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Fritz, Ildikó

2020

Document Version:
Publisher's PDF, also known as Version of record

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Epidemiological studies on the use of H1-antihistamines and survival in cancer

ILDIKÓ FRITZ
FACULTY OF MEDICINE | LUND UNIVERSITY

About the author

Ildikó Fritz is a molecular biologist with a licentiate degree in medical science and a cross-disciplinary background of studies that includes cognitive linguistics and cult fiction, as well as different areas (more or less) related to cancer biology. Ildikó finds writing about theirself in the third person odd, likes travel, creative writing and subverting the rule of three, and lives on the outskirts of Lund with their family. This doctoral dissertation is the result of their last three years of work at the Department of Cancer Epidemiology at Lund University.

Printed by Media-Tryck, Lund 2020 NORDIC SWAN ECOLABEL 3041 0903

LUND UNIVERSITY

FACULTY OF MEDICINE

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2020:87
ISBN 978-91-7619-949-7
ISSN 1652-8220

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Ildikó Fritz

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UNIVERSITY

DOCTORAL DISSERTATION

by due permission of the Faculty of Medicine, Lund University, Sweden.
To be defended at 1 pm on September 11th, 2020 in Segerfalksalen at BMC.

Faculty opponent
Olle Larkö

Organization: LUND UNIVERSITY	**Document name:** Doctoral dissertation
	Date of issue: September 11th, 2020
Author: Ildikó Fritz	**Sponsoring organization:** N/A

Title and subtitle:

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Key words: epidemiology; histamine H1 antagonists; histamine antagonists; chemotherapy; immunotherapy.

Classification system and/or index terms (if any): N/A

Supplementary bibliographical information: N/A		**Language:** English
ISSN 1652-8220		**ISBN** 978-91-7619-949-7
Recipient's notes	**Number of pages:** 65	**Price:** N/A
	Security classification: N/A	

Signature  Date 2020-08-10

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Front cover photo, of desloratadine tablets on a rock at Brännö, by Marcus Fritz.

Author photo on back cover by Hedvig Christensen.

Supervisor: Professor Håkan Olsson
Co-supervisors: Per Broberg and Philippe Wagner

Lund University, Faculty of Medicine Doctoral Dissertation Series 2020:87

ISBN 978-91-7619-949-7
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2020

For the two loves of my life, Gusti and Marcus.

"You start a question, and it's like starting a stone. You sit quietly on the top of a hill; and away the stone goes, starting others," *Mr Enfield, in* Strange Case of Dr Jekyll and Mr Hyde, *by Robert Louis Stevenson*

"I have never tried that before, so I think I should definitely be able to do that," *falsely attributed to Pippi Longstocking by Astrid Lindgren, actual source unknown*

"I'm sciencing as fast as I can!" – *Professor Farnsworth,* Futurama

Preface

As I write this in Copenhagen, where Swedes like me have just recently been allowed to enter, at the end of the serendipitous and far from straightforward road that led to this dissertation, the very air seems rife with symbolism for this former literature student. I shall endeavor to steer clear of the very lowest hanging fruits, despite the siren call for someone of my proclivities, and be content with acknowledging the strangeness of the times and the path that led here, and to note the enormous privilege inherent in an occupation where I can write a dissertation from the comfort and safety of (mostly) my own home during a global pandemic. My current situation, which is both stressful and privileged, is a (perhaps halting) metaphor for the duality this dissertation derives from: histamine has a dual role in cancer, and thus some antihistamines are associated with improved survival in several immunogenic cancers, and may have an application in cancer therapy.

Copenhagen, August 1st, 2020

Contents

List of papers

This dissertation is based on the following four papers, referred to in the text as Papers I-IV and included in full at the end.

I. **Fritz I**, Wagner P, Bottai M, Eriksson H, Ingvar C, Krakowski I, Nielsen K, Olsson H. Desloratadine and loratadine use associated with improved melanoma survival. *Allergy* 2020.

II. **Fritz I**, Wagner P, Broberg P, Einefors R, Olsson H. Desloratadine and loratadine stand out among common H_1-antihistamines for association with improved breast cancer survival. *Acta Oncologica* 2020.

III. **Fritz I**, Wagner P, Olsson H. Improved survival in tumors that respond to immune checkpoint therapy with use of H_1-antihistamines desloratadine and loratadine. *Submitted manuscript.*

IV. **Fritz I**, Wagner P, Olsson H. Quantifying the potential effect of desloratadine in cancer therapy. *Manuscript.*

Additional peer-reviewed papers not included in the dissertation

Fritz I, Olsson H. Lung cancer in young women in southern Sweden: a descriptive study. *Clinical Respiratory Journal* 2018.

Helgadottir H, Isaksson K, **Fritz I**, Ingvar C, Lapins J, Höiom V, Newton-Bishop J, Olsson H. Multiple primary melanoma incidence trends over five decades, a nation-wide population-based study. *Journal of the National Cancer Institute* 2020.

Abstract

Cancer often results from chronic inflammation, and anti-inflammatory medications are therefore potential candidates for drug repurposing for cancer therapy. H_2-antihistamines such as cimetidine have long been studied for their promise as cancer medications, but H_1-antihistamines have thus far not been studied widely for this purpose. We have previously shown an association with improved breast cancer survival for use of some H_1-antihistamines, and other studies have shown similar results for non-localized cancer, non-small cell lung cancer and ovarian cancer, while evidence is mounting that some H_1-antihistamines normally used to alleviate allergic reactions may also have anti-tumor effects. In the four studies that form the basis of this dissertation, we show that some of the most commonly used H_1-antihistamines in Sweden – desloratadine and loratadine – are associated with substantially improved survival for patients with melanoma (Study I), breast cancer (Study II) as well as several other immunogenic tumor types (Study III) and quantify the potential effect of a desloratadine intervention, showing that numerous lives may be spared should desloratadine be integrated into cancer therapy (Study IV). We suggest a desloratadine effect as the explanation of our findings, one that is likely immunological in nature, and call for randomized clinical trials of desloratadine as treatment of immunogenic cancers, and if effective, further studies to quantify and elucidate the mechanism.

Abbreviations

AML	**acute myeloid leukemia**
CMM	**cutaneous malignant melanoma**
CNS	**central nervous system**
CTCL	**cutaneous T cell lymphoma**
CTLA-4	**cytotoxic T-lymphocyte-associated antigen 4**
DDD	**defined daily dose**
ER	**estrogen receptor**
IgE	**immunoglobulin class E**
MCL	**mantle cell lymphoma**
NHL	**non-Hodgkin lymphoma**
NSCLC	**non-small cell lung cancer**
PD-1	**programmed cell death protein 1**
PDR	**the Swedish Prescribed Drug Register**
SCR	**the Swedish Cancer Register**
STAT3	**signal transducer and activator of transcription 3**
SweMR	**the Swedish Melanoma Register**
Th	**T helper**
Th1	**type 1 T helper**
Th2	**type 2 T helper**

Introduction

Drug repurposing for cancer therapy

Cancer is the second leading cause of death globally, with annual cancer deaths nearing 10 million (1). The leading cause of cancer death is lung cancer, followed by colorectal, gastric, liver, breast, esophagus and pancreatic cancer (1). Cancer therapy options can be severely limited depending on tumor type or subtype, and there is always a need for new and improved anti-cancer medication, especially for malignancies with dismal prognoses like pancreatic cancer (2). Repurposing of existing medication is a way to meet that need in a both time- and cost-effective manner (3, 4), and due to the similarities between the inflammatory and tumorigenic processes, anti-inflammatory medications are a promising place to start the search for cancer therapeutics among existing drugs (5).

Cancer and inflammation

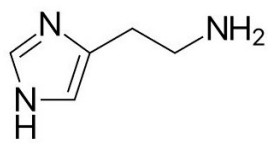
A possible link between cancer and inflammation was first shown in 1863, and since then, the similarities between the tumorigenic and inflammatory processes have been extensively mapped (6-8). Chronic inflammation, like cancer, is characterized by factors such as increased angiogenesis and inhibition of apoptosis, making the inflammatory microenvironment a tumorigenic microenvironment, favorable for the development of several types of tumors (7-9). Indeed, several inflammatory states, such as hepatitis, pancreatitis and inflammatory bowel disease, caused by infections, exposures and autoimmune diseases, have been shown to cause cancers in various organs (7, 10, 11). Both chronic inflammation and cancer are also associated with exhausted T cells (12).

