are included in the registry. While this includes the vast majority of prescription drugs used in Denmark, including drugs issued by hospital physicians, drugs used during admissions and drugs supplied directly by the hospital, are not covered by the registry. The recorded data include among other things the type of drug, date of dispensing, and the quantity. The dosing information and the indication for prescribing are not available. Drugs are categorized according to the Anatomic Therapeutic Chemical (ATC) index, a hierarchical classification system developed by the WHO [20], and the quantity dispensed for each prescription is given by the number and strength of the pharmaceutical entities (e.g., tablets), as well as the defined daily doses (DDD).

The Danish National Patient Register [14] contains nationwide data on all non-psychiatric hospital admissions since 1977 and both psychiatric and non-psychiatric outpatient contacts since 1995. Discharge/contact diagnoses have been coded according to ICD-8 from 1977 to 1993 and ICD-10 since 1994.

Statistics Denmark hosts registries on education and income [15,16]. The Population Education Register [15] contains information on nearly all adult Danes and provides the highest completed level of education, defined as that with the longest duration of schooling. The Income Statistics Register [16] contains more than 160 variables describing different aspects of income for all Danish citizens since 1970.

The Danish Civil Registration System [17] contains data on date of death and migration to and from Denmark, which allowed us to extract population controls and to keep track of all subjects.

Cancer outcomes

Across all projects included in this thesis, cancer outcomes were obtained using the Danish Cancer Registry [11,12]. This provides a distinct advantage regarding coverage, compared to studies based solely on e.g. general patient registries or pathology data. Further, it allows us to restrict to histologically verified cancers, which increases the general validity of the case material, while also allowing us to restrict to cancers of comparable histologies. In the literature, many studies use ‘all cancer’ as an outcome, which is effectively a combined endpoint, dominated by the most common cancers. Keeping in mind our understanding of cancer as a heterogeneous disease, the use of the ‘all cancer’-outcome should generally be avoided, as it is highly unlikely that a pharmaceutical compound should be revealed post-marketing as a general carcinogen. The slightly more refined identification of cancer cases according to organ site, e.g. using ICD-10 diagnostic codes, is increasingly common. However, in continuation of the
argument above, it is unlikely that any given drug should lead to the formation of
different types of cancers, even within the same organ. The differentiation between
histological subtypes is therefore often necessary for research purposes. In organs with
one dominant cancer type, e.g. colorectal cancer dominated by adenocarcinomas, this
issue will have limited implications. Conversely, consideration of histological subtypes is
a necessity for sites such as the lungs, where both adenocarcinomas, squamous cell
carcinomas, small-cell carcinomas, non-small-cell carcinomas, and carcinoids are
common [21]. Further, in context of signal detection, differentiation is of importance for
rare cancer outcomes. Consider carcinoids in the colon, which constitute a very small
proportion of all colon cancer cases [22]. When considering colon cancer as one
aggregate outcome, any associations specific to carcinoids will, regardless of strength, be
overlooked, as they will be diluted by the adenocarcinoma component of the outcome
definition.

In the papers presented in this thesis, we have used outcomes with specified histology
except for study I and to some extent III and VI. These three studies are, however, all
performed as a response to a safety signal, and the outcomes were therefore selected to
resemble those used in the previous studies. Regarding the potential association between
use of hypnotics and (all) cancer [I], the previous study by Kripke et al [23], provided
little information on risks stratified by cancer type. In our study, we provided
associations with single cancer types, although presented by organ system and
disregarding histology [I]. In the study of lithium’s potential association to urinary tract
cancers [III], we responded to a previous study by Zaidan et al. [24] reporting on all
renal tumors (including oncocytomas). While we used a combined endpoint of ‘upper
urinary tract cancers’, we also performed analyses restricted to the two constituents,
namely renal cancers (dominated by renal cell carcinomas) and cancers of the renal pelvis
or ureter (almost exclusively urothelial carcinomas). In the study of phosphodiesterase
inhibitors’ potential association with melanoma [VI], we performed no analyses of
individual melanoma subtypes. While somewhat less controversial, as melanoma is
generally considered to constitute an outcome in its own right, supplementary analyses
of individual melanoma subtypes could meaningfully have been performed, similarly as
to what was done in our study on hydrochlorothiazide and melanoma [X].

Sampling of cases and controls

Cases were defined using data from the Cancer Registry and based on the principles
outlined above. We further applied a range of exclusion criteria, although with some
variations between the individual studies. First, we restricted the case material to cancers
that were histologically verified. While the histology was not always used in the actual
analysis [I,III,VI], this was done to ensure the validity of the outcomes. Second, we for
some projects restricted to an age range of e.g. 18-85 years. Children and adolescents were excluded both as the (long-term) use of drugs is limited in this age range and as cancer outcomes are both rare and, should they occur, likely due to e.g. genetic dispositions more than exogenic exposures. The highest age range was excluded as the diagnostic work-up among the oldest old might differ from that among younger subjects. As such, the threshold for establishing a diagnosis might be higher among the oldest old and, more importantly, dependent on the individual’s general health status, potentially introducing bias. Third, we excluded cases with recent migrations, to ensure a sufficient capture of both exposure status and baseline covariates. Fourth, we excluded individuals with a previous cancer diagnosis, except non-melanoma skin cancer. This was done as a subsequent cancer in a different organ might be a matter of incorrectly diagnosed or registered metastases from the first cancer, which would lead to outcome misclassification. Further, the treatment of the primary cancer might lead to an increased risk of subsequent cancers [8]. Lastly, we excluded individuals with rare but strong risk factors for the cancer in question. As examples, we excluded persons with von-Hippel Lindau syndrome in the studies of renal cancers [II,III] and people with familial adenomatous polyposis from the study on colorectal cancer [IV].

