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2019

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

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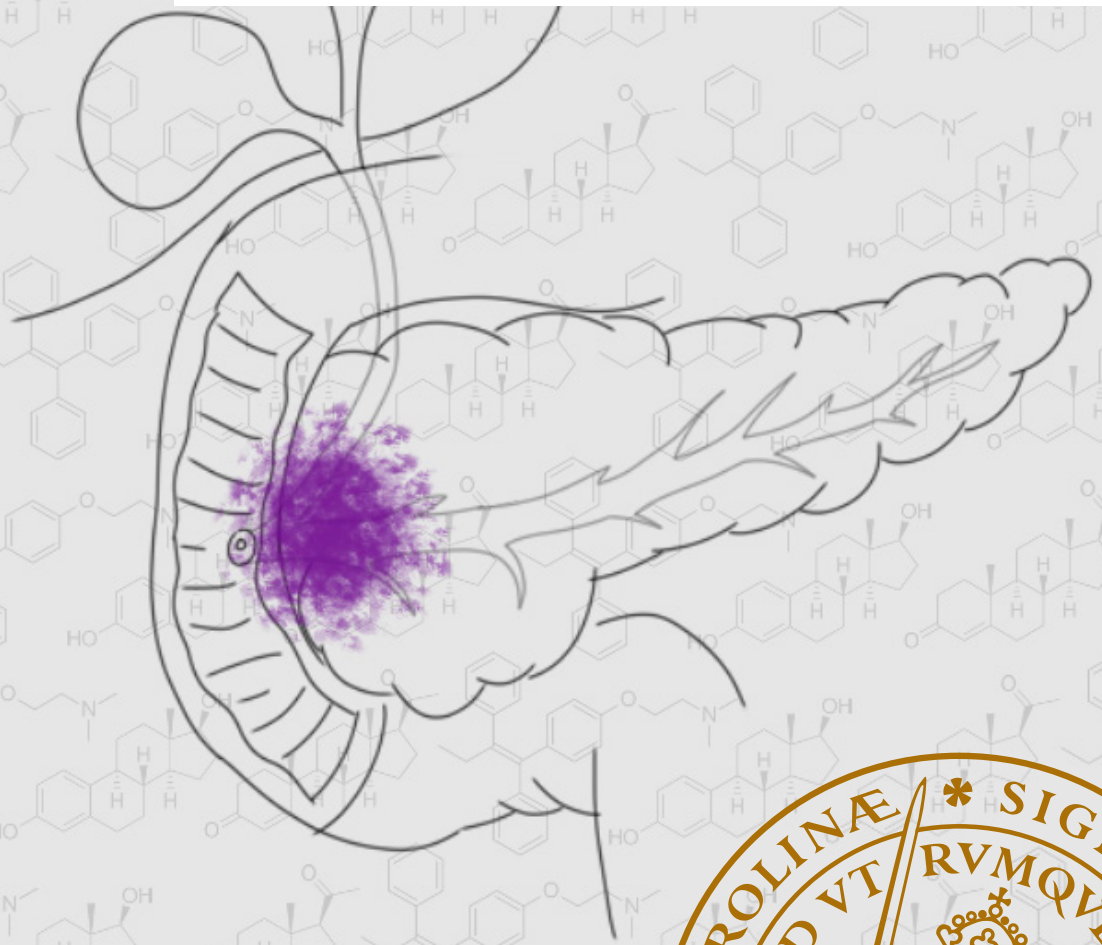

Sex, hormonal factors and pancreatic cancer

Gustav Andersson, MD

LUND
UNIVERSITY

DOCTORAL DISSERTATION

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To be defended in Hornbergssalen, Kulturen Restaurang & Konferens, Lund

Friday 17th May, 2019 at 9:30 am.

Faculty opponent
Björn Lindkvist, MD, PhD, Associate Professor
Department of Medicine, Sahlgrenska University Hospital, Gothenburg

Organization	Document name
LUND UNIVERSITY	Doctoral dissertation
Department of Clinical Sciences, Lund Divison of Oncology and Pathology Skåne University Hospital, Lund, Sweden	Date of issue May 17 2019
Author: Gustav Andersson	Sponsoring organization

Title: Sex, hormonal factors and pancreatic cancer

Abstract

Pancreatic cancer represents three percent of all incident cancer cases in developed countries, but stands the 7th most common cause of cancer related death. Worldwide, pancreatic cancer is more common among men, however in Sweden, the incidence ratio between sexes is levelling. Despite extensive research to map underlying risk factors, results are still largely inconclusive. Furthermore, there is a lack of knowledge regarding the influence of hormonal factors on risk and clinical outcome. A few studies have investigated the expression of female hormone receptors in pancreatic cancer, and others have reported beneficial effects of tamoxifen treatment in advanced pancreatic cancer, particularly in elderly women. The primary aim of this thesis was to investigate potential risk factors for pancreatic cancer, with particular reference to sex differences, and furthermore, to evaluate the presence and prognostic significance of hormone receptors in pancreatic and other periampullary cancers. Finally, based on the third paper, we composed a protocol for a clinical trial investigating the impact of tamoxifen treatment in women with advanced pancreatic cancer.

The thesis is based on the Malmö Diet and Cancer Study (MDCS), a prospective population-based cohort with 28 098 participants, as well as a retrospective cohort with 175 consecutive cases of resected pancreatic and other periampullary adenocarcinoma. Cox proportional hazards regression models were applied to study the potential associations between investigative baseline factors and risk of pancreatic cancer in the MDCS. Immunohistochemical expression of estrogen and progesterone receptors (ER, PR) was analysed on tumour tissue microarrays from the retrospective cohort.

Paper I confirms smoking as one of the most significant risk factors for pancreatic cancer, also proposing a greater risk increase among women.

Paper II demonstrates an increased risk of pancreatic cancer among women with high age at menarche and a lower risk among postmenopausal women with a history of ever using hormonal replacement therapy.

Paper III provides evidence of a prognostic interaction between stromal PR expression and *KRAS* mutation status in periampullary cancer, being particularly evident in women. More specificially, stromal PR positivity signified a prolonged survival in patients with *KRAS*-mutated tumours, and shorter survival in patients with *KRAS* wild-type tumours.

Paper IV is a protocol for a single-centre, randomized, double-blind, placebo-controlled, two-arm, phase II clinical trial, investigating the effects of tamoxifen treatment on survival and quality of life in women with advanced pancreatic cancer.

In summary, the thesis provides further evidence of tobacco smoking as one of the strongest risk factors for pancreatic cancer, with women being potentially more susceptible to these hazardous effects. Moreover, exogenous female hormones appear to have a protective effect, which is also in line with findings from some previous studies. The presence of ER and PR in the tumour-associated stroma in pancreatic and other periampullary adenocarcinoma, and the prognostic interaction between PR expression and *KRAS* status further supports that hormonal factors drive the pathogenesis and progression of these cancers. Based on the observations in Paper III, we will launch a randomized trial with tamoxifen treatment and control in women with advanced pancreatic cancer, that will also include relevant biomarker analyses.

Key words: Pancreatic cancer, sex, anthropometry, hormonal factors, tamoxifen treatment

Classification system and/or index terms (if any)

Supplementary bibliographical information		Language: English
ISSN and key title: 1652-8220		ISBN: 978-91-7619-776-9
Recipient's notes	Number of pages 86	Price
	Security classification	

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UNIVERSITY

This study was supported by grants from the Swedish Research Council, the Swedish Cancer Society, the Mrs Berta Kamprad Foundation, Governmental Funding of Clinical Research within the National Health Service (ALF), Lund University Faculty of Medicine and Skåne University Hospital Funds and Donations.