Hanahan and Coussens describe “both classic inflammation and more subtle involvement of immune cells in the tumor microenvironment” (9) and Dvorak has characterized tumors as “wounds that do not heal”, due to the similarities between tumor development and the wound-healing inflammatory response (6). However, while some have raised the question of inflammation as an immune response to tumor presence by the host, most of the evidence seems to be in favor of inflammation as a

consequence of oncogenetic mutations, and a process needed for tumor progression (10, 13). While the link between chronic inflammation and cancer is relatively straightforward, the role of specific mediators involved in the inflammatory response, like histamine, in tumorigenesis and cancer progression is anything but (14).

Histamine and its dual role in cancer

Histamine (Figure 1), an endogenous biogenic amine, was discovered more than one hundred years ago, and has been known to be involved in allergy and the inflammatory response for almost as long (15, 16). Histamine is synthesized from histidine by the enzyme histidine decarboxylase and stored in cytoplasmic granules in mainly mast cells and released when the cells are degranulated. Histamine can also be synthesized de novo at inflammatory sites by various cells, without being stored in granules. Histamine is present in all mammalian tissues, and found in high concentrations in the skin, connective tissue, lungs and much of the gastrointestinal tract. It acts as a neurotransmitter in the nervous system, as well as a local mediator in the skin, gut and immune system, by binding to four known types of receptors, histamine receptors H_1, H_2, H_3 and H_4, all of which are G-protein coupled receptors. (6, 7, 15-19) The histamine receptors, like some other members of the G-protein coupled receptor superfamily, are all constitutively active, able to activate intracellular targets even in the absence of histamine (20, 21). Histamine is released in large quantities by mast cells during allergic reactions, and causes smooth muscle constriction, vasodilation, endothelial permeability and sensory nerve stimulation by activation of histamine receptors (6, 7). Hanahan and Coussens describe histamine as a potent vascular mediator and mitogenic growth mediator (9).



HISTAMINE

Figure 1. Molecular structure of histamine.

(From a file in the public domain available from Wikimedia Commons at https://commons.wikimedia.org, accessed July 30, 2020 and attributed to Vaccinationist.)

Histamine and all of its receptors have been implicated in different aspects of tumor diseases, with histamine shown to stimulate tumor proliferation through H_1-receptors, promote tumor growth by negatively modulating immune cells through H_2-receptors, while also influencing the immune response against cancer and having an antiproliferative effect on cancer cells, having even been administered in clinical trials as combination cancer therapy. The H_1-receptor gene has been shown to be overexpressed in some tumor types and subtypes and associated with mainly – but not only – poor survival. This suggests that histamine and its receptors have dual roles in cancer, depending on histamine dose as well as tumor type. (7, 9, 14, 18, 22-37)

Antihistamines

Antihistamines are antagonists and/or inverse agonists of histamine receptors and are commonly used to treat allergic reactions and conditions such as allergic rhinitis, urticaria and motion sickness. Antihistamines are similar in structure to histamine (Figure 1 and Figure 2) and are categorized according to the histamine receptor type they bind to as H_1, H_2, H_3 or H_4. The antihistamines commonly used as allergy medications are all antagonists and/or inverse agonists of H_1-receptors, and here, unless specifically stated that $H_{2\text{-}4}$-antihistamines are being referred to or included, the term antihistamine can be taken to refer only to H_1-antihistamines.

H_1-antihistamines are classified as first- or second-generation: the first-generation, or classic, antihistamines have been in use since the 1940s, and as they readily cross the blood-brain barrier and bind to non-histamine receptors, they cause sedation and have a number of other side effects. Second-generation antihistamines were developed in the 1980s, and due to a higher specificity for H_1-receptors, a lower affinity for the H_1-receptors in the brain and non-histamine receptors, and a longer half-life, they have both fewer side effects such as sedation and need not be administered as often as the first-generation drugs. Second-generation antihistamines are also generally free from interactions with other drugs. (7, 16, 38)

Antihistamines include many different types of drugs, mostly amines. In this dissertation the six most common H_1-antihistamines used in our study populations of Swedish cancer patients are studied, namely cetirizine, clemastine, desloratadine, ebastine, fexofenadine and loratadine (Figure 2). Among those, clemastine is the only first-generation drug, and the only ethanolamine. Cetirizine is a piperazine, and the other four are all piperidines, together with other drugs such as astemizole and terfenadine. Desloratadine and fexofenadine are sometimes classified as third-generation antihistamines, as these compounds are active metabolites of the second-generation antihistamines loratadine and terfenadine respectively (7, 38).

Antihistamines inhibit the action of histamine by blocking the histamine receptors, or by inhibiting the activity of the enzyme histidine decarboxylase needed for histamine synthesis. Anti-inflammatory effects have also been shown for several H_1-antihistamines, particularly third- and second-generation drugs like desloratadine, fexofenadine and loratadine (38), thought to depend on the strong inverse agonism of these compounds, inhibiting even the basal signaling of the H_1-receptor (21, 38, 39). Desloratadine is the most potent antagonist and inverse agonist of the H_1-receptor among common antihistamines (38-40). Antihistamines desloratadine and ketotifen can also stabilize mast cell membranes, thereby preventing degranulation and the release of histamine from mast cells (7, 41, 42).

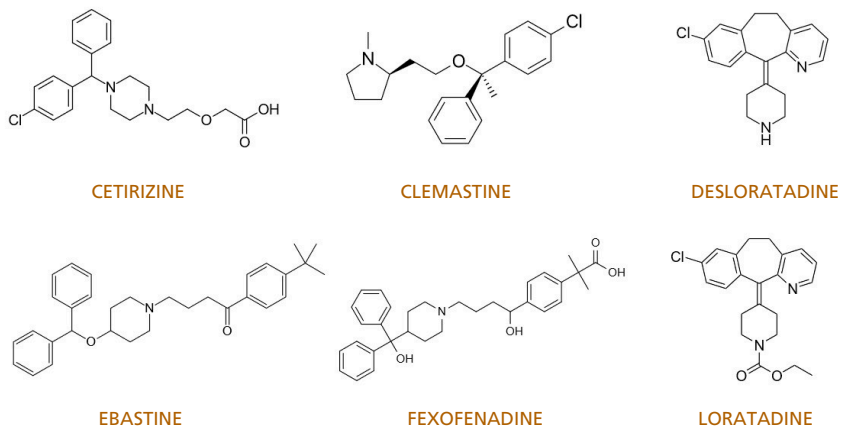


Figure 2. Molecular structure of common H_1-antihistamines. Molecular structures for antihistamines cetirizine, clemastine, desloratadine, ebastine, fexofenadine and loratadine. (Figure curated by the author from files in the public domain available from Wikimedia Commons at https://commons.wikimedia.org, accessed July 20, 2020 and attributed to Fvasconcellos, Fuse809, Harbin, JaGa and User:Mysid.)

Mast cells – Jekyll and Hyde in cancer

Mast cells are long-lived secretory leukocytes that were first described by Ehrlich in 1878, who also noted both their presence and particular abundance in tumors (7, 43). Mast cells are classified as "professional" histamine producers, producing large quantities of histamine that can be rapidly released (43). They derive from hematopoietic stem cells in the bone marrow, which give rise to mast cell progenitors that migrate into different tissues through the blood circulation, where they mature

and differentiate into granulated cells (7, 43). Granulated mast cells are degranulated after activating contact with antigens, releasing a vast array of immunomodulatory mediators including histamine, serotonin and vascular endothelial growth factor (43). Mast cells are among the first immune cells recruited to solid tumors, where several factors in the tumor microenvironment may activate their degranulation (7). Eissmann et al. have shown that the degranulation of mast cells leads to growth of gastric tumors (44), although Hayes et al. have suggested that basophils, another type of histamine-producing cells, may be involved, having shown that the release of histamine from basophils drives tumor growth in epithelial cells (45).

Mast cells have been shown to be associated with angiogenesis, tumor proliferation, immunosuppression and metastasis – suggesting theirs is a tumorigenic role – as well as with tumor and metastasis inhibition, immune cell recruitment and tumor cell disruption – suggesting mast cells have anti-tumor effects (7, 8, 19, 35, 36, 43, 44, 46-48). Mast cells in tumors are associated with poor as well as favorable prognoses in different studies, reflecting their dual involvement in variously pro- and anti-tumor responses (7, 47, 49-52). To signify this dual nature, authors Theoharides and Conti have dubbed mast cells "the Jekyll and Hyde of tumor growth"(36).