For each case, we identified a number of controls (ranging from 8 [I] to 100 [VIII]) with the same birth-year and sex as the case and assigned the controls the same index date as the case, i.e., the date of the case’s cancer diagnosis. We used risk-set sampling, thus maintaining the matching throughout the analysis (see below). The sampling of controls followed the general principle of treating cases and controls alike, to ensure that a control was eligible for sampling as a case, had the control received a cancer diagnosis on the date of sampling. As such, the same exclusion criteria that were applied for the cases were also applied for the controls. This ensures that the odds ratios (ORs) obtained from the analyses are unbiased estimates of the incidence rate ratios (IRRs) that would have been obtained if the study had been conducted as a cohort study within the same population [25].

**Exposure ascertainment**

A central challenge in the conduct of pharmacoepidemiological studies is to identify not only the right people that are at risk of a given outcome under a given exposure, but also the time during which this increased risk is present. In the case of cancer as the outcome of interest, this is complicated by the concepts of induction and latency [26], with the first referring to the time between the initiation of the carcinogenic process and the development of a cluster of cancer cells, and the latter referring to the time between this formation and the actual detection, i.e., diagnosis. As such, it is not meaningful to directly compare ‘currently exposed’ people to unexposed people, as your current use of
The optimal choice of time window depends on multiple factors. A full account of this vastly complex subject goes beyond the scope of this text. However, three factors will be briefly discussed. First, and most importantly, the choice of exposure model should aim for mimicking the (potential) underlying biological effect, taking into account the characteristics of the specific cancer in question. As such, if the potential mechanism for a carcinogenic effect is that the drug leads to chronic inflammation and that cancer in question has a considerable induction/latency period, then the time at risk should start a long time after initiation of drug treatment. Including time during ‘early’ exposure in the model, effectively dilutes the comparison with person-time that is not at an increased risk, thereby attenuating any observed associations. Conversely, some drug-cancer associations show a very short time until onset [27–29], and ignoring recent exposure will in these situations abolish the study’s capability of detecting an association. Similar considerations need to be given to the amount of time since drug cessation after which an individual is no longer considered to be at an increased risk. Regrettably, for the majority of drug-cancer studies the potential biological mechanism is unknown or, at best, a hypothesis. Further, the induction/latency period is unknown for many cancers. As a result, the optimal time window for the assessment of drug-cancer associations is largely unknown [30], and care should therefore be taken to apply different cut-offs and definitions within the single study, in order not to overlook any potential associations.

Second, when estimating exposure, the risk of reverse causation needs to be considered. Reverse causation, also called ‘protopathic bias’, is a concern as early symptoms of the
cancer, as well as the increased health care utilization leading up to the diagnosis, might lead to an increased drug use. As an example, a markedly increased risk of bladder cancer is seen in the first six months after initiation of drugs used against overactive bladder, likely because the drug was prescribed for symptoms of the cancer in question [31]. We have described how, for most cancers, this phenomenon can be handled using a lag-time of no more than six months [32].

Lastly, the actual use pattern of the drug in question needs to be taken into account. Consider as an example the use of phosphodiesterase inhibitors such as sildenafil [VI]. The majority of users have a very low cumulative exposure, at least compared to the exposure that would normally be considered necessary for any drug to exert a carcinogenic effect. As such, the study of risks associated with this limited use might, from a strict biological perspective, be of little relevance. However, it needs to be considered that these are the exposure levels that the patients are subjected to, and it is thus, from a public health perspective, more relevant to consider in detail the risk these patients are subjected to, than that of the (very few) long-term users.

To exemplify the considerations above, consider the studies on use of hypnotics [I] and lithium [III, IV]. In the former [I], the safety concern related to relatively small amounts of drug and thus we applied a main exposure criterion of 500 DDDs of hypnotic use within a period of -5 to -1 years from the index date. Further, we applied a new user design, to limit exposure misclassification. In the studies on lithium use [III, IV], a drug that is continuously dose-adjusted and typically used for a very long period of time, we used a main exposure criterion of ≥5 years of cumulative exposure, using all the available prescription data (back to 1995), while disregarding exposure in the last year prior to the index date.

Analysis

The analyses all conformed to standard case-control methodology. As we had used risk-set sampling, conditional logistic regression was used, i.e., stratified on the single risk-set consisting of one case and its corresponding controls.

All studies applied a main exposure criterion, e.g. ≥5 years of cumulative exposure [III,IV], that was defined prior to the conduct of the study. Further, all studies included dose-response analyses, which, while presented slightly differently throughout the papers, should generally also be considered part of the main analysis. The dose-response analyses serve different purposes. Most importantly, when evaluating the potential causality of an observed association, the presence (or absence) of a dose-response pattern is a strong argument for (or against) a causal relationship. Further, the dose-response pattern to some extent allow for an evaluation of confounding. If the dose-
response curve does not originate from zero, i.e., if even very limited exposure is also associated with an increased (or decreased) cancer risk, this argues strongly against causality, instead pointing to confounding as a potential explanation.

The adjustment for confounders naturally varied between studies, but generally follows the principles applied in all pharmacoepidemiological studies. All drugs and diseases that could conceivably be associated (either positively or negatively) with exposure to the drug while also being either directly or indirectly associated with the risk of the given cancer was included. Further, risk factors for the given cancer were also included, regardless of their association to the exposure. While not exerting any confounding effect, this reduces the variation in the material.