Cover by Gustav Andersson

Department of Clinical Sciences, Lund
Division of Oncology and Pathology

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2019:47

ISBN 978-91-7619-776-9
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2019

"Keep exploring. Keep dreaming. Keep asking why.
Don't settle for what you already know.
Never stop believing in the power of your ideas, your imagination, your hard work to change the world."
- Barack Obama

Table of Contents

Thesis at a glance

Paper	Objective	Method	Patients	Results/Conclusion
I	Investigate the associations of sex, lifestyle factors and pre-diagnostic anthropometry with risk of pancreatic cancer.	Cox proportional hazards regression model.	The Malmö Diet and Cancer Study (MDCS), a prospective population-based cohort, encompassing 11 063 men and 17 035 women recruited between 1991 and 1996.	Smoking was the strongest risk factor for pancreatic cancer, with occasional and environmental smoking also being significant risk factors in women, but not in men. WHR was the only anthropometric factor associated with risk.
II	Investigate the associations of reproductive factors and hormone use with risk of pancreatic cancer in women.	Cox proportional hazards regression model.	All women in the MDCS.	Higher age at menarche was a significant risk factor for pancreatic cancer, and ever use of HRT, particularly estrogen-only regimen, was a protective factor.
III	Investigate the expression and prognostic significance of female hormone receptors ER and PR, and their relation to tumour mutational status in periampullary cancer.	ER and PR expression was assessed by IHC on TMAs.	A retrospective cohort of 175 consecutive cases with pancreatic and other periampullary adenocarcinoma, resected by pancreaticoduodenectomy in Lund and Malmö University hospitals between 2001 and 2011.	ER and PR were mainly expressed in the tumour-associated stroma. A significant prognostic interaction was found between PR expression and *KRAS* mutation status.
IV	Investigate the effects of tamoxifen tretment in women with advanced pancreatic cancer, with analysis of baseline tumour characteristics and on-treatment biomarker analysis.	A single-centre, randomized, double-blind, placebo-controlled, two-arm, phase II clinical trial. Treatment with 40 mg of tamoxifen or placebo.	150 women with advanced pancreatic cancer not eligible for further systemic chemotherapy.	

List of papers

The thesis is based on the following papers, and referred to in the text by their Roman numerals:

I. **Andersson G**, Wennersten C, Borgquist S, Jirström K, Pancreatic cancer risk in relation to sex, lifestyle factors and pre-diagnostic anthropometry in the Malmö Diet and Cancer Study. *Biology of Sex Differences* 2016;7:66

II. **Andersson G**, Borgquist S, Jirström K, Hormonal factors and pancreatic cancer risk in women: The Malmö Diet and Cancer Study. *International Journal of Cancer* 2018;143:52-62

III. **Andersson G**, Lundgren S, Heby M, Nodin B, Elebro J, Jirström K, Clinical significance of stromal ER and PR expression in periampullary adenocarcinoma. *Submitted*

IV. **Andersson G**, Borgquist S, Hall P, Jirström K, Heby M, Tamoxifen in women with advanced pancreatic cancer: A randomized, double-blind, placebo-controlled trial. *Manuscript*

Abbreviations

5-FU	5-fluorouracil
AJCC	American Joint Committee on Cancer
AR	Androgen receptor
ASR	Age-standardized incidence rate
AUS	Abdominal ultrasound
BMI	Body mass index
BRCA1	Breast cancer gene 1
BRCA2	Breast cancer gene 2
CA19-9	Carbohydrate antigen 19-9
CHA	Common hepatic artery
CIS	Carcinoma in-situ
CNS	Central nervous system
CRT	Chemoradiotherapy
CT	Computed tomography
ctDNA	Circulating tumour DNA
CTLA-4	Cytotoxic T-lymphocyte-associated antigen 4
DFS	Disease-free survival
DHT	Dihydrotestosterone
DM	Diabetes Mellitus
dMMR	Defective mismatch repair
DNA	Deoxyribonucleic acid
E2	17β-estradiol
EGFR	Epidermal growth factor receptor
EPIC	European Prospective Investigation into Cancer and Nutrition
ER	Estrogen receptor
ER-α	Estrogen receptor alpha

ER-β	Estrogen receptor beta
ER+	Estrogen receptor positivity
ERCP	Endoscopic retrograde cholangiopancreatography
ESPAC	European Study Group for Pancreatic Cancer
ETS	Environmental tobacco smoke
EUS	Endoscopic ultrasound
FAMMM	Familial atypical multiple mole melanoma
FAP	Familial adenomatous polyposis
FDA	The U.S. Food and Drug Administration
FNA	Fine-needle aspiration
FOLFIRINOX	Folinic acid, 5-FU, irinotecan and oxaliplatin
FPC	Familial pancreatic cancer
GemCap	Gemcitabine, capecitabine
GTP	Guanosine triphosphate
HBOC	Hereditary breast-ovarian cancer
HBV	Hepatitis B virus
hENT1	Human equilibrative nucleoside transporter 1
HER2	Human epidermal growth factor receptor 2
HGD	High-grade dysplasia
HNPCC	Hereditary nonpolyposis colorectal carcinoma
HR	Hazard ratio
HRT	Hormone replacement therapy
I-type	Intestinal type
IDF	International Diabetes Federation
IHC	Immunohistochemistry
IPMN	Intraductal papillary mucinous neoplasia
KRAS	Kirsten rat sarcoma viral oncogene homolog
Lag-3	Lymphocyte activation gene 3
MCN	Mucinous cystic neoplasia

MDC	Multidisciplinary conference
MDCS	Malmö Diet and Cancer Study
mFOLFIRINOX	Modified FOLFIRINOX regimen
mOS	Median overall survival
MRCP	Magnetic resonance cholangiopancreatography
mRFS	Median recurrence-free survival
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
NGS	Next generation sequencing
NorPACT	Nordic Pancreatic Cancer Trial
OC	Oral contraceptives
OR	Odds ratio
OS	Overall survival
OTS	Ovarian-type stroma
PanIN	Pancreatic intraepithelial neoplasia
PARP	Poly(ADP-ribose) polymerase
PB-type	Pancreatobiliary type
PD-1	Programmed cell death protein 1
PD-L1	Programmed death-ligand 1
PDAC	Pancreatic ductal adenocarcinoma
PFS	Progression-free survival
PGR	Progesterone receptor gene
PR	Progesterone receptor
PR+	Progesterone receptor positivity
PV	Portal vein
RCT	Randomized controlled trial
RFS	Recurrence-free survival
RNA	Ribonucleic acid
RR	Relative risk

SERM	Selective estrogen receptor modulator
siRNA	Small interfering ribonucleic acid
SMA	Superior mesenteric artery
SMV	Superior mesenteric vein
TCGA	The Cancer Genome Atlas
TGF-α	Transforming growth factor alpha
TGF-β	Transforming growth factor beta
TKI	Tyrosine kinase inhibitor
TMA	Tissue microarray
TNM	Tumour node metastasis
WHO	World Health Organization
WHR	Waist-hip ratio

Populärvetenskaplig sammanfattning

Pankreascancer är en tumörsjukdom som utgår från bukspottkörteln (pankreas), ett organ som i friskt tillstånd har en rad olika funktioner. Pankreas är belägen mitt i buken och är sammankopplad med första delen av tarmkanalen, i vilken den utsöndrar enzymer, ämnen som hjälper till med matsmältningen. Utöver en viktig roll vid matsmältningen utsöndrar pankreas en mängd olika ämnen direkt till blodbanan, bland annat insulin som hjälper kroppens celler att ta upp socker för att användas som energi, och på så vis reglerar pankreas även kroppens blodsockernivå.

År 2017 drabbades 1442 svenskar av pankreascancer, motsvarande 2 % av all cancer som diagnostiserades under detta år. Antalet fall av pankreascancer ökar i västvärlden och från att tidigare ha varit vanligare hos män är andelen kvinnor som drabbas i dag lika stor. Trots att pankreascancer fortfarande är en förhållandevis ovanlig sjukdom är det den fjärde vanligaste orsaken till död orsakad av cancer, då endast några få procent överlever mer än fem år efter diagnos. De som drabbas har alltså i många fall mycket dystra utsikter. De senaste decennierna har det gjorts betydande medicinska framsteg som förbättrat överlevnaden vid många andra typer av cancer, men inte för pankreascancer. Vad är då orsakerna till att just denna cancersjukdom är så dödlig?