Tumor immunogenicity and immune checkpoints

As with mast cells, and many concepts within immunology, Ehrlich was the first to hypothesize the immune system's ability to repress cancer growth. This concept has now been defined as tumor immunogenicity – the ability of a tumor to induce an immune response that can prevent its growth. (53) Successful tumors are thought to arise through immunoediting, meaning the selection of clones that can evade the immune system, and tumor cells adopt a variety of responses to avoid immune detection (12, 53). Immune checkpoints – inhibitory receptors crucial in protecting against autoimmunity and tissue damage from excessive immune responses – provide tumors with immune resistance mechanisms, and by the blockade of immune checkpoints, the anti-tumor immune responses can function. Important immune checkpoint receptors are cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1). Both of these receptors are known to be upregulated by exhausted T cells in chronic inflammation and cancer, and both inhibit T cell activity. (12, 54, 55) This is utilized in cancer immunotherapy, where activation of tumor immunity is often done by immune checkpoint inhibition (54). The first immune checkpoint inhibitor to be approved for treatment in cancer was the anti-CTLA-4 antibody ipilimumab, which has been proven to confer a survival benefit to patients with melanoma (12, 54). It has since been followed by anti-PD-1 and other immunotherapies, with these and many more in clinical trials for treatment of various immunogenic cancers (55-63).

T helper cells in cancer and allergy

Important in both the inflammatory and immune responses are the T helper (Th) cells, lymphocytes characterized by the surface expression of CD4, that have received their name as they aid B cells in antibody production (64). A major function of Th cells is the secretion of a vast array of cytokines that mediate inflammatory and immune responses (54, 65). Th cells develop into different subtypes, with the major ones being type 1 Th (Th1) cells and type 2 Th (Th2) cells that release different cytokines, and thereby regulate different types of immune responses. The Th1-response is important for the defense against intracellular pathogens and for the cytolytic response and can lead to chronic inflammation and autoimmunity, whereas the Th2-response is important for the defense against extracellular pathogens and for IgE secretion, and can lead to allergic conditions (64, 66).

Histamine has been shown to affect the Th1/Th2 balance, skewing toward a Th2-response by turning dendritic cells into Th2-promoters (67), upregulating Th2-attractors, downregulating Th1-effectors (68) and disrupting the cytolytic response, thereby diminishing anti-tumor immunity (69). Myeloid-derived suppressor cells, also involved in the Th2-skewing immune response, are promoted by histamine, and interactions between mast cells and myeloid-derived suppressor cells can "serve as a bridge between allergic inflammation and tumorigenic host responses" according to Martin et al. (69). Tosolini et al. analyzed Th cell subpopulations in colorectal cancer and found that patients with a high Th1-response expression had a longer disease-free survival, whereas the Th2-response expression was not associated with any prognosis prediction (70).

Allergy and cancer

Unsurprisingly, the link between cancer and allergy is also complex and contradictory. Studies have shown variously increased, decreased or unaltered risks of developing different cancers for those with allergy and atopy. Overall, allergic conditions do not seem to protect against cancer. (71-89) Allergic conditions may protect against tumors through increased immunosurveillance as a result of a heightened immune response and through prophylaxis, as well as be detrimental by causing chronic persistence of inflammatory cells such as mast cells that create a beneficial milieu *for* tumors and by skewing the Th-response. The risk of developing a tumor depends on both the type of allergic condition and the type of tumor in question. (74, 76, 79, 80, 82, 83, 86-88) Some have raised questions of reverse causality with regard to the decreased risk of certain cancers associated with allergic conditions, for example non-Hodgkin

lymphoma and glioma, where immunosuppression resulting from the cancer may in fact be responsible for the allergic response reported (71, 83, 86).

The picture is even more complex when it comes to cancer prognosis and allergy: in their case-control study on atopy and melanoma risk and prognosis, Marasigan et al. found that while there was an inverse association between atopy and melanoma risk, there was no difference in progression or survival among melanoma patients with or without atopy, suggesting that the heightened immunosurveillance in those with atopy may protect against tumor development, without conferring any survival benefit for those that do develop tumors (82). Other studies have found associations with decreased progression and improved cancer survival for individuals with allergic conditions and markers, and still others show no survival difference (88, 90-98). As with cancer risk, progression and survival depend on the tumor type, organ and the allergic condition(s) in question (88, 94-96).

Further clouding the picture is the widespread use of medications, such as antihistamines, to alleviate symptoms of allergic conditions, that may confound the association of allergies with cancer risks and prognoses, and many studies even define allergic conditions among participants by proxy through use of medications such as antihistamines (76, 86, 99).

Antihistamines and cancer risk

Prompted in part by reports on increased cancer risk associated with the use of antidepressants (as antihistamines are structurally similar to antidepressants), as well as the findings in an early study on antihistamines (100) that could not be reproduced (101), where certain antihistamines were found to promote tumor growth, several epidemiological studies were done to determine whether antihistamine use was associated with increased cancer risk, recurrence or second primary tumors (102-104). Neither study on breast cancer found any association with increased breast cancer risk for antihistamine users (102, 103), and no association with recurrence or second primary tumors for antihistamine users with breast cancer, colon cancer or melanoma were found (104). A study from 1989 on cancer risk and prescription drug use found a significantly decreased breast cancer risk for users of the antihistamine hydroxyzine and a significantly decreased overall cancer risk, as well as decreased lung and pancreatic cancer risks, for users of meclizine, but increased risks of some cancers were also found for those and other antihistamines in the same study (105). More recently, associations with increased risk for both non-Hodgkin lymphoma and glioma with long-term antihistamine use have been found (106-108), while a 2019 study on antihistamine use and ovarian cancer incidence showed no altered risk of ovarian cancer for antihistamine users (109).

H_1-antihistamines as cancer therapy

Even though it has long been known that tumors maintain an inflammatory microenvironment, this knowledge has only been applied relatively recently in finding and developing anti-inflammatory agents with potential in cancer therapy, though antihistamines as anti-tumor medications have been suggested as early as the 1970s (110, 111). Among anti-inflammatory medications, antihistamines are particularly good candidates, and H_2-antihistamines, cimetidine chief among them, have shown a great deal of promise as adjuvant drugs for the treatment of several gastrointestinal cancers, with a Cochrane review calling for large-scale trials of cimetidine as a colorectal cancer treatment (112).

Despite this, H_1-antihistamines have not been studied in relation to cancer therapy to nearly the same degree, though they are among the most ubiquitously used medications globally, and are increasingly sophisticated, affordable and safe. Most importantly, they have much lower toxicity than conventional chemotherapeutics, and taken together, these properties make H_1-antihistamines ideal candidates in the search for novel cancer therapeutics. In a review published in 2017, Faustino-Rocha et al. argue that given the low toxicity of antihistamines compared with chemotherapeutics, "the study of their use as adjuvants for conventional therapy is warranted" (7), while Massari et al. point to the fact that as both histamine and antihistamines are approved for human use, "the gap between experimental work and potential clinical application" is reduced (18).

Early *in vitro* and *in vivo* studies on H_1-antihistamines in relation to cancer were inconclusive, with some authors variously presenting no effects on tumors (100, 113), tumor growth promotion and decreased survival (22, 23, 100) and anti-tumor effects (111, 114, 115) of the – nearly only first-generation – H_1-antihistamines studied. Since then, evidence that certain H_1-antihistamines may have anti-tumor effects that could be utilized in the management of several tumor diseases has been mounting, with *in silico*, *in vitro* and *in vivo* studies showing promising results, particularly regarding astemizole (116-130), loratadine (131-135) and terfenadine (32, 126, 127, 131, 134, 136-138) as well as cetirizine (45), clemastine (139), cinnarizine (140, 141), cyproheptadine (142-145), desloratadine (139), ketotifen (146), meclizine (147) and 2-(3-fluorophenyl)histamine (33). These studies have found that antihistamines have selective, dose-dependent cytotoxicity against tumor cells, and can induce apoptosis and inhibit tumor proliferation, growth and migration. A recent study, published this year, shows anti-tumor effects of desloratadine on bladder cancer cells *in vitro* (148), and another study published online just this July shows *in vivo* effects of combination treatment with loratadine for colorectal cancer (149).

When our research group first reported an association with improved survival with use of common H_1-antihistamines among Swedish women with breast cancer in 2015

(150), to the best of our knowledge it was the first finding of the sort, as previous studies in the field have utilized cell lines and animal models. Recently, two Danish studies with somewhat similar approaches to ours have also shown an association with improved survival with use of common H_1-antihistamines in non-small cell lung cancer and non-localized cancers (151) as well as in ovarian cancer (152).

See Table 1 for some of the accumulated evidence for anti-tumor effects of H_1-antihistamines known to date.