A range of supplementary analyses and sensitivity analyses were applied. While many were tailored specifically to the individual study, e.g. the deliberately flawed sampling to mimic a previous study [I], a few general principles need to be mentioned here. First, we performed subgroup analyses on age categories, sex and other restrictions, e.g. to those with low comorbidity score or those without diabetes. These analyses are generally carried out to look for potential effect modifiers [33]. Second, analyses were carried out according to cancer stage. This mainly facilitates an evaluation of the potential for surveillance bias. If an increased risk is confined to low-grade (localized) cancers, as was e.g. seen in the study of use of phosphodiesterase inhibitors and risk of melanoma [VI], this indicates that use of the drug is associated with a lower threshold for establishing the diagnosis. Third, the use of lag-time was varied in sensitivity analysis. The choice of lag-time, as discussed above, is often arbitrarily defined, as the true risk window is unknown. As such, applying varying cut-offs for lag-time is necessary to assess how this analytical choice affects the estimates. Lastly, whenever possible, analyses were carried out for other drugs with similar indications, e.g. valproic acid in the study of lithium [III] and other antihypertensives in the studies on hydrochlorothiazide [VIII-X]. Such analyses enable a direct evaluation of the potential for confounding by indication.

Screening algorithm
The screening study [VII] is generally based on the same principles as outlined above. Nevertheless, some aspects of this study need to be considered separately.

The basic principle is that of a multiple case-control study, as described by Shapiro [34]. In brief, this entails conducting a large number of case-control studies, while varying both the outcome and the exposure of interest. In keeping with the above considerations regarding outcome definitions, 99 different cancer outcomes were defined, by taking into account both the organ site and histology. For each of these 99 cancers (outcomes), we performed a case-control study for all drugs or drug classes (exposures) that had 10
or more exposed cases either observed or expected based on the proportion of users
among the controls. Here, exposure refers to ‘long-term use’, defined somewhat
arbitrarily as 8+ prescription fills for the drug or drug class in question. Matching was
performed (1:10) on sex and birth year and analyses were carried out comparing ‘long-
term use’ to never-use, while adjusting for Charlson comorbidity index [35] and level of
education.

We defined signals as drug-cancer associations that in the initial analyses either returned
an OR lower than 0.67 or higher than 1.50 or that had the higher boundary of its 95%
confidence interval lower than 0.83 or the lower boundary higher than 1.20. Such signals
then underwent further evaluation. In this second step, we required specificity and
evidence of a dose-response pattern. Specificity was tested by dividing the point estimate
of the given signal by the OR associating the drug or drug class in question to all cancers
(as a combined outcome). We required that the resulting ratio was outside the range of
0.83-1.20. The dose-response criterion was assessed by restricting to exposed individuals
(cases and controls) and estimating the incremental OR per prescription fill (while
capping exposure at 50 prescriptions). Signals were considered to meet this criterion if
the p-value for this incremental OR was below 0.10. Signals that passed both criteria
were, as agreed prior to the conduct of the study, published in full.
RECAP OF STUDIES

This section provides a summary of the 10 studies included in the thesis, focusing on the background for conducting the single study and the main conclusions and interpretation. The six single studies and the screening study are summarized individually, while the three studies on hydrochlorothiazide and skin cancer are described collectively. For further details, please see the corresponding full papers supplied in the appendix.

Benzodiazepines and (all) cancer [I]

In 2012, Kripke et al. published a cohort study on the use of hypnotics and risk of all cancer and all-cause mortality [23]. Regarding cancer, an up to 35% increased risk was reported. The International Agency for Research on Cancer (IARC) have generally not found hypnotics to be associated with an increased risk of cancer [36]. Further, we found several potential flaws in the methodology applied by Kripke et al. [37]. However, considering the widespread use of hypnotics, the public health impact of a carcinogenic effect of these drugs would be substantial. We therefore conducted a case-control study on the use of hypnotics and risk of cancer.

Using a new user design, we identified 149,360 cancer cases that were matched to 1,1947,729 population controls. Long-term use of hypnotics was associated with an adjusted OR of 1.09 (95%CI 1.04-1.14). The marginally increased overall risk was driven by cancers of the stomach, esophagus, liver, lung, pancreas, and kidney (ORs ranging from 1.35-1.81). Generally, cancers associated with tobacco use showed a higher risk compared to other cancers (OR 1.15 vs. 1.01). Lastly, in a sensitivity analysis mimicking one of the flaws that we proposed played a central role in the findings by Kripke et al., we obtained an estimate identical to that of Kripke, namely an OR of 1.35 (95%CI, 1.28-1.41).

When taking into consideration that users of hypnotics are known to have a higher use of tobacco [38,39] and alcohol [40] compared to the background population, we interpreted the slightly elevated risks as evidence of a null association. This interpretation was not accepted by Kripke [41]. As highlighter by Kripke [41], concurrent studies by Kao et al. also found an increased risk of cancer associated with use of hypnotics [42,43]. However, these studies seems to apply a similar, and thus flawed, methodology as that of Kripke et al. Recently, we have revisited the association in methodological work that included partial adjustment for lifestyle confounders, which attenuated the slight association further [44].
Statins and renal cell cancer [II]

Statins have received considerable interest for their potential chemopreventive properties, primarily on the basis of laboratory findings [45–47]. While the epidemiological evidence is generally conflicting [48,49], a US cohort study reported a markedly reduced risk of renal cell carcinoma [50], which sparked considerable urological interest in this potential association. To this end, we conducted a case-control study on the use of statins and risk of renal cell carcinoma.