Ett stort bekymmer är dessa tumörers förmåga att motstå cellgifter, och att de ständigt anpassar sina egenskaper för att överleva och fortsätta växa. Ett ännu större problem är att cancern ofta upptäcks alltför sent, det vill säga när den vuxit sig så stor att det inte längre är möjligt att operera bort tumören. Endast en femtedel av patienterna kan opereras, men risken för återfall är mycket hög. Majoriteten av patienterna befinner sig vid tidpunkten för diagnos alltså redan i en så kallad palliativ situation, det vill säga att sjukdomen är obotlig. Den enda idag tillgängliga behandlingen för dessa patienter är cellgifter, som tyvärr inte har någon markant effekt och dessutom är förenat med mycket biverkningar.

I litteraturen finns ett stort antal riskfaktorer rapporterade, men många av dessa har inte kunnat bekräftas med särskilt hög tillförlitlighet. Risken att insjukna i pankreascancer ökar successivt med stigande ålder, vilket är väldokumenterat då vi i Sverige sedan 1958 följer landets cancerstatistik noggrant och på så vis enkelt kan se hur fördelningen av pankreascancer ser ut i olika åldersgrupper. Vidare är ärftlighet en stark riskfaktor som bedöms ligga till grund för upp till 10 % av all pankreascancer. Sjukdomar som diabetes och inflammation i pankreas (pankreatit)

har i flera studier visats öka risken för pankreascancer, men de kan också orsakas av cancern i sig. När en pankreascancer upptäcks har den ofta vuxit till sig långsamt under många år, utan att ge några tydliga symptom, och det kan därför vara svårt att fastställa huruvida de första cancercellerna uppkom innan eller efter debuten av andra sjukdomar. Detta kan vara ett stort bekymmer när forskare försöker hitta äkta samband mellan två faktorer, så kallade kausala samband.

Man har också kunnat koppla ett antal livsstilsfaktorer till risk att utveckla pankreascancer, av vilka rökning har absolut störst inverkan. Flera studier har visat att en stor andel av alla pankreascancerfall (10 - 30 %) kan tillskrivas rökning. Ytterligare livsstilsfaktorer som med relativt stor säkerhet ökar risken är hög alkoholkonsumtion och fetma. Fetma mäts ofta i ”Body Mass Index” (BMI) och är en så kallad antropometrisk faktor, det vill säga ett mått på kroppskonstitution. Andra antropometriska faktorer är längd, vikt, midje- och stussmått, samt förhållandet mellan midje- och stussmått. Många studier har undersökt sambandet mellan kroppskonstitution och risk för pankreascancer, men resultaten har inte varit helt samstämmiga.

Syftet med detta avhandlingsarbete var därför att undersöka, och skapa en bättre förståelse kring vilka faktorer som kan öka risken att drabbas av pankreascancer. Vidare var syftet att utreda potentiella könsskillnader, samt att ta reda på vilken roll hormonella faktorer spelar för risken att insjukna och för sjukdomsförloppet.

Som underlag till de första studierna i avhandlingen användes ett stort datamaterial från en studie kallad Malmö Kost och Cancer-studien (MKC). MKC-studien pågick under 1990-talet och totalt insamlades information om bland annat social situation, livsstilsfaktorer och tidigare sjukdomar från 28 098 invånare i Malmö. Dessa gav sitt godkännande till att forskare efter studiens avslut fortlöpande skulle få tillgång till information från exempelvis cancerregister och dödsorsaksregister.

I avhandlingens första arbete undersöktes effekterna av rökning, alkoholkonsumtion och antropometriska mått på risken att utveckla pankreascancer. Från avslutandet av MKC-studien fram till och med 31 december 2015 insjuknade 163 av studiedeltagarna i pankreascancer. Risken att insjukna vara högre för både kvinnor och män som vid studiestart uppgett sig röka regelbundet. Hos kvinnor, men inte män, sågs även en samband mellan sporadisk rökning samt passiv rökning på arbetet och en ökad risk. Det sågs inget samband mellan alkoholkonsumtion och pankreascancer. Av alla antropometriska faktorer var endast en hög midje-/stussmåtts-kvot (så kallad äppelfetma) kopplad till en högre risk.

Kvinnor föreföll således vara mer känsliga för de cancerframkallande effekterna av rökning, vilket man även sett tendenser till i tidigare studier kring både pankreascancer och lungcancer. Enligt Folkhälsomyndigheten har andelen rökare i den svenska befolkningen gradvis minskat under de senaste årtionden. Under 1980-

talet var andelen rökare hos män tydligt högre än hos kvinnor, men idag ses inte längre någon större skillnad mellan könen. Vidare insjuknar kvinnor nu lika ofta som män i pankreascancer.

Då kvinnors hormonella profil skiljer sig från mäns undersöktes i det andra arbetet betydelsen av hormonella faktorer för risken hos kvinnor att insjukna i pankreascancer. Även här användes MKC-studien med 17 035 kvinnor som underlag. De faktorer som undersöktes var kopplade till fertilitet, barnafödsel och hormonbalans, så som ålder vid första och sista mens, ålder för första barnafödseln, antal barn, antal amningsmånader, intag av preventivmedel i tablettform och intag av hormontillskott efter klimakteriet.

Studien visade att kvinnor som fått sin första mens vid ung ålder hade lägre risk att utveckla pankreascancer. Vidare hade kvinnor som efter klimakteriet tagit hormontillskott en betydligt minskad risk, framför allt om dessa tillskott endast innehöll östrogen.

Östrogen är ett könshormon som kan påverka kroppens celler genom att skicka signaler via östrogen-receptorer. Receptorer är en form av mottagare som uttrycks på eller i cellerna, och som kan aktivera olika processer i cellerna när de kommer i kontakt med signalsubstanser från cellens omgivning.

Tredje arbetet syftade därför till att undersöka om det fanns något uttryck av hormonreceptorer i tumörceller från periampullära tumörer, ett samlingsnamn för pankreascancer och andra närbesläktade tumörer i samma område, och huruvida förekomst av dessa receptorer påverkade överlevnaden för patienterna. Som underlag till denna studie studerades tumörer från 175 individer, vilka alla opererats bort vid Lunds och Malmös Universitetssjukhus mellan 2001 och 2011. Förekomst av receptorer för könshormonerna östrogen och progesteron (ett annat könshormon) i tumörerna analyserades i ljusmikroskop med hjälp av antikroppsbaserad, så kallad immunhistokemisk infärgning.

Uttryck av båda receptorerna observerades i cirka en tredjedel av tumörerna, och då framför allt i stödjevävnaden omkring tumörcellerna, det så kallade stromat, men inte i själva tumörcellerna. Resultaten visade också ett samband mellan uttryck av progesteronreceptorn och långtidsöverlevnad, vilket var gynnsamt eller ogynnsamt beroende på om tumörerna samtidigt uppvisade en viss genmutation (DNA-skada). Detta samband var särskilt tydligt hos kvinnor.

Hormoner och hormonreceptorer tycks således spela en viktig roll för såväl risken att drabbas av pankreascancer som för sjukdomens förlopp. Detta är ett känt fenomen vid några andra cancerformer, framför allt bröstcancer, och kan utnyttjas vid behandling av sjukdomen. Tamoxifen är ett hormonmodulerande läkemedel som ofta används vid behandling av bröstcancer och verkar genom att hämma

cancercellernas tillväxt. Det finns äldre studier som visat att tamoxifen även förlänger överlevnaden hos patienter med pankreascancer.