Table 1. Evidence of anti-tumor potential of H_1-antihistamines

Antihistamine	Cancer type/ tumorigenic process	Type of study	Reference	Year
Astemizole	Angiogenesis	*In vivo*	Downie et al.(121)	2008
		In vitro	Lyu et al.(128)	2018
	Breast	*In vitro*	Ouadid-Ahidouch et al.(129)	2004
		In vitro	Roy et al.(130)	2008
		In vitro	García-Becerra et al.(122)	2010
		In vitro	García-Quiroz et al.(123)	2012
		In vivo	García-Quiroz et al.(124)	2014
		In vitro	García-Quiroz et al.(125)	2019
	Cervical	*In vitro*	Díaz et al.(120)	2009
		In vitro	Chávez-López et al.(117)	2014
	Leukemia (AML)	*In silico + in vitro*	Laverdière et al.(127)	2018
	Liver	*In vitro*	Chávez-López et al.(118)	2015
	Lung	*In vitro*	Chávez-López et al.(119)	2017
	Lung (NSCLC) + non-localized	Register + *in vitro*	Ellegaard et al.(151)	2016
	Melanoma	*In vitro*	Jangi et al.(126)	2006
	Ovarian	Register + *in vitro*	Verdoodt et al.(152)	2020
	Prostate	*In vitro*	Bernal-Ramos et al.(116)	2017
Cetirizine	Epithelial	*In vivo*	Hayes et al.(45)	2020
Cinnarizine	Lymphoma + multiple myeloma	*In vitro*	Schmeel et al.(141)	2015
	Uveal melanoma	*In silico*	Fagone et al.(140)	2017
Clemastine	Lymphoma (CTCL)	*In vitro*	Döbbeling et al.(139)	2013
Cyproheptadine	Leukemia + multiple myeloma	*In vivo*	Mao et al.(144)	2008
	Liver	*In vitro*	Feng et al.(142)	2015
	Lymphoma (MCL)	*In vitro*	Paoluzzi et al.(145)	2009
	Multiple myeloma	*In vitro*	Li et al.(143)	2013
	Ovarian	Register + *in vitro*	Verdoodt et al.(152)	2020
Desloratadine	Bladder	*In vitro*	Ma et al.(148)	2020
	Breast	Register	Olsson et al.(150)	2015
	Lymphoma (CTCL)	*In vitro*	Döbbeling et al.(139)	2013
	Ovarian	Register + *in vitro*	Verdoodt et al.(152)	2020
Ebastine	Breast	Register	Olsson et al.(150)	2015
	Lung (NSCLC) + non-localized	Register + *in vitro*	Ellegaard et al.(151)	2016
	Ovarian	Register + *in vitro*	Verdoodt et al.(152)	2020
Fexofenadine	Breast	Register	Olsson et al.(150)	2015

Table 1. *Continued*

Antihistamine	Cancer type/ tumorigenic process	Type of study	Reference	Year
Ketotifen	Breast + fibrosarcoma	*In vitro*	Kim et al.(146)	2014
Loratadine	Colorectal	*In vitro* + *in vivo*	Chen et al.(131)	2006
	Colorectal + gastric	*In vitro* + *in vivo*	Chen et al.(132)	2017
	Colorectal	*In vivo*	Lin et al.(149)	2020
	Colorectal + prostate + glioblastoma	*In vitro*	Soule et al.(135)	2010
	Lung (NSCLC) + non-localized	Register + *in vitro*	Ellegaard et al.(151)	2016
	Mast cell	*In vitro*	Hadzijusufovic et al.(134)	2010
	Ovarian	Register + *in vitro*	Verdoodt et al.(152)	2020
	Pancreatic	*In vitro*	Desai et al.(133)	2019
Meclizine	Colorectal	*In vitro*	Lin et al.(147)	2007
Terfenadine	Colorectal	*In vitro*	Chen et al.(131)	2006
	Leukemia (AML)	*In silico* + *in vitro*	Laverdière et al.(127)	2018
	Liver	*In vitro*	Lampiasi et al.(136)	2007
	Lung + pancreatic	*In vitro*	Varbanov et al.(138)	2019
	Mast cell	*In vitro*	Hadzijusufovic et al.(134)	2010
	Melanoma	*In vitro*	Jangi et al.(126)	2006
		In vitro	Jangi et al.(32)	2008
		In vitro	Nicolau-Galmés et al.(137)	2011
	Ovarian	Register + *in vitro*	Verdoodt et al.(152)	2020

However, among the H_1-antihistamines showing promise as new cancer treatment, both astemizole and terfenadine have been withdrawn due to their cardiotoxicity (4), and cinnarizine, while still available, has several side effects, some quite severe, including drug-induced parkinsonism (153), which rather limit their suitability, compared with other, commonly used and safer antihistamines. As Izumi-Nakaseko et al. note, having evaluated the proarrhythmic potential of astemizole to ascertain its suitability for cancer therapy, the proarrhythmic dose of astemizole is lower than the anti-cancer dose (154).

The mechanism(s) behind the anti-cancer effects and associations shown for H_1-antihistamines are largely unknown, though it is clear that whatever anti-tumor effects certain H_1-antihistamines may exert, these are not solely dependent on H_1-receptor inhibition, as some studies have shown this experimentally (127, 137), and not all H_1-antihistamines were associated with improved survival in register-based studies (150-152).

Aims and approach in Studies I-IV

Contrary to many of the existing studies on antihistamines with a potential in cancer therapy, focused in large part on the withdrawn medications astemizole and terfenadine, or uncommon antihistamines, our focus has instead been on six H_1-antihistamines commonly used in Sweden: cetirizine, clemastine, desloratadine, ebastine, fexofenadine and loratadine (Figure 2). All, bar clemastine, are second- or third-generation drugs, with a high selectivity and affinity for the H_1-receptor, little to no CNS effects or interactions with other drugs. Desloratadine has the highest H_1-receptor affinity, anti-inflammatory properties, and no reported interaction with cardiac potassium channels, and is approved for use in both adults and children. (38)

Our hypothesis was that there may be a potential role for some, or all, of these antihistamines in cancer therapy, and for this purpose, we set out to study the mortality of antihistamine users and non-users among patients with different cancers. Initially, we focused on breast cancer, then in our first study (reported on in Paper I), we focused on melanoma. Our second study (reported on in Paper II) was again focused on breast cancer, and in our third study (reported on in Paper III), we compared several immunogenic and non-immunogenic cancer types in a meta-analysis, trying to establish whether the potential effect may be immunological. In our fourth study (reported on in Paper IV), we try to quantify the potential effect of desloratadine as cancer therapy for immunogenic cancer types. See Figure 3 for an overview of the studies and papers included in this dissertation.

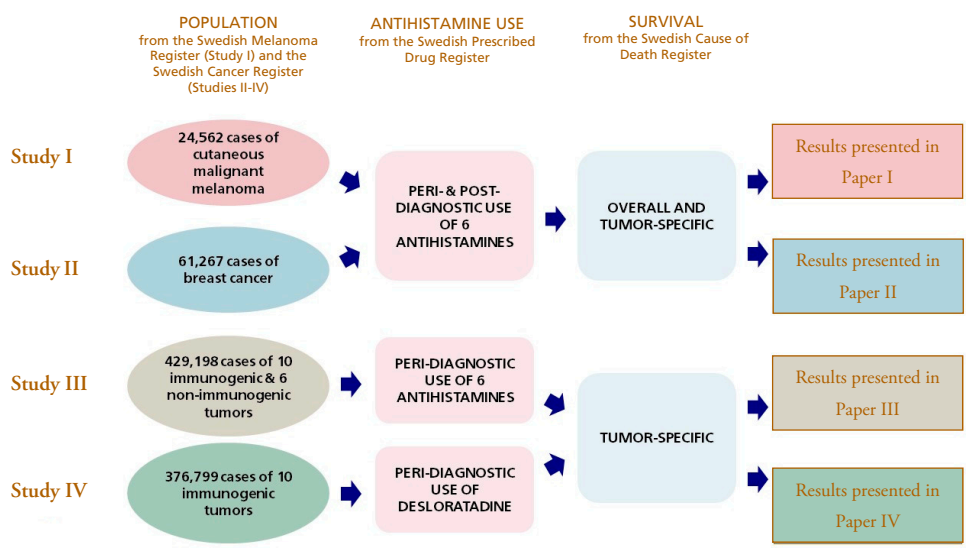


Figure 3. Overview of the studies and papers included in the dissertation. Types of tumors, antihistamine use and mortality analyzed in Studies I-IV presented in Papers I-IV.

Methodology

As shown in Figure 3 above, the studies included in this dissertation and reported on in Papers I-IV are all similarly structured, with a population selected based on cancer diagnoses, and data on antihistamine use and deaths for that population added and analyzed using Cox regression. All four studies included here are register studies. The methodology used for each study is more extensively described in the corresponding paper at the end of this dissertation, and what follows below is an overarching description of the materials and methods used.