We identified 4606 renal cell carcinoma cases that was matched to 46,060 population controls. Overall, no association was seen between long-term use of statins and renal cell carcinoma, with an adjusted OR of 1.06 (95%CI 0.91-1.23). No substantial variations were seen in subgroup or sensitivity analyses.

We thus found no evidence of any important chemopreventive effect of use of statins towards renal cell carcinoma. As pointed out in an accompanying editorial [51], the lack of data on lifestyle confounders might realistically bias our results upwards, that is mask a protective effect of statin use. However, our analyses of the potential magnitude of this bias does not indicate that any major chemopreventive effect has been overlooked [II].

Lithium and upper urinary tract cancers [III]

In 2014, a case-series by Zaidan et al. raised the question whether use of lithium was associated with a markedly increased risk of renal cancers [24], which was in line with a similar previous study by Rookmaker et al. [52]. Considering the known nephrotoxic effects of long-term lithium treatment [53,54], such an association was considered plausible. We therefore conducted a case-control study on the use of lithium and risk of upper urinary tract cancers.

We included 6477 upper urinary tract cancer cases that were matched to 259,080 population controls. Long-term use of lithium was observed among 0.22% of cases and 0.17% of controls which yielded an adjusted OR of 1.3 (95%CI 0.8-2.2) for upper urinary tract cancer. No substantial variations were seen in subgroup or sensitivity analyses, although the interpretation of these analyses was generally hindered by limited statistical power. Assuming a causal association, the point estimate of an OR of 1.3 would correspond to one additional cancer case per 12,364 person-years spent as a long-term user. The corresponding value using the upper limit of the 95% confidence interval (2.2) yielded an estimate of 3091 person-years.

Lithium users might have a lower threshold for undergoing renal imaging compared to non-users [55]. Further, individuals suffering from bipolar disorder are known to smoke
more than the general population [56]. Both effects would bias our estimate upwards, and we thus interpret the slightly elevated, although statistically insignificant, result as a null-finding. Further, even under the assumption of a causal association corresponding to the upper value of the 95% confidence interval, absolute effect sizes were small. A similar conclusion was reached by another Danish group applying a cohort design to the same data material [57].

Lithium and colorectal adenocarcinoma [IV]

Lithium has been shown to accumulate in the colonic epithelium via the Na⁺ channel ENaC [58,59] and its use has in rodent models been shown to lead to colon tumor growth [60]. Further, lithium inhibits the Wnt-signaling pathway, that plays a role in oppressing tumor growth [61]. We therefore conducted a case-control study to assess whether use of lithium is associated with an increased risk of colorectal cancer.

We identified 36,248 colorectal adenocarcinoma cases that were matched to 362,480 population controls. Long-term lithium use was associated with an OR of 1.13 (95%CI 0.89-1.43) for colorectal cancer. No substantial variation was seen in subgroup and sensitivity analyses, with the exception that a slightly increased risk was observed for tumors located in the distal part of the colon (OR 1.52, 95%CI 1.05-2.20), while no association was seen for tumors of the proximal colon (OR 1.01) or the rectum (OR 0.80).

The overall interpretation is that of a null-finding, also keeping in mind the potential upwards bias from smoking and alcohol use that were not accounted for. However, the results raise a possibility of an association specific to the distal part of the colon. While ENaC has been shown to be expressed with a gradient towards the distal colon in rodents [62,63], it is unknown whether the same is true in humans. Furthermore, the lack of an apparent association with rectal adenocarcinomas speaks against this interpretation. An alternative explanation is that of a surveillance effect, with lithium users undergoing earlier or more intensive medical workup than non-users. This might affect proximal and distal estimates differently, as distal cancers has been shown to present earlier than proximal cancers [64].

Tricyclic antidepressants and glioma [V]

Some drugs have been shown to affect glioma risk [65–67]. One such drug class is tricyclic antidepressants, that in a study by Walker et al. was associated with a protective
effect towards glioma [68]. To investigate this further, we performed a case-control study on the use of tricyclic antidepressants and risk of glioma.

We included 3767 glioma cases that were matched to 75,340 population controls. Long-term use of tricyclic antidepressant was associated with glioma by an OR of 0.72 (95%CI 0.41-1.25). There was some evidence of a dose-response pattern, although this was hindered by limited statistical power and thus did not reach statistical significance. Similar analyses for use of selective serotonin reuptake inhibitors showed no apparent associations.

The general lack of statistical power severely hampered our ability to interpret our findings. However, our results were generally in full accordance with the previous study by Walker et al. [68]. Taken together, the results are therefore still indicative of a protective effect of tricyclic antidepressants towards glioma, although future studies are needed to confirm this.

**Phosphodiesterase inhibitors and melanoma [VI]**

In 2014, Li et al. reported an increased risk of melanoma associated with use of the phosphodiesterase inhibitor sildenafil [69]. It has previously been shown that a large proportion of melanomas contain mutations that leads to suppression of phosphodiesterase enzyme 5A, which again leads to melanoma cell invasion and metastasis. As phosphodiesterase inhibitors infer a direct pharmacological inhibition of the same enzyme, the hypothesis that their use infers an increased risk of melanoma has biological merit. To this end, we conducted two case-control studies, using Danish and US data, on the association between use of phosphodiesterase inhibitors and risk of melanoma.