Det fjärde och avslutande arbetet är därför ett protokoll för en studie som ska utforska effekterna av tamoxifen hos kvinnor med pankreascancer som är i ett så sent skede av sjukdomen att inga andra behandlingsalternativ finns att tillgå. Till skillnad mot tidigare studier kommer denna även att analysera olika typer av så kallade biomarkörer, exempelvis hormonreceptorer i tumörvävnaden samt hormonella faktorer i blodet. Förhoppningen är att den studie som nu utformas ska visa positiva effekter av tamoxifen och även ge information kring vilka egenskaper hos tumörerna som krävs för att patienterna bäst ska kunna tillgodogöra sig dessa gynnsamma effekter.

Introduction

Pancreatic cancer

The term “pancreatic cancer” is most commonly referring to a tumour originating in the ductal cells of the exocrine pancreas, more specifically known as pancreatic ductal adenocarcinoma (PDAC). Moreover, although unusual, it could be used in a more general manner, indicating any cancer arising in the pancreatic gland. However, the vast majority of all pancreatic neoplasms arise in the exocrine pancreas, and 90 - 95 % of them are PDACs (1, 2). Therefore, throughout this thesis, the term pancreatic cancer will be used and referring to PDAC.

Approximately 75 % of pancreatic cancers arise in the pancreatic head (caput), and the remaining portion is evenly distributed between the body (corpus) and the tail (cauda) (3). Furthermore, there are several precursor lesions eventually evolving into pancreatic cancer, such as intraductal papillary cystic neoplasia (IPMN), pancreatic intraepithelial neoplasia (PanIN) and mucinous cystic neoplasia (MCN) (4). Tumours can also derive from the endocrine pancreas, but these neoplasms constitute a widely different disease and will not be deeply discussed in this thesis (5).

Periampullary adenocarcinoma

Pancreatic cancer is the most common adenocarcinoma in the periampullary region, however, adenocarcinomas in this area can also originate from the duodenum, the ampulla or papilla of Vater, or the extrahepatic lower biliary tracts. On the other hand, due to its prognostic value, these tumours are more commonly classified according to their morphology into intestinal-type (I-type) or pancreatobiliary-type (PB-type) tumours. The superior prognosis for patients diagnosed with I-type tumours compared to PB-type tumours is supported by several studies (6-9). An illustration of the four types of periampullary adenocarcinoma and the two morphological subtypes is shown in Figure 1.

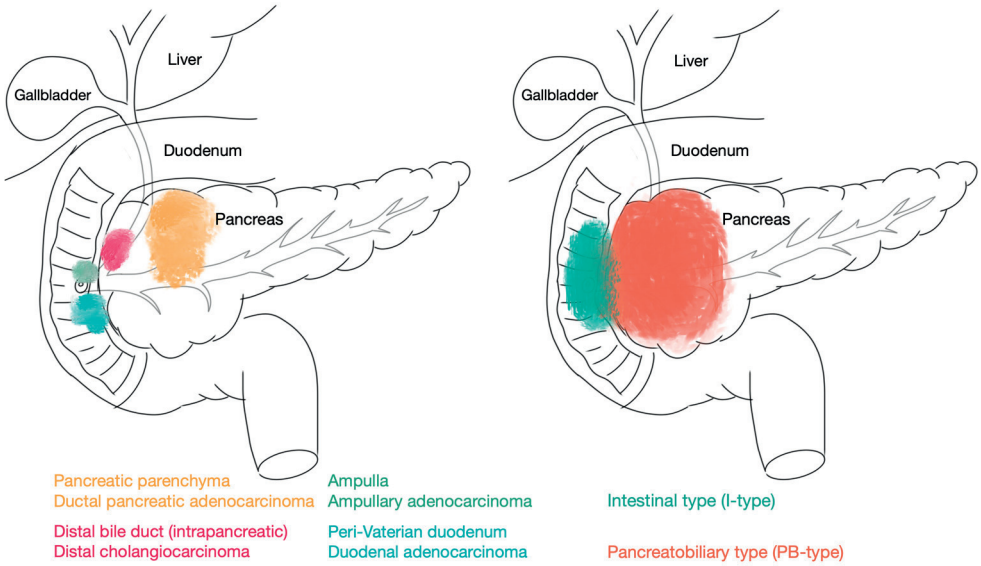

Figure 1
Illustration of the four different types of periampullary adenocarcinoma (left) and the morphological subtypes (right).

Epidemiology

Worldwide

In 2018, pancreatic cancer was estimated to be the 12th most common cancer worldwide, representing nearly 3 % of all incident cancer, non-melanoma skin cancer excluded. It was however the 7th most common cause of death by cancer, reflecting the utterly poor prognosis of the disease. Furthermore, there is a predominance for pancreatic cancer in men, with 243 033 men and 215 885 women being diagnosed in 2018, resulting in a male to female incidence ratio of 1.1:1 (10).

Low versus high income countries

The corresponding ranks for incidence and mortality in low income versus high income countries during 2018 were 18th and 13th versus 9th and 3rd, respectively. Furthermore, the crude and age-standardized incidence rates (ASR) were estimated to 1.0 and 1.9 versus 18.0 and 7.8 cases per 100 000 inhabitants, in low versus high income countries, which emphasizes that pancreatic cancer is indeed a disease of health and welfare, not affected by better access to superior health care (11).

Sweden

According to the most recent report, *Cancer in numbers 2018*, by the National Board of Health and Welfare (12), 1 321 new cases of pancreatic cancer (661 men and 660 women) were diagnosed in Sweden in 2016, and, accordingly, it was the 9th most common cancer in both sexes. With a crude incidence rate of 13.3 cases per 100 000 inhabitants, the incidence rate was highest among 75- to 79-year-olds, for both sexes, with approximately 70 and 65 cases per 100 000 men and women, respectively.

In an updated report of cancer incidence statistics in 2017 (13), there were 1 442 incident cases (719 men, 723 women), moving pancreatic cancer to the 8th most common cancer in both men and women. The crude incidence-rate had risen to 14.2 and 14.4 cases per 100 000 men and women, respectively.

For 2018, the World Health Organization (WHO) (11) reported an estimated number of 1993 new cases (1 020 men, 973 women) in Sweden, moving pancreatic further up, to the 7th and 6th most common cancer among men and women, respectively. It was also estimated to be the 4th most common cause of cancer related death in Sweden in 2018.

Conclusively, these reports demonstrate that pancreatic cancer is swiftly climbing the ladder of the most common cancers in Sweden, and further, the sex-related difference seen on a worldwide basis no longer applies to Sweden.

Aetiology

Endogenous risk factors

Endogenous risk factors are individual characteristics that are generally not possible to influence by modification of environmental factors and life-style habits, but that are controlled by life-time, heredity or other known and unknown circumstances.

Age

The risk of pancreatic cancer is gradually increasing with age, as demonstrated by the annual cancer statistics reports published by the Swedish National Board of Health and Welfare. The incidence and incidence rate is highest among 70 - 74 year old and 75 - 79 year old individuals, respectively, and varies somewhat between sexes depending on age category (13).

Heredity

Family history of pancreatic cancer is one of the most evident factors associated with an increased risk, which has been demonstrated in several studies. Hereditary pancreatic cancer is estimated to account for 1 - 10 % of all cases (14-16), and it has been estimated that the most common syndromes associated with an increased risk account for 15 - 20 % of those cases; Peutz–Jeghers syndrome, familial atypical multiple mole melanoma (FAMMM), hereditary breast–ovarian cancer (HBOC), Lynch syndrome/hereditary nonpolyposis colorectal carcinoma (HNPCC), hereditary pancreatitis and familial adenomatous polyposis (FAP) (17). Furthermore, familial pancreatic cancer (FPC) is another rare tumour syndrome, describing families with two or more first-degree relatives with histologically confirmed adenocarcinoma of the pancreas, that is not correlated to the aforementioned inherited tumour syndromes (18).