Registers and data

In Studies I-IV we have utilized some of the excellent health care registers here in Sweden, namely the Swedish Cancer Register, hereafter referred to as SCR; the Swedish Cause of Death Register; the Swedish Prescribed Drug Register, hereafter referred to as PDR; and the Swedish Melanoma Register, hereafter referred to as SweMR. We also used data on education level from The Longitudinal Integrated Database for Health Insurance and Labor Market Studies, or LISA, where data from a register of the education of the Swedish population is available. See Table 2 for an overview of the registers used.

Table 2. Registers included in Studies I-IV

Name	Administered by	Includes	Type	Data since	Completeness	Used in
The Swedish Melanoma Register	Regional Cancer Center Southeast Sweden	Cases of cutaneous malignant melanoma diagnosed in Sweden	Quality register	1990	~99%	Study I
The Swedish Cancer Register	The National Board of Health and Welfare	Cases of cancer diagnosed in Sweden	Mandatory	1958	96%(155)	Studies II, III & IV
The Swedish Prescribed Drug Register	The National Board of Health and Welfare	Dispensed prescribed pharmaceuticals in Sweden	Mandatory	July 1st 2005	~100% for most variables	Studies I-IV
The Swedish Cause of Death Register	The National Board of Health and Welfare	Causes of death for the deceased in Sweden	Mandatory	1961	98-99%	Studies I-IV
The Longitudinal Integrated Database for Health Insurance and Labor Market Studies	Statistics Sweden	Socioeconomic data on all adolescents and adults in Sweden	Database with data from 11 registers	1990	99.3% for data on education level	Study I

All these registers have comprehensive coverage of the entire population, and certain inevitable loss of data notwithstanding, in theory, all cancer cases are recorded in the SCR, and all cutaneous malignant melanomas in SweMR, all dispensed prescriptions are noted in the PDR, and all deaths and their causes are recorded in the Swedish Cause of Death Register. The registers can all be linked through the Swedish personal identity numbers assigned to all individuals entered in the Swedish Population Register, and this was done at The National Board of Health and Welfare, where data was pseudo-anonymized (a key being kept for a limited time by The National Board of Health and Welfare) before being delivered.

Definitions, classifications and variables

As some of the data regarding individuals in these registers derives from the Swedish Population Register, where only data on an individual's legal gender exists, this has been used in our studies a proxy for sex to adjust for sex-specific differences in our survival analyses. Throughout this dissertation and the included papers, therefore, the terms "woman" and "man" refer to individuals whose legal gender is female and male respectively.[1]

We have defined antihistamine use in two ways in our studies; peri-diagnostic use and post-diagnostic use (see the "Statistical analyses" section below for a more precise description of these definitions). Pre-diagnostic antihistamine use has not been considered here, although for some antihistamine users who received their diagnoses toward the end of the study periods, such data was available. However, considering the high average age of a tumor at diagnosis (median latency time for a breast tumor has been approximated at 22 years (156)), we have little to no data on pre-tumor antihistamine use for most individuals, and the data we have therefore does not allow for such analyses. Throughout all studies, only use of the six most common H_1-

[1]A note on sex and gender: in Swedish, the word "kön" means both sex and gender, and the two meanings are commonly conflated because of this lack of linguistic differentiation. In registers, the variable "kön" denotes legal gender, which is here used as a proxy for sex assigned at birth, as there is a considerable overlap. Currently, only two options exist in Sweden for "kön" legally, meaning that this variable is to be seen as a rough proxy, as it neither allows for intersex individuals or for non-binary people to be recognized for who they are, and individuals under the broader trans umbrella may have their "kön" in registers correspond to their assigned sex at birth or their legal gender. First and foremost, this results in a human rights issue for the numerous individuals in the LGBTQIA+ community whose identities are not recognized. It also creates a conundrum for those of us using register data for research purposes, as what we may want to know about the individuals in our study populations may be variously related to their physiological or anatomical characteristics such as presence or absence of certain organs or bodily features, gender identity or assigned sex at birth, and what we have is a single, binary variable.

antihistamines used in our patient population is considered antihistamine use, as the low number of users of other H_1-antihistamines precluded analyses of these.

In Study III, we define immunogenicity of tumors based on known responses to immune checkpoint therapy. While some of the classifications we have made are based on weaker evidence, namely regarding pancreatic and prostate cancer (56, 58, 61, 157), there is ample evidence of response to immune checkpoint therapy for the rest of the tumor types we have classified as immunogenic: bladder, breast, colorectal, gastric, kidney and lung cancer, Hodgkin lymphoma and melanoma (57, 60, 62, 63). Based on preclinical studies, it may be argued that ovarian cancer is immunogenic, however, as patients with ovarian cancer have failed to show response to immune checkpoint therapy in clinical trials (59), we have chosen to classify ovarian cancer as non-immunogenic. The low heterogeneity measure for desloratadine supports our groupings.

Statistical analyses

We used Cox regression analyses in all four studies to analyze antihistamine use and mortality, with time-to-event (death, or censoring due to migration or end of study) as outcome (158, 159). Cox regression, or the Cox proportional hazards model, is commonly used in survival analysis, as it allows for the assessment of associations between several covariates and survival (158, 160).

In Studies I and II, we analyzed both peri- and post-diagnostic antihistamine use, and both overall and tumor-specific mortality, whereas only tumor-specific mortality and peri-diagnostic antihistamine use were analyzed in Studies III and IV.

Peri-diagnostic use is here defined as dispensed prescriptions of any of the six studied antihistamines in the year surrounding the cancer diagnosis in question (six months pre-diagnosis to six months post-diagnosis). The small proportion of individuals who used more than one of the six antihistamines during the time in question were defined as users of the antihistamine for which they had the most dispensed defined daily doses (DDDs).

Post-diagnostic antihistamine use was defined in a cumulative manner, as the total number of dispensed DDDs of any of the six antihistamines from diagnosis until a specific time during follow-up. Time-varying covariates were used for post-diagnostic analyses (158, 159), allowing for the cumulative use to change over time as antihistamine use continues, and for individuals to provide data as both non-users and users, as well as concomitant use of several antihistamines. Cumulative DDDs were analyzed by log transformation. In Study II, the post-diagnostic analysis was extended to include lag from zero to five years in six-month intervals for four of the studied

antihistamines. The lag was added in order to evaluate whether any potential effects of antihistamines were delayed in time, and to exclude any end-of-life alterations in use due to poor health or other reasons (161).

Analyses were stratified for patient age in Study II, for patient age and gender in Study I and Study III (although some analyses were crude), and for patient age, gender and calendar year of diagnosis in Study IV. Statistical analyses were performed using R (162), and P-values less than 0.05 were considered statistically significant.

Methodological advantages and limitations

There are, as always, both advantages and limitations associated with our chosen data and methods. Some limitations we can adjust or compensate for, and some we cannot.

While register-based research is not without its drawbacks, the main advantage in each of our studies is that, since we used nation-wide population registers, our target population overlaps our study population to the highest possible degree, leading to great generalizability of results. Another advantage in our studies is that by the very nature of our study designs, the antihistamines we evaluate for possible clinical potential are already in use in the patient populations in question.

A major limitation is our inability to adjust for the underlying indications for which the antihistamines are prescribed, to exclude any confounding by indication. However, with the possible exception of clemastine, which can be prescribed to counteract side effects of chemotherapy (151), there is no known association with being prescribed a certain antihistamine for a certain indication that may be connected to cancer mortality. Other studies have shown no overall association with altered cancer prognosis for those with allergic conditions (see "Allergy and cancer" above), and as discussed above, some studies have even classified allergic conditions by antihistamine use (82, 88, 90-98). While the inability to adjust for the indications in question is a limitation in our studies, we do not believe that we have any significant confounding by indication, as there seem to be both increased and decreased risks associated with the indications, use of different antihistamines is not associated with uniform risks of cancer and survival (150-152), and, importantly, we do not have a single common indication for all antihistamine users in our studies. The *in vitro* studies on primary cells and cancer cell lines showing anti-tumor effects of certain antihistamines listed in Table 1 also support the hypothesis that there may be true associations between antihistamine use and cancer survival.

As discussed in the note on sex and gender above, a limitation in register-based studies such as these is that only the binary variable for legal gender is available. While trans

and intersex individuals are not estimated to make up a large proportion of the population (163, 164), this nevertheless creates unnecessary misclassifications.

As data on prescription-free drug use at the individual level is not recorded in Swedish registers, we cannot consider the use of these antihistamines without prescriptions. Three of the studied antihistamines (cetirizine, ebastine and loratadine) have been available without a prescription throughout our study periods, which precludes us from being able to appreciate the full exposure in the studied populations with regard to those three drugs. Fexofenadine was made available without a prescription in Sweden in 2011, and desloratadine in 2014, while clemastine is not available prescription-free. Therefore the full or nearly full exposure of the studied populations to those three antihistamines can be appreciated in these studies.