We identified 7045 Danish and 2972 US melanoma cases. In Denmark, the overall adjusted OR for high-use of phosphodiesterase inhibitors was 1.22 (95%CI 0.99-1.49). The corresponding US finding was an OR of 0.95 (95%CI 0.78-1.14). No apparent dose-response pattern was found, although the two highest exposure strata in the Danish data (200-499 and 500+ tablets) returned ORs of 1.44 and 1.47, respectively. Some variation was observed according to melanoma stages, with the highest estimates obtained for localized melanoma in the Danish data and in situ melanoma in the US data.

The overall interpretation of the two studies is that they provide little support for a causal association between use of phosphodiesterase inhibitors and melanoma. The slightly increased risk estimates are likely explained by more frequent health-care contacts among users of phosphodiesterase inhibitors, and thus better opportunity for
establishing of a melanoma diagnosis, than among non-users. This is supported by the finding that the slightly increased risks were only observed for in situ and localized melanoma.

Three other papers, one based on Swedish data [70] and two based on CPRD data [71,72], was published in close proximity to our study. These generally arrived at similar conclusions, although the study by Matthews et al. also pointed to differences in sun exposure patterns as a potential explanation for the marginally increased risks [72]. The studies were recently summarized in a meta-analysis, that also arrived at the conclusion that the apparent association is unlikely to be causal [73].

**Screening for drug-cancer associations [VII]**

As argued in the introduction to this text, traditional pharmacovigilance systems, primarily based on spontaneous reporting, has limited possibilities to detect cancer occurrence as adverse events. Besides doing stand-alone studies when concerns are raised regarding the safety of a specific drug, a more systematic use of health care data seems feasible, especially when leveraging the Danish health care registries. To this end, we constructed a nationwide screening algorithm to identify associations between use of prescription drugs and risk of cancer.

Based on 278,485 incident cancer cases, 22,125 different drug-cancer pairs underwent evaluation. After the initial round of testing, 4561 signals were identified. After applying criteria for specificity and dose-response relationship, 1020 drug-cancer associations remained. These 1020 signals were published in full.

From the list of associations, it is evident that a considerable proportion are explained by confounding from factors associated with the underlying condition being treated. As an example, drugs used against chronic obstructive pulmonary disease associate strongly with risk of lung cancer. However, the identified associations also include drug-cancer pairs with strong apparent associations were no immediate explanation could be found. Thus, the list of associations provides a potentially valuable repository of hypotheses that can be addressed in future studies.

**Hydrochlorothiazide and skin cancer [VIII-X]**

One of the strongest drug-cancer associations that were identified during the screening study was the association between use of an amiloride/hydrochlorothiazide combination and risk of squamous cell carcinoma of the lip [VII]. Amiloride has not previously been associated with an increased risk of cancer [74]. However, hydrochlorothiazide, one of
the most common drugs in the Western world [75,76], was in 2013 classified as ‘possibly carcinogenic to humans’ (group 2B) by the International Agency for Research on Cancer (IARC) [9], primarily based on a previous US study linking its use to an increased risk of lip cancer [77]. The suggested mechanism pertains to hydrochlorothiazide’s photosensitizing properties [78]. To investigate this further, we performed three separate case-control studies of the association between use of hydrochlorothiazide and risk of lip cancer [VIII], non-melanoma skin cancer [IX] and malignant melanoma [X].

For lip cancer (n=633 cases), we found a clear dose-response relationship with an OR of 7.7 (95% CI 5.7–10.5) for cumulative use of ≥100,000 mg hydrochlorothiazide. Regarding non-melanoma skin cancer, a weak, dose-dependent association was seen for basal cell carcinoma (n=71,553 cases), increasing to an OR of 1.54 (95% CI 1.38–1.71) in the highest exposure category of ≥200,000 mg hydrochlorothiazide. A stronger dose-response relationship emerged for squamous cell carcinoma (n=8629 cases), with an OR of 7.38 (95% CI 6.32–8.60) in the ≥200,000 mg category. Lastly, for melanoma (n=19,273 cases), a marginally increased risk with hydrochlorothiazide use was seen (≥50,000 mg: OR 1.22; 95% CI 1.09–1.36), resulting from increased ORs for nodular (OR 2.05; 95% CI 1.54–2.72) and lentigo melanoma (OR 1.61; 95% CI 1.03–2.50). Corresponding analyses for other antihypertensive agents, including the chemically related bendroflumethiazide, yielded neutral associations for all of the studied outcomes.

Taken together, the three studies strongly support the hypothesis that hydrochlorothiazide is carcinogenic to humans. Subsequently, we have shown that hydrochlorothiazide is also associated to an increased risk of Merkel cell carcinoma and malignant adnexal skin tumors [79]. Thereby, hydrochlorothiazide has now been shown to be associated to all UV dependent skin cancers. The strength of the associations, the specificity to hydrochlorothiazide compared to other drugs used for similar indications, the plausible biological mechanism, and the fact that a similar association has been seen in another setting [77] altogether satisfies Hill’s criteria for making causal inferences based on epidemiological observations [80]. At present, several additional studies regarding hydrochlorothiazide’s association to skin cancer is ongoing, as is an evaluation by the Pharmacovigilance Risk Assessment Committee (PRAC) within the European Medicines Agency [81].
CONCLUSIONS AND PERSPECTIVES

In this section, some of the major challenges that are particularly pronounced for pharmacoepidemiological drug-cancer studies will be discussed. Specifically, the pursuit of false hypotheses will be discussed, including how methodological less sound analyses can contribute to this. Further, the potential for methodological improvements is discussed was well as the use of hypothesis-free screening as an approach to detect safety issues.