Diabetes Mellitus

Several studies highlight diabetes mellitus (DM) as a risk factor for pancreatic cancer (19-21), however, only Elena et al. restricted their investigation to patients with type 2 DM, while the other based their results on unknown proportions of type 1 and type 2 DM. Nevertheless, due to the origin of pancreatic cancer, it is obvious that these patients will develop disease related DM at some point during the progression of their cancer (22-24). Consequently, DM seems to be both a cause and an outcome of pancreatic cancer, making it difficult to provide evidence of causal relationships.

Pancreatitis

Similar to the issue that pancreatic tumours may cause DM, they may also cause pancreatitis. The evidence of an association between pancreatitis and risk of pancreatic cancer is however rather strong. In a meta-analysis by Raimondi et al. (25), reviewing 22 studies investigating the impact of pancreatitis on pancreatic cancer risk, unspecified pancreatitis was shown to increase the relative risk (RR) by 5.1, while chronic pancreatitis increased the risk by 13.3, hereditary pancreatitis by 69 and tropical pancreatitis, in one single study, by 100. Furthermore, two large pooled case-control studies reporting odds ratios (OR) of 5.6 (26) and 7.2 (27) in patients with a history of unspecified pancreatitis, have also demonstrated significant trends of decreasing ORs with increasing time since diagnosis of pancreatitis.

Obesity and other anthropometric factors

The influence of anthropometric factors on pancreatic cancer risk has been a subject of discussion for many years, however concerning several factors, no definite consensus has yet been reached. Numerous studies report significant associations

between high body mass index (BMI) and risk of pancreatic cancer in both women and men (28-31), with no significant differences between sexes. Several biological theories for this correlation have been presented, and one plausible explanation could be that, as a consequence of obesity, the increased insulin resistance and elevated blood levels of circulating insulin increase the risk of neoplasms to arise through growth stimulation of pancreatic tissue (29).

There is also a suggested positive association of waist circumference (19, 29), waist-hip ratio (WHR) (29, 31) and height (32) with risk of pancreatic cancer. Furthermore, rapid weight loss has been demonstrated to correlate with an increased risk (33), however, whether this is a causal relationship is debatable since weight loss is also one of the most common traits of the disease. Moreover, any evidence of sex differences regarding these less thoroughly examined anthropometric factors is lacking.

Metabolic syndrome

The metabolic syndrome represents a cluster of factors associated with cardiovascular disease and type 2 DM. In the most recent update by the International Diabetes Federation (IDF) in 2006, metabolic syndrome is defined as central obesity (described by ethnicity specific values of waist circumference) plus two or more of the following factors: raised triglycerides, reduced HDL cholesterol, raised blood pressure and raised fasting plasma glucose.

In a meta-analysis from 2011, Rosato et al. (34) reported an increased risk of pancreatic cancer among individuals with metabolic syndrome (OR = 2.1). However, in a similar analysis the following year, including only a selection of the studies included by Rosato et al., Esposito et al. (35) demonstrated that this association was only applicable to women (RR = 1.6), and that it was significantly stronger in women compared with men. Of note, these studies partly applied the previous definitions of metabolic syndrome.

Reproductive factors

Several reproductive factors have also been suggested to correlate with risk, however, results are conflicting. A decreased risk has been observed in women with; a higher age at menarche (36, 37), a higher age at menopause (38), a higher number of children (39, 40), a lower age at first childbirth (39, 41), a higher number of total months breastfeeding (42) and intact ovaries (38). Of note, studies demonstrating contradictory findings, or no associations of aforementioned reproductive factors and pancreatic cancer risk, are approximately just as many (36, 38-40, 42).

Exogenous risk factors

Tobacco smoking

Smoking is the most important exogenous risk factor for pancreatic cancer, being responsible for 11 - 32 % of all pancreatic cancer cases (43). In a recent large meta-analysis (44), current and former smokers were reported to have a RR of 1.8 and 1.2, respectively, versus never smokers. Moreover, there seems to be a clear dose-response association of the number of cigarettes smoked per day, number of years smoking and total cigarettes smoked, with risk of pancreatic cancer, and a gradually decreasing risk with each year since quitting in former smokers (45). The risk in former smokers has been estimated to be comparable to the risk for never smokers 10 - 20 years after smoking cessation (44, 46).

The potential influence of sex on the association between smoking and risk of pancreatic cancer has been addressed in a few studies, proposing that the impact on risk is stronger in women than in men (19, 47, 48), while only Silverman et al. found a significant interaction between sex and duration of smoking. On the other hand, in two larger pooled analyses by Lynch et al. in 2009 (49) and Bosetti et al. in 2012 (50), there were no significant interactions between sex and smoking.

Another important aspect of smoking is the association with risk of pancreatitis, particularly its chronic form. In a recent meta-analysis, Ye et al. (51) reported a 3-fold risk of chronic pancreatitis among ever smokers versus never smokers.

Passive tobacco smoking

The impact of environmental tobacco smoke (ETS), i.e. passive exposure to smoke, has not yet been thoroughly investigated. In a meta-analysis by Zhou et al. published in 2012 (52), there was no significant impact of exposure to ETS during childhood, at home or at work during adulthood, on risk of pancreatic cancer, however, they did not investigate the association of a combination of the aforementioned ETS exposures with risk. In the European Prospective Investigation into Cancer and Nutrition cohort (EPIC), never smokers with daily exposure to ETS both during childhood and at home and/or at work during adulthood, were reported to have an increased risk, with a hazard ratio (HR) of 3.8 (48). Potential sex differences concerning the association between exposure to ETS and pancreatic cancer risk do not seem to have been previously investigated.

Smokeless tobacco use – Moist snuff – Snus

Moist snuff, in Sweden called snus, is a smokeless tobacco product, sold and used in rather few countries, which has been given much attention during the past decades due to the scarce knowledge of its potential carcinogenic effects. One of the issues studying the impact of snus use on cancer risk is the major confounder smoking. In

a Swedish study, Luo et al. (53) reported a RR of 2.0 for pancreatic cancer in ever-users of snus compared with never-users of any tobacco products, and a significant dose-response relationship with increasing amounts of snus used. Additionally, a Norwegian study (54) reported a RR of 1.7 for ever-users of snus compared with never-users of snus. Both of these studies did however have limitations regarding the classification of non-smokers, as well as the study methodology, which may have caused residual confounding by smoking (55). Snus is predominantly used in men, which most likely explains the lack of studies investigating potential sex differences regarding its impact on pancreatic cancer risk.

Tobacco-specific nitrosamines, found in both tobacco smoke (56) and snus (57), have been suggested to have carcinogenic effects in the pancreas, along with numerous other substances, however, in snus it has been proposed to be the most important factor for the increased risk of pancreatic cancer seen in ever-users of snus (57).

Alcohol consumption

Alcohol consumption is closely related to smoking and other risk behaviours, and furthermore, there is an established association with both acute pancreatitis and chronic pancreatitis (58). It is therefore rather problematic to assess the causal relationship between alcohol consumption and risk of pancreatic cancer, and hitherto published studies are inconclusive. However, in the summary review of meta-analytical studies, by Maisonneuve et al. (43), a consumption of > 3 glasses of any alcoholic beverage, or ≥ 30 g of alcohol daily, was estimated to increase the risk of pancreatic cancer by 1.2 compared with no or occasional intake (< 1 drink/day). None of the studies referred to therein found any significant difference between men and women, however, it is emphasized that there is a clear difference in alcohol habits between the sexes, at least reported habits, rendering very small subgroups of women with heavy alcohol drinking habits (59-62).