To evaluate potential outcome misclassification due to under-recording of tumor-related deaths, we included analyses of both tumor-specific and overall deaths in Studies I & II, as while there is no reason to doubt that a death that has been noted as such in the Swedish Cause of Death Register is in fact tumor-related, autopsies are not routinely performed in Sweden, and the true number of tumor-specific deaths may be higher than what is known. Having seen that our results did not alter to any significant degree due to potential outcome misclassification, we analyzed only tumor-specific deaths in the subsequent two studies.

Another issue that arises when only tumor-specific mortality is considered is that of competing risks: if antihistamine use has an effect on other causes of death (for instance, if the anti-inflammatory properties of the drugs confer a survival benefit due to decreased risk of cardiovascular death), the association we see when we examine only tumor-specific death (which cannot occur for someone who has died of cardiovascular disease) may be influenced by the true association with cardiovascular death through competing risks (159, 165).

Unmeasured risk factors affecting both the outcome and cancer incidence may cause a type of bias where cancer becomes a collider if the exposure also influences cancer incidence (166-169). As Cespedes Feliciano et al. report, "a risk factor that increases disease incidence will increase disease-specific mortality" (166). Here, we rely on the previous work by Hemminki et al., Nadalin et al. and others, presented above in the sections "Allergy and cancer" and "Antihistamines and cancer risk", showing no increased overall cancer risk for those with allergic conditions or users of antihistamines, and that both decreased and increased risks of different types of cancer can be seen (75, 103), indicating that there need not be any such systematic bias in our studies. We also did an analysis of desloratadine use and cancer risk (not presented here) using controls from another study, where we found no increased risk of cancer with desloratadine use. We did not have enough events to exclude this as the reason for not finding any increased risk, but the analysis further suggests that this type of bias may not affect our studies. Also, the anti-tumor effects of antihistamines seen on cancer cells from different

tissues (presented in Table 1 above) further suggest that this type of bias is not a major issue here.

As immortal time bias, or survivor treatment selection bias, can be an issue in survival analysis of treatment effects (170, 171), time-varying covariates were used in our post-diagnostic analyses, so as to account for the changes in exposure status for different individuals (158, 171). For that same reason, study start was set to six months following diagnosis for the peri-diagnostic analyses.

Results

As few studies have thus far been done where antihistamine use and survival in cancer is analyzed, this dissertation advances the understanding of whether the potential anti-tumor effects of some antihistamines reported in previous studies correspond to any real-life survival difference for antihistamine users with tumor diseases.

The main findings of the studies included in this dissertation are as follows:

1) use of desloratadine and loratadine is associated with a survival benefit in several tumors (Studies I-III);
2) the tumors where use of these antihistamines is associated with improved survival can all arguably be classified as immunogenic (Study III); and
3) hundreds of thousands of lives may be spared globally with a desloratadine intervention (Study IV).

These results are presented in brief below, and more extensively in Papers I-IV at the end of this dissertation.

Initial results

In the initial analyses of breast cancer mortality (that would ultimately result in Study II), we treated the H_1-antihistamine users as one group and compared their mortality to that of non-users of antihistamines and found no statistically significant difference in survival between the groups (Figure 4). However, there is an indication of increased survival for antihistamine users, that may become significant given a longer study time.

In all subsequent analyses, we examined the survival of antihistamine users separately by each antihistamine.

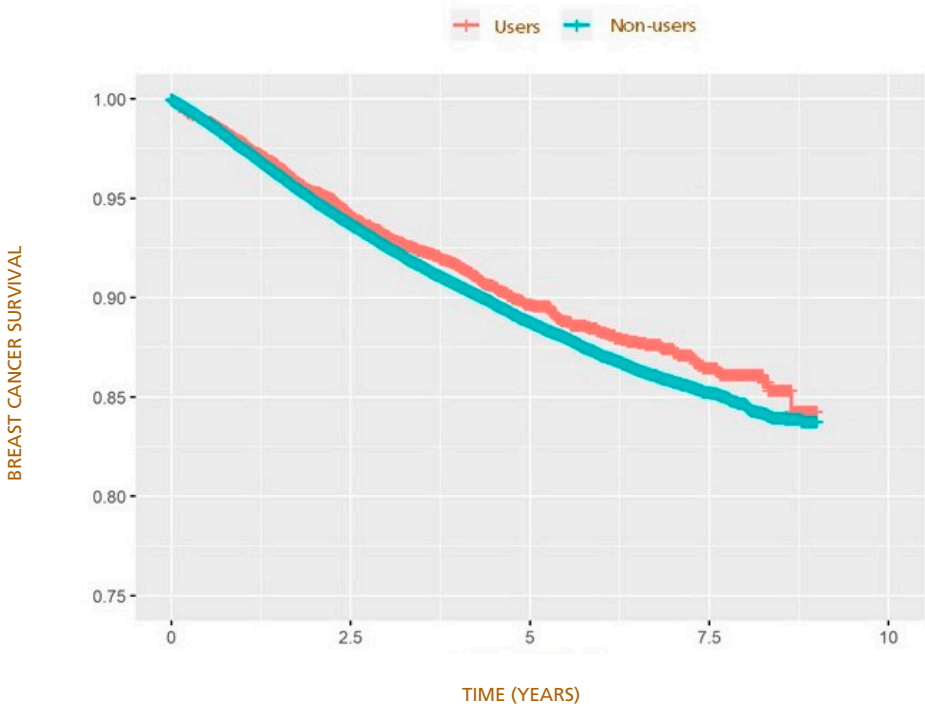


Figure 4. H_1-antihistamine use and breast cancer-specific survival. Survival probability of women diagnosed with breast cancer 2006-2013 who used cetirizine, clemastine, desloratadine, ebastine, fexofenadine and loratadine peri-diagnostically (n=5,001), compared with non-users of those antihistamines (n=55,284).

Study I

In Study I, we compared the mortality of antihistamine users with cutaneous malignant melanoma (CMM) to that of non-users of antihistamines with the same disease, and saw that there was an association with improved survival for use of desloratadine and loratadine – both in the crude analyses and when adjustments were made for various tumor and patient characteristics; when melanoma-specific and overall mortality was analyzed; and when antihistamine use was defined as use in the year surrounding diagnosis as well as post-diagnostically. Use of the other four studied antihistamines was not associated with any significantly altered melanoma survival. (Paper I, Tables 1 & S2-4 and Figure S1). We also saw a markedly improved survival for desloratadine users among both younger and older patients (defined as 65 and below, and above age 65, respectively), such that older desloratadine users with CMM appeared to have mortality rates similar to those of non-users in the younger age group (Paper I, Figure 1).

We analyzed the risk of developing a second primary CMM, and while the results were not statistically significant, there does appear to be a reduced risk of developing a second primary CMM for users of desloratadine and loratadine (Paper I, Figure S2).

Study II

In Study II, similar in approach to Study I but with a focus on breast cancer, we compared the mortality of antihistamine users with breast cancer to that of non-users of antihistamines with the same disease. Here, we saw that survival in breast cancer was improved for women who used desloratadine, ebastine and loratadine. Both peri- and post-diagnostic antihistamine use was analyzed, as well as both overall and breast cancer-specific survival. Subgroup analyses were made based on estrogen receptor (ER) status (using prescribed treatment as a proxy for ER-positive disease) and menopausal status (using age 50 as a proxy for menopause). The association with improved survival was most consistent across analyses for desloratadine, although in some cases seemed most pronounced for ebastine. (Paper II, Tables 2-5 & S1-2 and Figure 1.)

Study III

In Study III, we did a meta-analysis of the mortality of antihistamine users compared to non-users with ten immunogenic and six non-immunogenic tumor types (grouped according to any known response to immune checkpoint therapy). We classified bladder, breast, colorectal/anal, gastric, kidney, lung, pancreatic, and prostate cancer together with melanoma and Hodgkin lymphoma as immunogenic, whereas

brain/CNS, liver/biliary tract, ovarian, thyroid and uterine cancer and non-Hodgkin lymphoma were classified as non-immunogenic cancers. Desloratadine use was associated with an improved tumor-specific survival for all immunogenic but no non-immunogenic tumors (Paper III, Figure 1). Loratadine use was associated with improved tumor-specific survival for some of the immunogenic tumors as well as for ovarian cancer (Paper III, Figure 2). Cetirizine use was associated with improved survival in gastric, pancreatic and ovarian cancer, while use of clemastine, ebastine and fexofenadine was not associated with any improved cancer survival (Paper III, Figures S1-4).