Scientific dissemination

As evident from the papers included in this thesis, the scientific literature provides a steady stream of new hypotheses linking together specific drugs with specific cancer outcomes. Such hypotheses stem from many different places, such as spontaneous adverse event reporting and case reports, laboratory studies, re-analysis of clinical trials as well as epidemiological studies, a larger proportion of which might suffer from limited statistical power and at times major methodological flaws. One mechanism that might at least to some extent explain this pattern is the phenomenon of publication bias, that is, that small scale papers are more likely to be published if they report a potential but hitherto unrecognized safety issue with a given drug. Regardless of mechanism, the need for larger and more methodologically robust studies is constant, to reassure clinicians and patients of the safety of the drugs in question, and thus ensure the rational use of the drugs available to us.

It is, however, evident that the publication of one or few larger studies is not necessarily sufficient to put a topic to rest. This is amply illustrated by the controversy regarding the risk of cancer associated with use of benzodiazepines. Despite us highlighting in letters to the editor, the methodological issues in the work by Kripke [37] and our study showing no excess risk [I], at least beyond what can be explained by residual confounding, later studies [42,43], also using questionable methodology, have found risks in the order of those reported by Kripke et al. By use of self-citation and cherry-picking of references, these studies signal, at least to those that do not have sufficient time to perform structured literature searches, that there is an emerging consensus on the issue, i.e., that use of benzodiazepines infer an increased risk of cancer. Another, more recent, example includes the potential carcinogenic effects of lithium, which, in our studies, was quite clearly refuted. In a recent Taiwanese study published in the British Journal of Psychiatry [82], this hypothesis is now reversed, as lithium was found to be associated with a statistically significant decreased risk of all cancer (HR 0.735, 95%CI 0.554-0.974) including evidence of a dose-response pattern. However, several methodological shortcomings hinder the interpretation. Besides the ill-advised use of a
composite ‘all cancer’ outcome, this study classifies individuals at baseline into cohorts, depending on their future use of drugs, that is, users of lithium that at a later point starts using antipsychotics are never allowed into the cohort of lithium users. In addition, the dose-response analysis is performed by estimating the cumulative amount of drug filled and treating this as a baseline variable, thereby providing people with longer duration of follow-up a higher propensity of ending up in the higher exposure strata. Despite limited biological plausibility and seemingly flawed methodology, a new epidemiological hypothesis is thus born, and, as highlighted by the authors, “future studies will be needed” [82].

The publication of small-scale studies with flawed methodology is not the only contributing factor in keeping alive such hypothesis. Another important factor is the skewed interpretation of otherwise sound studies that is sometimes put forward by readers with limited epidemiological insight. The interpretation of the main result of our study on use of benzodiazepines [I] as ‘evidence for harm’ [41] is an example of this. Another, potentially more impactful, example is related to the putative association between use of phosphodiesterase inhibitors and risk of melanoma. Our study concluded, along with three other large-scale studies, that there was a weak association, but that it was unlikely to be causal, an interpretation supported by a long list of carefully executed supplementary analyses. This conclusion was also reached in a recent meta-analysis by Loeb et al. [73]. However, simultaneously with the publication of the Loeb meta-analysis, another meta-analysis by Wang et al. was published [83]. Despite being based on the four papers summarized above, Wang et al. base their conclusion solely on the point estimates obtained from their meta-analysis. With blatant disregard for the supplementary analyses performed, and ultimately conclusions reached, within the individual papers, they therefore conclude that there is evidence that phosphodiesterase inhibitor use is associated with an increased risk of melanoma [83]. And, of course, this warrants further study in the future [83]. A joint letter by the authors of all four studies have openly criticized the conclusion reached by Wang et al. (the letter is no longer available due to PubMed Commons being shut down in February 2018). However, besides providing a classic example of authors relying too much on statistical significance in their interpretation of epidemiological studies [84], the paper by Wang et al. also risks reviving a hypothesis that four teams of dedicated epidemiologists had done their very best to put to rest.

Solutions that will address the issues pointed out above are not immediately obvious. Most likely, this will require a more fundamental restructuring of the academic publishing traditions [85,86], with ramifications that goes far beyond the narrow field of drug-cancer studies. In the meantime, it is an important, albeit time-consuming, duty of established researchers to address such controversies. Strategies include, but is not limited to, seeking to increase the methodological quality of work within the field by
writing up 'best practice' guidelines [87], the writing of 'letters to the editor' [37], performing systematic reviews to summarize the literature on a given hypothesis (Kristensen, *in press*), making yourself available as a reviewer for scientific journals as well as with (critical) comments as part of the press coverage of new studies [88], and, of course, to perform new and better studies of putative associations.

**Methodological developments**

By many accounts, the field of drug-cancer association studies is still in its infancy. One potential reason is the fact that long term outcomes such as cancer requires data sources of a certain age and with limited ‘churn’ [89,90] in order to provide a reasonable amount of follow-up for the single individual. While some data sources meeting this requirement have existed for some time, e.g. the Danish health data databases [12–14,17] or the Kaiser Permanente database in the US [91], others have only recently emerged. Take as an example the Swedish Prescribed Drug register [92], which was established in 2005. It has therefore only recently achieved an age where it begins to contribute meaningfully to drug-cancer studies. Another example is Medicare Part D in 2006, which has facilitated the use of Medicare data in the conduct of drug-cancer studies [93,94].