Oral contraceptives

Reports on the associations between use of oral contraceptives (OC) and risk of pancreatic cancer are inconsistent. While several studies demonstrate an increased risk with long-duration OC use (41, 63), others claim an inverse association (39). In a meta-analysis by Tang et al. in 2015 (64), there was no association between OC use and risk of pancreatic cancer when pooling the RRs from all selected studies, however, when limiting the analysis to cohort studies only or to studies adjusting for smoking, diabetes and BMI, there was a positive relationship between ever use, compared with never use, of OC and risk.

Hormone replacement therapy

Hormone replacement therapy (HRT) is mainly prescribed to women with troublesome perimenopausal symptoms, either going through menopause naturally or from oophorectomy, and most commonly includes estrogen and progesterone combined in this setting (65, 66). However, it can also be administered after hysterectomy, as estrogen-only therapy. The number of studies investigating the influence of HRT use on risk of pancreatic cancer are limited, and have in general failed to demonstrate any associations. However, Lee et al. (63) suggested a protective effect, in particular in ever users of estrogen-only therapy and Lujan-Barroso et al. (67) reported a reduced risk only in hysterectomized users of HRT.

Other factors

Blood group

The risk of pancreatic cancer is reported to be increased in individuals with blood groups A, B or AB, with HRs of 1.3, 1.7 and 1.5 respectively, compared to blood group 0 (68).

Allergy

A decreased risk of pancreatic cancer has been demonstrated among individuals with a history of allergy, in particular among those with atopy related allergy. A suggested explanation for this association is that a hyperactive immune system provides an improved surveillance and protection against emerging cancer cells in the pancreas (69).

Physical activity

The associations between physical activity and risk of pancreatic cancer appear to be rather weak, however, consistent physical activity over a time period of more than 10 years may decrease the risk marginally (70).

Diet

In 2017, Zheng et al. (71) performed a systematic review of case-control and cohort studies investigating the associations between dietary patterns and risk of pancreatic cancer. They reported significant associations of animal products, starch rich products, and Western dietary patterns with an increased risk of pancreatic cancer, while dietary patterns encompassing higher intake of fruits and vegetables, vitamins and fibres, and the “Prudent diet”, were associated with a reduced risk. With another method to classify diet patterns, they concluded that a high quality dietary pattern conferred a protective effect against development of pancreatic cancer.

Coffee

There are several reports investigating the associations between coffee intake and risk of pancreatic cancer, however, results have been inconclusive. In a large meta-analysis from 2011, Turati et al. (72) could not demonstrate any impact of coffee consumption on risk of pancreatic cancer, irrespective of the volume of coffee intake.

Infections

Helicobacter pylori infection has been suggested to correlate with an increased risk of pancreatic cancer (73), the results are however inconclusive, with studies reporting no associations (74), an inverse association in Eastern countries (75), or associations only in never smokers and in individuals with low risk alcohol consumption (76).

In a meta-analysis, Luo et al. (77) demonstrated that previous, chronic and acute hepatitis B virus (HBV) infection was associated with an increased risk of pancreatic cancer compared to "never exposure to HBV".

Genetic alterations

Pancreatic cancer is a molecularly heterogeneous disease harbouring several genetic alterations, with the oncogene *KRAS* and the tumour suppressor genes *TP53*, *SMAD4/DPC4* and *CDKN2A* being most commonly mutated (2). Amplification of the *HER2* gene and activation of the *KRAS* gene are often the first occurring mutations in the carcinogenesis of pancreatic cancer, followed by inactivation of the *CDKN2A* gene, and later also loss of function of *TP53*, *SMAD4/DPC4* and *BRCA2* (78).

Activating mutations in *KRAS* are reported in approximately 70 - 90 % of pancreatic cancers (79, 80) and represent a key event in the carcinogenic initiation (78). The frequency of *KRAS* mutations in ampullary, distal bile duct and duodenal adenocarcinoma are considerably lower, with percentages of 30, 21 and 35 %, respectively (80). The role of the *KRAS* protein, when bound to guanosine triphosphate (GTP), includes regulation of cell behaviour such as proliferation, differentiation, cell migration and apoptosis (81). Activating *KRAS*-mutations can however occur in healthy tissue without causing carcinogenesis, and may thus alone not be sufficient for cell transformation (82).

Mutation of the *TP53* generally means inactivation of the gene, which is reported in 49 % of pancreatic cancers (80). An inactivating mutation in this gene causes loss of the tumour supressing properties of normal *TP53* protein, e.g. its anti-

proliferative effects, but may also cause an increased metastatic potential by gain-of-function activity (83).

Approximately 18 % of pancreatic cancers harbour inactivating *SMAD4*/*DPC4* mutations (80), which may lead to enhanced oncogenic signalling of transforming growth factor beta (TGFβ) (84). The metastatic properties may also be enhanced through loss of *SMAD4*/*DPC4* (85).

The reported mutation frequency of *CDKN2A* in pancreatic cancer is 14 % (80), and the properties of the two most well-known proteins products of this gene; p16 and p14arf, which modulate the cell cycle, may be affected by the mutation (86).

Clinical presentation and diagnostics

Due to the location of the pancreas, tumours arising in this area often present very late, and with quite unspecific symptoms. These are likely the two main reasons for the delayed diagnosis in the majority of the patients, but in light of the low incidence, screening would not be feasible in the general population. The most common symptoms are abdominal discomfort or pain, anorexia, early satiety, xerostomia, weight loss, constipation, dyspepsia and jaundice (87).

Radiology

There are several diagnostic instruments which can detect pancreatic cancer, however, they all have strengths and weaknesses. Abdominal ultrasound (AUS) is a non-invasive method, which is highly dependent on the patient's body constitution, tumour stage and the operator's experience, and therefore has a reported sensitivity of anywhere between 50 - 90 % (88).

Endoscopic ultrasound (EUS), via the stomach and duodenum, provides improved visualization compared with AUS, and also allows the operator to achieve fine-needle aspirations (FNA) from the tumour, which contributes to an increased sensitivity (87 %) and specificity (96 %) to diagnose a solid mass in the pancreas (89).

Accordingly, EUS-FNA has become the recommended endoscopic method of choice to obtain tumour tissue and has to a great extent replaced endoscopic retrograde cholangiopancreatography (ERCP) with brush cytology, a method that has a higher rate of post-operative complications and an inferior success rate compared to EUS (89). ERCP is however still an available instrument used for diagnostics, as well as in the palliative situation to, for example, relieve patients of jaundice by insertion of a stent into the biliary tree (90).

Computed tomography (CT) scan is however the most commonly used diagnostic tool, and with a pancreas-specific protocol and intravenous contrast, the CT generally provides sufficient information on tumour size and invasion of the surrounding vessels (91). Thereby, it offers a possibility to stage the tumour, and additionally, potential metastases can be detected, which is important information, since metastatic disease disqualifies the patient from resection of the primary tumour.

Magnetic resonance imaging (MRI) and magnetic resonance cholangiopancreatography (MRCP) are more modern techniques which have gradually become more common in the field of pancreatic cancer diagnostics. MRI and MRCP are however still most frequently used in situations where additional information is needed. For example, MRI is superior to CT when; the suspected tumour is small, there is hypertrophy in the pancreatic head or the pancreatic tumour is isoattenuating (88). MRCP is reported to be as sensitive as ERCP (92) and is further a better method than CT and MRI to help distinguish between pancreatitis and pancreatic cancer (93).

Biochemical markers

There is a plethora of omics studies on potential diagnostic, prognostic and predictive pancreatic cancer biomarkers (1). Preferably, to be suitable for screening purposes, a biomarker should be detectable in blood or other easily accessible body fluids, such as urine. However, there are currently no such biomarkers available that have sufficient sensitivity, specificity and reproducibility, and at a reasonable cost.

Carbohydrate antigen 19-9 (CA19-9) is the most extensively studied biomarker in blood, and it is a sometimes valuable complementing factor to strengthen the probability of a pancreatic cancer diagnosis. The sensitivity and specificity of roughly 80 % is however insufficient for this marker to be used more widely in screening and early detection (94). Furthermore, CA19-9 is frequently used to monitor regression and progression during treatment. Obstruction of the biliary tree causing cholestasis may increase CA19-9 levels, leading to potential false positive values.