Study IV

In Study IV, using the same population and initial methodology as in Study III, we focused on the immunogenic tumor types where we showed an association with improved survival with desloratadine use in Study III. Here, we used the tumor-specific mortality of desloratadine users and non-users of antihistamines, adjusted for age, gender and calendar year of diagnosis, and quantified the potential effect of desloratadine through a simulated desloratadine intervention in the whole population, by treating the population as first desloratadine users and then non-users, resulting in a survival difference of 2.5% for the immunogenic tumor types together, and corresponding to an additional 855 individuals of the incident annual cases with immunogenic tumors in Sweden that may survive beyond five years. For gastric and pancreatic cancer and Hodgkin lymphoma, the risk differences were 9-11%. Using the calculated survival difference we estimated that an additional 226,250 individuals of those with incident annual cases of immunogenic tumors may survive beyond five years in the global population. Using projections for cancer cases in low- and middle-income countries in 2030, we found that an additional 125,000 lives may be spared annually in low- and middle-income countries in ten years' time with a desloratadine intervention. (Paper IV, Results.)

Discussion

Antihistamines, including H_1-antihistamines, have been suggested as anti-cancer drugs since the 1970s, and with the work presented in this dissertation, we fill in some of the important gaps between the existing evidence from *in silico*, *in vitro* and *in vivo* studies and the threshold of evidence needed for the initiation of randomized clinical trials of H_1-antihistamines for cancer therapy, using register data on the entire Swedish population.

We have found that desloratadine and loratadine stand out among common H_1-antihistamines for their association with improved survival in melanoma, breast cancer and other immunogenic cancers, and that a potential desloratadine intervention could spare hundreds of thousands of lives. Our findings show a promising potential for desloratadine and loratadine as cancer therapy, and we propose an antihistamine effect, that may be immunological in nature, as an explanation of our findings.

Points related to our combined findings in Studies I-IV are discussed in brief here, while the results of the specific studies are discussed in more detail in Papers I-IV at the end of this dissertation.

The case for an antihistamine effect

The first, and most obvious, objection to our findings is the question of whether the association we show is merely an effect of a heightened immune response for those with allergic conditions that positively influences cancer survival, or some other confounding by indication, whereby those with allergic conditions have an improved cancer survival in the diseases we have studied. As presented in the section "Methodological advantages and limitations", there are several reasons as to why we argue that some confounding by indication would not explain our findings. The recent study on anti-tumor effects of desloratadine on bladder cancer cells in particular supports our findings regarding desloratadine (148). In addition to what is discussed above, not all studied antihistamines are associated with improved cancer survival in our studies, further supporting that the observed association may be true. Our lag analyses also show that the potential effect of some of the antihistamines increases with a longer duration and larger cumulative dose, something that cannot be readily explained if allergic rhinitis or

atopy or some other indication had an effect on mortality. All of this suggests that our findings are not merely due to some unknown protective effect of allergy or other conditions for which antihistamines are prescribed.

While we cannot adjust for allergic conditions, or other indications for which antihistamines are prescribed, similarly in studies where the cancer survival of those with allergies are studied, antihistamine use cannot readily be adjusted for, as antihistamines are used by a large proportion of those with allergic conditions. What we *can* do, however, is present a case for further studies and clinical trials of certain antihistamines in cancer therapy, where both the best- and worst-case scenarios may result in conclusive proof of either an antihistamine or allergy effect.

The proposed antihistamine effect

No overall survival benefit was seen when all antihistamine users with breast cancer were treated as a group (Figure 4), and therefore we recommend that any study assessing risks and prognoses associated with antihistamine use analyze the drugs separately. Most probably, the reason that no overall survival benefit was seen in our initial analyses is that use of clemastine (and possibly cetirizine) may influence survival negatively, perhaps reflecting the dual nature of histamine in cancer.

We propose an antihistamine effect that may in fact be greater than what we can show here, as we unfortunately cannot correct for the likely dilution due to prescription-free use of loratadine throughout the study periods, and of desloratadine from 2014 (as well as possibly that of ebastine, fexofenadine and cetirizine to some extent).

As desloratadine is the active metabolite of loratadine, it is possible that the observed association of improved survival with loratadine use that we show in Papers I-III is due to a desloratadine effect. Alternatively, a heterogeneity among tumor types with regard to the proposed antihistamine effect may explain the differences seen.

In Study IV, our calculation is based on the use of desloratadine to treat common allergies and other indications for which desloratadine is currently prescribed and does not take into account either duration of use or dosage. Therefore our quantification is in all likelihood an underestimation of any actual desloratadine intervention, after randomized clinical trials have shown what would constitute optimal dosage and duration. The full antihistamine effect may also be greater than what we propose in Paper IV, as loratadine, ebastine, and possibly even cetirizine and fexofenadine may have anti-tumor effects in some cancers where desloratadine is not as effective, but we have only quantified the possible desloratadine effect. Our group of non-users also includes those who use antihistamines without a prescription, leading to a potential

underestimation of the survival difference. That the survival can still be estimated at 2.5% for all tumor types together is promising.

Immunotherapy against CTLA-4 and PD-1 are important in the treatment of advanced metastatic melanoma now, as is treatment with BRAF and MEK inhibitors, but no CMM patients included in Study I received immunotherapy, and only a few were treated with BRAF and MEK inhibitors, and any survival benefit of desloratadine or loratadine together with modern melanoma and other immunogenic cancer therapy remains to be seen. It could be greater than what we can show here, as antihistamines may work synergistically with, or even potentiate the effects of, modern immuno- and chemotherapeutics (127, 138, 151).

An immunological mechanism?

Our first notion of the most likely mechanism behind the potential effect of desloratadine and loratadine is that it is immunological in nature. Histamine, mast cells and other cells and mediators on which desloratadine and loratadine have an effect are involved in many aspects of inflammation and the immune response to tumors. That all the tumors where we saw an association with improved survival in Study III can be classified as immunogenic lends support to this notion.

The potential effect of desloratadine and loratadine may rely on the blockade of H_1-receptors (27, 28) through the potent antagonism (38, 40) and inverse agonism of desloratadine (38, 39) inhibiting both the basal and histamine-induced signaling of the receptors. It may also have to do with the inhibition of histamine synthesis and secretion, or depend on the stabilizing effect of desloratadine on mast cells (41, 42). It may involve counteracting the histamine-promoted immunoregulatory activity of myeloid-derived suppressor cells (69), or the disruption of the histamine-mediated skewing away from the Th1-dependent cytolytic response (67-70). The effect may also involve the CTLA-4 or PD-1 pathways (12, 54), STAT3 signaling (139, 172), or the paradoxical, basophil-mediated, role of IgE in cancer described by Crawford et al. and Hayes et al. in two recent papers (45, 173).

While we believe an immunological effect is the most likely explanation of our results, non-immunological effects, such as an effect on lysosomes or some unknown novel effect, may also explain our results. Others have postulated that the potential mechanism involves lysosomal cell death (174), as lysosomes are more abundant and larger in tumor cells than in other cells of the same tissue (151, 152, 175). Class-II lysosomotropic drugs (including cationic amphiphilic antihistamines like desloratadine and loratadine) may restore sensitivity of multi-drug resistant cancer cells to chemotherapeutics. Due to this, and a severalfold selectivity for cancer cells, these drugs have been proposed by Kuzu et al. as “a novel approach for killing cancer cells without

affecting normal ones". (176) Any synergy or additive effects remain to be seen whatever the mechanisms involved may be.

An immodest proposal

We realize the unorthodox nature of our call for randomized clinical trials, based on our findings and the other evidence in support of a potential anti-tumor effect of antihistamines. However, due to the nature of the circumstances, where replication is not only impossible and impractical, and the direness of the diagnoses where desloratadine may have an effect on survival, the delays caused by waiting for results of extensive preclinical studies might also prove fatal for severely ill patients. The situation is already very unusual in that the drugs we propose for repurposing are already in use in the patient population in question. As these antihistamines are already in use among the patients, there is very little risk associated with initiation of clinical trials, while the gain, should our hypothesis prove correct, may be great, as we show in Paper IV.

Conclusions

In Studies I & II, we found that the common H_1-antihistamines desloratadine and loratadine are associated with increased survival in melanoma and breast cancer, and in Study III we saw that this pattern holds for other immunogenic tumors as well, especially with regards to desloratadine, leading us to the conclusion that desloratadine and loratadine may prove useful in cancer therapy for patients with immunogenic tumors. In Study IV we quantify this potential desloratadine effect, and show that numerous lives may be spared, concluding that the rationale for clinical trials, particularly of desloratadine in cancers that are difficult to treat and deadly, is strong.