There has been limited methodological focus on long term outcomes. The reason for this field having received less priority compared to immediate outcomes is likely closely related to the data issue outlined above, and to the fact that immediate outcomes by many other regards are more approachable than long-term outcomes. Contrary to the issue with availability of suited data sources, which is a problem that is to a large extent self-correcting with time passing, the need for methodological development requires time and resources allocated to it. An example of a methodological topic that is currently debated is the use of the new user design [95] possibly coupled with an active comparator design. The new user active comparator design is increasingly heralded as the ‘gold standard’ in observational studies [96], mainly due to its ability to protect against the phenomenon of ‘depletion of susceptibles’ [97] and as use of an active comparator can control for unmeasured confounding [98]. However, the appropriateness of these design choices in the context of long-term outcomes such as cancer is not well established. As illustrated by our study on benzodiazepines and cancer, a new user design can be used when the suspicion of a carcinogenic effect is tied to shorter term exposure [I]. However, in studying the long-term risks of lithium exposure [III], restriction to new users would have severely impeded our ability to assess carcinogenic risks, due to lack of data, and further have provided limited reassurance to clinicians and patients, that were specifically concerned about risks associated with very long-term exposure [24,55]. When considering the wide range of questions that need to
be addressed, it seems important to maintain a methodologically pluralistic approach [99].

Other issues are more specific to the conduct of studies with cancer as an outcome. This includes the choice of an appropriate lag-time period. Ideally, this should be decided based on in-depth knowledge of both the biology of the specific tumor and the mechanism underlying the carcinogenic (or chemopreventive) effect of the drug in question. However, such knowledge will rarely, if ever, be available, and even if it were, no consensus exists on how to incorporate it into exposure ascertainment algorithms. Solid pharmacological reasoning is crucial to address these issues. A common epidemiological practice is to perform analyses based on observed patterns of exposure, e.g. analysis of cumulative use divided into tertiles of the observed use. While this provides some reassurance for the safety of a drug under the most common exposure patterns, this can, in the face of a heavily skewed use, result in analyses of drug amounts that are, from a pharmacological point of view, highly unlikely to infer an increased risk of cancer. As an example of this, the otherwise robust analysis by Loeb et al. [70] investigating the association between use of phosphodiesterase inhibitors and risk of melanoma applied a dose-response analysis where use of as little as 24 tablets categorized a user as being in the highest exposure strata. In the face of insufficient data to guide cutoffs for dose categories, pharmacological reasoning should be applied. As an example, a study of the potential association between use of prolactin-inducing antipsychotics and risk of breast cancer used an upper exposure strata corresponding to use of ≥3,000mg of olanzapine [100]. When doing a similar Danish study [101], we found that the vast majority of users of antipsychotics had used very limited amounts, with four in five users having a cumulative use lower than 5,000mg of olanzapine. However, based on pharmacological reasoning, we still applied a pre-defined main exposure criteria of ≥10,000mg of olanzapine and furthermore performed analysis with dose strata up to ≥50,000mg of olanzapine [101].

The definition of cancer outcomes constitutes another challenge. We have argued that the use of ‘all cancer’ as an outcome is not appropriate and that additional differentiation may also be necessary according to subtypes within specific organs such as lungs and ovaries with several distinct histological cancer subtypes [87]. However, in the future, this might be taken a step further, as increasing knowledge of molecular profiles of cancer will likely provide even more detailed classification of cancer outcomes [102,103].

Lastly, a challenge central to the conduct of drug-cancer studies is the lack of available data on core risk factors for cancer such as smoking [104], alcohol intake [105], and obesity [106]. While these are important risk factors for some cancers, it is important to remember that they are not universally and equally associated with all cancers, and, more importantly, they are not always associated with exposure to the drug under scrutiny,
which is a requisite for them to exert confounding effects in a given study. Nevertheless, drug users do often differ from non-users on these parameters, such as the higher prevalence of smoking among benzodiazepines users [38,39] and lithium users [56]. Consequently, tactics to handle such (unmeasured) confounding are often required in drug-cancer studies. Often, the potential confounding effect from smoking, alcohol, and obesity is simply debated in the discussion section of a paper, e.g. as in our study on use of benzodiazepines, were we discuss the likelihood of residual confounding, which ultimately led us to conclude that the marginally increased risk should be viewed as a null finding. However, it has been argued that we need to quantify more directly such potential confounding [107,108]. Moving towards that, we conducted a study of the potential chemopreventive effects of disulfiram [109], a drug that is used to treat alcohol abuse, and thus obviously riddled with the potential for residual confounding. In that study, we used survey data [110] to describe how long-term users compared to short-term users (the main comparison in our study) regarding smoking, alcohol consumption and BMI. Another approach was applied in our study of the association between statin use and risk of renal cell carcinoma [II], where we abstracted data on the prevalence of overweight [111] to quantify the potential for residual confounding from these factors, using the method described by Schneeweiss [107]. Taking it one step further, we have recently illustrated how the survey data can be utilized for so-called propensity score calibration [107,108] and thus be used for direct adjustment for lifestyle confounding in Danish case-control studies of cancer outcomes [44]. In our example, we showed an additional attenuation of the association between use of benzodiazepines and cancer risk upon adjustment for lifestyle confounding [44]. While the strategies discussed so far all applies to the Danish health registries, another approach is to utilize data sources in other countries. One example of this is our study of the association between use of phosphodiesterase inhibitors and risk of melanoma [VI]. The use of US data from Kaiser Permanente [91] not only allowed for comparison with a country with markedly different sun exposure patterns and inclusion of in situ melanomas, but also allowed analyses directly adjusted for health care utilization that illustrated the potential for bias stemming from health-seeking behavior. Another data source that can nicely complement the Danish data sources is the Clinical Practice Research Datalink [112], primarily based on general practice data, which was recently illustrated in our study of the association between use of the topical calcineurin inhibitors tacrolimus and pimecrolimus and risk of skin cancer [113]. While many different strategies to assess the potential for residual confounding from lifestyle factors are thus available, they remain underutilized in drug-cancer studies. A more rigorous assessment of this issue holds promises of more qualified conduct and interpretation of such studies in the future.
Screening studies