In 2009, Harsha et al. (95) composed a summary of potential biomarkers for pancreatic cancer and reported a total of 2 516 genes, overexpressed at the mRNA, mRNA and protein, or protein level only.

Concerning biomarkers for improved prediction of response to systemic therapies, expression of the protein human equilibrative nucleoside transporter 1 (hENT1) has been reported to be a robust predictor of response to gemcitabine-based chemotherapy (96). Furthermore, high expression of hENT1 has been demonstrated to signify an improved OS in patients with pancreatic cancer receiving gemcitabine

(97, 98). In a study by our research group on periampullary adenocarcinoma (99), hENT1 expression was found to be an independent predictor of a prolonged recurrence-free survival (RFS) in patients with I-type tumours treated with adjuvant chemotherapy, regardless of regimen, but was not prognostic in PB-type tumours.

Clinicopathological factors

There are several clinicopathological factors used to describe the characteristics of a pancreatic cancer, some more commonly used than others.

Tumour node metastasis

The magnitude and spread of a pancreatic cancer, i.e. the tumour stage, is most commonly defined by the tumour node metastasis (TNM) system, which is described in the Cancer Staging Manual compiled by the American Joint Committee of Cancer (AJCC). Until recently, T was defined by the largest diameter and anatomic spread of the primary tumour as follows; T1: ≤ 2 cm, T2: > 2 cm, T3: extension outside the pancreas without involvement of the celiac axis or superior mesenteric artery (SMA) and T4: involvement of the celiac axis or SMA and defined as unresectable. Further, N and M referred to the absence (N0 and M0) or presence (N1 and M1) of regional lymph node metastases and distant metastases, respectively (100). However, based upon reports demonstrating "size only" to be a superior prognosticator compared to size and tumour spread combined (100, 101), and a prognostic impact not only of the presence, but also of the number of regional lymph node metastases (100, 102, 103), the T and N definitions were adjusted for the 8th Edition of the AJCC Cancer Staging Manual (100, 104). The T1 - T3 stages now refer to tumour size only (T1: ≤ 2 cm, T2: > 2 cm ≤ 4 cm and T3: > 4 cm), and T4 has been slightly modified to also include invasion of the common hepatic artery (CHA), and the definition "unresectable" has been removed. Further, N is now more specifically defined by the number of regional lymph node metastases (N0: Node negative, N1: 1 - 3 and N2: ≥ 4 regional lymph node metastases).

Moreover, the different combinations of T, N and M are jointly interpreted as the stage of the tumour disease, which is described with roman numerals (I - IV) and Latin alphabetic letters (A and B). A summary of the 7th and 8th edition of the TNM classification system for pancreatic adenocarcinoma is shown in Table 1.

Of note, the TNM classification system varies somewhat between the different origins of periampullary tumours. The T-stage definitions for tumours of the distal bile duct and pancreas are similar, with size-based T1 - T3, and identical T4 definitions, i.e. invasion of the celiac axis, SMA or CHA. This T4 definition is also

shared by ampullary adenocarcinoma, however, in these tumours T2 defines a tumour invading the muscularis propria of the duodenum, and T3 a tumour invading the pancreas. For duodenal adenocarcinoma, T2 is defined as invasion of the muscularis propria, T3 as invasion of the mesentery or retroperitoneum, and T4 as invasion of other organs or structures (104).

Table 1
Summary of the 7th and 8th AJCC staging systems for pancreatic adenocarcinoma. (Adapted from (100))

Seventh edition of the AJCC staging system		Eighth edition of the AJCC staging system	
Primary tumour (T)			
T1	Localised to the pancreas. Largest diameter ≤ 2 cm.	**T1**	Localised to the pancreas. Largest diameter ≤ 2 cm.
T2	Localised to the pancreas. Largest diameter > 2 cm.	**T2**	Localised to the pancreas. Largest diameter > 2 cm and ≤ 4 cm.
T3	Spread outside the pancreas. No involvement of celiac axis or SMA.	**T3**	Localised to the pancreas. Largest diameter > 4 cm.
T4	Involvement of celiac axis or SMA (unresectable).	**T4**	Involvement of celiac axis, SMA or CHA.
Regional lymph nodes (N)			
N0	Absence of regional lymph node metastases.	**N0**	Absence of regional lymph node metastases.
N1	Presence of regional lymph node metastases.	**N1**	1 - 3 regional lymph node metastases.
		N2	≥ 4 regional lymph node metastases.
Distant metastases (M)			
M0	Absence of distant metastases.	**M0**	Absence of distant metastases.
M1	Presence of distant metastases.	**M1**	Presence of distant metastases.
STAGE			
IA	T1, N0, M0	**IA**	T1, N0, M0
IB	T2, N0, M0	**IB**	T2, N0, M0
IIA	T3, N0, M0	**IIA**	T3, N0, M0
IIB	T1 - T3, N1, M0	**IIB**	T1 - T3, N1, M0
III	T4, N0 - N1, M0	**III**	T1 - T4, N2, M0 or T4, N1 - N2, M0
IV	T1 - T4, N0 - N1, M1	**IV**	T1 - T4, N0 - N2, M1

Differentiation grade

Pancreatic tumours are heterogenous and the differentiation grade of a tumour is therefore often based on the poorest differentiation grade seen in a significant portion of the tissue sample (105).

Invasion of the vascular and lymphatic system

Additional features recorded at the evaluation of tumour tissue specimens are invasion of microscopic blood vessels and lymphatic vessels, which, if present, have been shown to correlate with an impaired prognosis in periampullary adenocarcinoma (9).

Perineural growth and invasion of peripancreatic fat

In several reports, perineural invasion has been associated with a shorter survival in patients with pancreatic cancer (106). Tumour growth into peripancreatic fat is a more uncommonly assessed variable, although it has been associated with a shorter survival in periampullary adenocarcinoma (107), as well as in pancreatic cancer (108).

Resection margins

Approximately 30 % of all patients with resected pancreatic cancer have an isolated local recurrence without confirmed metastases (109), proposing that residual tumour tissue is often present after surgery, despite a visually radical tumour resection. Therefore, the resection margins are examined microscopically, and most commonly reported as R1 if there is 1 mm or less between cancer and any margin, and as R0 if this distance is more than 1 mm. However, this definition is not fully established since some pathologist communities recommend that R1 should be defined as presence of cancer at definite resection margin (110). Nevertheless, resection margin status is reported to be an important prognosticator for pancreatic cancer, and according to the firstly mentioned classification system, the survival time of patients with R0 resected tumours is significantly improved compared to patients with R1 resection. Furthermore, among R1 resected cases, there is a significant survival benefit if the resection margin is 0 - 1 mm compared to 0 mm. The reported proportions of R1 resected tumours vary a lot throughout the literature, ranging between 17 - 85 %, which is suggested to be a result of inconsistent definitions of resection margins and a lack of standardized pathological examination routines (110).

Treatment

Being a highly chemo- and radiotherapy resistant malignancy, pancreatic cancer is extremely complicated to treat. Additionally, in contrast to many other types of solid cancers, neither targeted therapies nor immunotherapy have proven to be efficient, and there is also a profound lack of predictive biomarkers to assist the oncologist in the choice of therapy.

When a patient presents with pancreatic cancer, his/her case is discussed at a multidisciplinary conference (MDC), generally with a surgeon, oncologist, pathologist and radiologist being present. At the MDC, the decision is firstly made on whether the patient is eligible for surgical tumour resection or not, thus with a curative intention. In some cases, the tumour is assessed to be borderline resectable, suggesting a potentially curative setting if downstaging of the tumour with chemotherapy is successful. In the majority of the cases, however, the tumour is already locally advanced, and/or has given rise to distant metastases at the time of diagnosis (111), which implicates that the setting of the patient care will be solely palliative, potentially including administration of chemotherapy and sometimes radiotherapy to hopefully prolong survival.