Future perspectives

The conclusions of our studies point toward two main areas for future research:

1) randomized clinical trials of desloratadine and loratadine (and perhaps other H_1-antihistamines such as ebastine) as additional treatment for immunogenic cancers in particular; and
2) future studies to elucidate the nature of any anti-tumor effect.

While we hypothesize that the potential effect of desloratadine and loratadine may be immunological in nature, as the cancers where their use is associated with increased survival are also cancers that respond to immune checkpoint inhibition, the nature of the potential effect and the exact mechanism remain to be found. Studies on tumors and normal tissue from antihistamine users and non-users with immunogenic cancers should be undertaken to better understand what mechanisms may be involved in the proposed antihistamine effect. Studies of animal models will also shed light on the nature of the association that we have found. However, the lack of a reliable immunocompetent *in vivo* model for melanoma (177, 178), arguably one of the most interesting cancers for a desloratadine intervention, where interactions with anti-CTLA-4 and anti-PD-1 could be studied, further supports our call for clinical trials.

Verbaanderd et al. point out some of the challenges involved in drug repurposing and highlight the case of the H_2-antihistamine cimetidine, which, despite positive results in clinical trials and a Cochrane systematic review calling for large-scale clinical trials (112), still has not been adopted into clinical practice anywhere (179). Pantziarka et al. discuss the need for clinicians to "facilitate repurposing by supporting clinical trials and trial applications when they occur" in a 2018 editorial, and end their piece by asking "scientists, citizens, doctors, and patients to join forces, and voices, in support of repurposing old drugs for new indications" (3). Here at the end of this dissertation, I echo their plea, and the by now decades-old suggestion that certain H_1-antihistamines could have a role in cancer therapy.

Populärvetenskaplig sammanfattning (Summary in Swedish)

Cancer är en stor grupp sjukdomar med gemensamma karaktäristika som ohämmad tillväxt av celler. Cancersjukdomar är den näst vanligaste dödsorsaken i världen, och orsakar årligen nästan 10 miljoner dödsfall globalt, och ca 22 500 dödsfall i Sverige. Lungcancer är den cancerform som orsakar flest dödsfall, åtföljd av tjocktarms-, magsäcks-, lever- och bröstcancer samt cancer i matstrupen och bukspottkörteln.

Behandlingsmöjligheterna skiljer sig avsevärt för olika cancerformer, och det finns ett stort behov av nya verksamma läkemedel, särskilt när det gäller svårbehandlade och mycket dödliga tumörer som bukspottkörtelcancer. Genom vad som kallas för läkemedelsompositionering, eller rätt och slätt läkemedelsåteranvändning, då redan existerande, godkända läkemedel får ett nytt användningsområde, kan detta behov tillgodoses både tids- och kostnadseffektivt.

Eftersom uppkomsten av cancersjukdomar har många likheter med inflammatoriska tillstånd kan detta utnyttjas i jakten på nya cancerläkemedel. Flera antiinflammatoriska läkemedel har mycket riktigt visat sig vara verksamma även mot tumörsjukdomar. Vanliga allergiläkemedel som antihistaminer tillhör gruppen antiinflammatoriska läkemedel, och flera studier har visat att vissa antihistaminer har lovande tumörhämmade egenskaper.

I tre av de fyra delarbetena som ligger till grund för den här avhandlingen har vi – genom att samköra data från Svenska Melanomregistret, Cancerregistret, Läkemedelsregistret och Dödsorsaksregistret – undersökt om användning av några av de vanligaste allergiläkemedlen i Sverige är förknippade med överlevnadsfördelar för patienter med melanom (delarbete 1), bröstcancer (delarbete 2) samt dessa och ytterligare fjorton tumörsjukdomar (delarbete 3).

Vi fann att användning av framförallt två av de sex antihistaminer vi studerat (desloratadin och loratadin) är förknippad med ökad överlevnad för patienter

med melanom och bröstcancer, och att desloratadinanvändning är förknippad med ökad överlevnad i ytterligare åtta tumörsjukdomar: cancer i bukspottkörteln, lungorna, magsäcken, njurarna, prostatan, tjocktarmen och urinblåsan, samt Hodgkins lymfom.

Eftersom alla dessa tumörtyper också har gemensamt att de svarar på behandling med immunoterapi – där kroppens eget immunförsvar används för att hämma tumörtillväxten – misstänker vi att den potentiella effekten av desloratadin och loratadin troligen är immunologisk, och möjligen inbegriper de processer som även immunoterapin utnyttjar för att bekämpa tumörer. Stämmer vår hypotes kan det också medföra en ytterligare ökad överlevnad om dessa antihistaminer ges tillsammans med de nya immunoterapier som tagits fram de senaste åren.

I det fjärde delarbetet har vi uppskattat effekten av desloratadin som möjlig cancerbehandling, och beräknat att ytterligare ca 855 individer av de årligen insjuknade i de tio immunogena tumörsjukdomarna i Sverige, och ca 226 250 individer globalt, skulle kunna överleva med desloratadinbehandling. Det återstår att se vad den faktiska siffran hamnar på efter optimal behandlingsdos och -längd, men klart står att desloratadin är ett läkemedel med lovande potential för återanvändning. Desloratadin är säkert, billigt, och beprövat, och bör studeras vidare och ingå i kliniska prövningar som ett cancerläkemedel.

Acknowledgments

A long and serendipitous road led to my involvement in the projects that resulted in this dissertation, and I am sincerely grateful to everyone who helped me along the way, university colleagues and academically unaffiliated friends alike. If you're reading this looking for mention of your name without finding it, please know that any omission is due to the stress that comes with writing a dissertation under unusual circumstances, not a lack of gratitude.

First and foremost, my most heartfelt thanks to **Håkan Olsson**, my main supervisor, who gave me the enviable opportunity to work with projects of my choosing, and who has been encouraging, challenging and guiding me throughout it all. Your ability to stay focused among manifold ventures and remain constructive even in adversity is something I can only hope to partially emulate. Thank you for proving that even among sharp elbows, kindness can prevail, and that research, while frustrating at times, is, above all, fun.

Secondly, sincere thanks to my former and current co-supervisor, respectively, **Per Broberg** and **Philippe Wagner**, for all the support and statistical know-how, without which there would not be very much to present in these pages. If I am to judge based on the two of you (although you'd certainly both object to the sample size), statisticians are the friendliest and most patient people on the planet. Per, thank you for explaining things when everything to do with epidemiology was new to me while being great company. Philippe, I have more to thank you for than what can be listed here, but I'm especially grateful for your long-distance support during the trials and tribulations of manuscript submission and revision, for the pep talks during thesis-writing, and for being the sort of person with whom work calls never feel like work.

Many thanks to **all my co-authors** for their input and cheerful support regarding the manuscripts and to **every past and present colleague at the Departments of Cancer Epidemiology and Oncology** who have helped me with issues big and small; scientific, computer-related or administrative.

Thank you to **all my weird and wonderful friends** for cheering me on, distracting me or commiserating with me, depending on what the situation called for. I am lucky to have so many great people in my life. Particular thanks are due to **Andrés**, **Emma** and (the other) **Philippe**, **Simon** and **Tove**, who helped out with various things and listened to me talk about antihistamines, cancer and the peer review process in what I'm certain must have been excruciating detail. Thank you to (the other) **Håkan** for the last-minute help.

Thank you for the photo for the back cover, **Hedvig**, and for being the little sister I never had for nearly two decades now.

Many thanks are also due to my mother, **Maria**, who has driven me to and from work much more often than what is reasonable, while being the one who taught me to value logic and reason; my sister, **Melinda**, who is (still) always there for me when I need her to be; my brother, **Attila**, who promised he will actually read this dissertation without skipping to this bit; and my father, **Tamás**, who taught me things unrelated to science, but just as important.

Lastly, and most importantly, my own little family:

Marcus – all PhD students should have the kind of support you have provided me with throughout the (by now many) years (I was sciencing as fast as I could!), though I very much doubt that there are any others quite this lucky. The words "thank you" are silly and woefully insufficient in this context: I owe you everything and couldn't and wouldn't have done this (or many of the other slightly bananas schemes over the years) without you. Not every aspect of our life together for the last two decades has been fun, but most of it has been wonderful, and I wouldn't change a thing. I hope we can continue to make others shake their heads at many more slightly bananas schemes to come.

Gusti – the last time I wrote and defended a dissertation, I was pregnant with you, and the six years between these two theses have been the greatest adventure of my life. Words cannot express my gratitude, nor the immensity of my love and pride. You are the shiniest of shinies, eclipsing everything else, and being your parent is my single greatest privilege, purpose and achievement. JAG ÄLSKAR DIG. DU ÄR BÄST.

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