The screening study [VII] and the confirmatory studies of the association between hydrochlorothiazide use and risk of skin cancer [VIII-X], provides ‘proof of concept’ for hypothesis-free screening of secondary health care data as a mean to identify previously unrecognized drug-cancer associations. Much work, however, remains to be done to fully realize the potential of screening. This concerns refinement of even the most basic steps of screening, including finetuning of the criteria that is used to define signals that undergo further analyses and the development of new internal tests that is used to qualify signals before they undergo manual consideration [114]. The latter is important, as the high output of screening studies requires a significant amount of post-processing [115]. One approach to tackling the challenges associated with the post-processing of signals is to establish closer collaboration between screening initiatives for drug-cancer associations. One might either compare directly the output from the screening studies in Denmark [VII] and the US [91], to identify signals that are seen in both databases, that could then receive higher priority moving forward. One challenge with this approach would be the different drug utilization patterns between Denmark and the US, as risks associated with drugs that are only rarely used in one of the two countries cannot be assessed in this way. Another approach could be to establish a similar database in another similar country, e.g. within Scandinavia, that could then be used as a secondary data source, that is, that signals from the Danish screening study underwent further characterization in an external database, and only signals that were confirmed would receive further attention. Establishment of such a setup using Norwegian data sources is currently ongoing.

Another challenge in relation to screening studies that is still to some extent unresolved is the potential issues with re-using the same data sources for subsequent confirmatory studies [116]. The principal concern is that it is futile to use the same data to confirm a hypothesis as the data that the hypothesis was originally derived. While fully acknowledging this concern, there are other aspects that needs to be considered in this regard, which can be illustrated by the case of hydrochlorothiazide and skin cancer. First, we need to consider the very basic concept of clarity. The results obtained in screening studies are necessarily very crude, with limited and non-specific confounder adjustment and little output besides a single measure, e.g. an OR, and perhaps few additional details, such as a p-value obtained in dose-response analyses or the like. Regardless of concerns over re-use of data, there is no reason to rely on such crude measures when interesting signals appear. For this reason alone, potential signals should always be refined in tailored analysis, all the while keeping in mind that a refined signal does not constitute an independent confirmation. For the hydrochlorothiazide and skin cancer association, the tailored analyses not only provided much more detailed dose-response analyses, but also included similar analyses for other antihypertensive and diuretic drugs with similar
indications as hydrochlorothiazide. Second, the point estimate obtained in a screening study will rarely be the only aspect to consider when interpreting a potential safety issue. Rather, one should also consider prior knowledge, including results from previous studies as well as any potential biological mechanism. For the hydrochlorothiazide signal, the previous study by Friedman et al. [77], finding a similar association, and the established photosensitizing properties of hydrochlorothiazide [78] both provided strong support for the signal to constitute something worth investigating further. Third, the concept of ‘orthogonal predictions’ [117] needs to be considered. In brief, the idea of orthogonality here refers to other hypotheses that are (often strongly) biologically associated with the hypothesis under scrutiny but with little or no statistical association. If such orthogonal hypothesis can be devised and tested, they provide an opportunity to rise above the potential for fallacies created by statistical coincidences, although systematic confounding can still be at play. This concept is used in several analyses in the three follow-up studies on hydrochlorothiazide. The signal from the screening study was specifically attributed to the combination of hydrochlorothiazide and amiloride. The orthogonal hypothesis would thus be that a similar signal should be seen with hydrochlorothiazide use outside the combination with amiloride. As this was the case [VIII], it speaks strongly against the signal being a chance finding. Similarly, the initial signal was specific for squamous cell carcinoma of the lip, and another orthogonal hypothesis would thus be to see whether hydrochlorothiazide was also associated with an increased risk of squamous cell carcinoma of the skin at other parts of the body, which we confirmed in a subsequent study [IX]. Such considerations about reuse of data, which has recently been summarized and expanded upon [118], provide strong support for the conduct of tailored studies to follow up on signals generated in screening studies, even within the same database.

**Concluding remarks**

With my work concerning drug-cancer associations, including the ten analyses presented in this thesis, I have strived to improve upon our understanding of cancer as a potential side effect to drug treatment and of how this can be analyzed when using large-scale registry-based data. As argued above, there is still a considerable amount of work to be done in refining our methodological approaches, in addressing emerging hypotheses, in fully elucidating newly established associations, e.g. that of hydrochlorothiazide and skin cancers, and in disseminating our findings effectively. All of this, perhaps the latter in particular, is necessary to move towards the overarching aim of all pharmaco-epidemiological research: Ensuring the best, safest, and most rational use of drugs.
REFERENCES


APPENDIX: PAPERS I-X