Surgical resection

A surgically performed radical resection of the cancer lesion stands the only treatment option with curative potential, but, unfortunately, the proportion of patients diagnosed with pancreatic cancer who meet the requirements for surgical resection is only approximately 15 - 20% (111). The most common procedure is pancreaticoduodenectomy, which was described as early as in 1898, but first performed in 1912. The method was however improved by Allen Whipple in 1935, from whom it has also been named the Whipple procedure (112), and generally, the procedure includes removal of the pancreatic head, the common bile duct, the gallbladder, segments of the duodenum and the distal portion of the stomach (113).

Thus, roughly 80 % of the patients present with locally advanced tumours, which are not possible to resect surgically. Tumour infiltration of the portal vein (PV) and/or superior mesenteric vein (SMV) is one of the greatest issues, and was previously considered an absolute contraindication for the procedure, however, with improved surgical techniques, venous resection and reconstruction have become possible and more commonly practiced, showing similar results on outcome compared with patients undergoing pancreaticoduodenectomy of tumours without venous engagement (114). Arterial involvement is far more problematic since these tumours often infiltrate the celiac plexus as well, thus making arterial resection and reconstruction technically complicated and rarely successful (115, 116).

Conclusively, the criteria for resectability are; no involvement of the celiac artery, CHA and SMA, involvement of the PV and SMV being less than half of their circumference, sufficient portal/splenic vein confluence and absence of metastases outside of the routine surgical area (117). However, in rare occasions, exceptions are made regarding the distant metastases criteria, e.g. in patients with single liver metastases responding well to primary or neoadjuvant chemotherapy, where the primary tumour may be resected irrespective of residual liver disease, which has been reported to improve survival (118).

Chemotherapy

Accordingly, chemotherapy can be administered as neoadjuvant treatment in the preoperative setting for downstaging of borderline resectable tumours (119), as adjuvant treatment in the postoperative setting to eliminate potential local tumour residuals or micro-metastases (120), or, most commonly, in the palliative setting with the purpose to suppress symptoms and prolong the patient's life (121). A summary of the most commonly used chemotherapeutic drugs is shown in Table 2.

Table 2
Summary of common chemotheraputic drugs in the treatment of pancreatic cancer

Chemotheraputic drug	Characteristics
Fluorouracil (5-FU)	A pyrimidine analogue and antimetabolite which blocks thymidylate synthase, causing lack of the DNA nucleoside thymidine and thereby cell death. To increase the effect of 5-FU, folinic acid/leucovorin/calcium folinate is added (122).
Oxaliplatin	A platinum based drug that forms cross links in the DNA. This hinders DNA replication and transcription, and thereby inhibits DNA synthesis (123).
Irinotecan	A cytotoxic drug, which inhibits the enzyme topoisomerase I, causing DNA to break and thereby cell death (124).
Liposomal irinotecan (nal-Iri)	Irinotecan with the addition of a liposomal construct, which increases the circulation time of irinotecan, causing a prolonged release of the drug (125).
Gemcitabine	A cytidine analogue and antimetabolite, which inhibits the nucleotide excision repair system of DNA (126).
Nab-paclitaxel	Paclitaxel: A cytoskeletal taxane based drug, which targets tubulin, causing inhibition of mitosis and thereby apoptosis. Paclitaxel is hydrophobic and therefore requires toxic solvents to be intravenously administrable. When bound to albumin (Nab-paclitaxel) the toxicity of the drug decreases and it can be administered without exposure to solvents (127, 128).
Capecitabine	An orally administered prodrug to 5-FU, which gradually converts into 5-FU by thymidine phosphorylase in the liver (129).
S1 (Teysuno®)	An orally administered drug containing tegafur (prodrug of 5-FU), gimeracil (blocks DPD*, and thereby inhibits 5-FU degradation) and oteracil (blocks OPRT**, and thereby inhibits the production of 5-FU in the stomach, which in turn decreases gastrointestinal toxicity) (130).

*Dihydropyrimidine dehydrogenase, **Orotate phosphoribosyltransferase

Neoadjuvant chemotherapy

Neoadjuvant treatment is currently not standard of care for resectable tumours, however several ongoing studies are evaluating the effect of neoadjuvant chemotherapy compared to surgery-first, e.g. the Nordic Pancreatic Cancer Trial (NorPACT). The current role of neoadjuvant chemotherapy is downstaging of borderline resectable tumours, and sometimes locally advanced tumours, however, due to the absence of randomized controlled trials (RCT) comparing different preoperative regimens, there is no specific substance that is more strongly recommended. The most commonly used regimens in the neoadjuvant setting are "FOLFIRINOX", which is a combination of folinic acid, 5-FU, irinotecan and oxaliplatin, or a gemcitabine-combination, supported by evidence of a beneficial effect from those regimens in the treatment of metastatic disease (131). Hence, patients with borderline resectable tumours, showing good response to neoadjuvant chemotherapy with successful downstaging of the tumour, can have their tumours resected, and there is growing evidence of a similar survival among these patients compared with patients undergoing surgery upfront (132). Furthermore, postoperative morbidity and mortality appear to be similar, or even lower, among patients receiving neoadjuvant chemotherapy compared with patients who do not (133, 134), and the latter of those studies also reports a better OS for patients receiving perioperative chemotherapy (neoadjuvant + adjuvant) compared to those only receiving adjuvant chemotherapy. Additionally, the OS among patients who have their tumours resected is more than twice the OS among those who have unresectable tumours (132).

Adjuvant chemotherapy

The use of adjuvant chemotherapy in pancreatic cancer is markedly more common than that of neoadjuvant therapy. All patients subjected to primary tumour resection are offered adjuvant treatment, with the standard therapy of choice consisting of gemcitabine plus capecitabine (GemCap), based on the results from the ESPAC 4 trial in 2016 (135). However, in the recent PRODIGE 24-ACCORD/CCTG PA trial, a modified regimen with FOLFIRINOX (mFOLFIRINOX), not including the 5-FU bolus and with a lower irinotecan dose, was compared to gemcitabine, showing a superior outcome on survival, with a median OS (mOS) of 54.4 months compared to 35.0 months for gemcitabine (136). Accordingly, instead of GemCap, mFOLFIRINOX may be considered in the adjuvant setting for patients with adequate performance status, however, confirmatory studies are warranted, in particular due to the very long mOS in the gemcitabine arm of this study, which is remarkable.

Concerning adjuvant treatment of adenocarcinoma of the ampulla and distal bile duct, there is an evident lack of studies addressing this topic, and treatment of these periampullary cancers is mainly based on studies on pancreatic cancer (137, 138).

The ESPAC 3 trial did however investigate the effects of adjuvant 5-FU or gemcitabine compared to no chemotherapy in periampullary adenocarcinoma, and demonstrated an improved survival among patients receiving any of these two regimens (139). Of note, the response to gemcitabine differs between I-type and PB-type tumours, and therefore it has been suggested that subdivision of these morphological subtypes is of great importance in trials on adjuvant treatment in periampullary adenocarcinoma (140). Furthermore, it may be clinically relevant to consider distal bile duct adenocarcinoma as a completely different entity, which thus should be analysed separately from the other periampullary adenocarcinomas. Based on a recent systematic review of reports on adjuvant treatment for resected biliary tract cancer, the recommendation from the ASCO Clinical Practice Guideline is that these patients should be offered adjuvant treatment with capecitabine for 6 months (141).

Treatment of duodenal adenocarcinoma has often been studied in combination with adenocarcinoma of the jejunum and ileum. One study has reported improved disease-free survival (DFS) among patients receiving any adjuvant therapy compared to those not receiving adjuvant therapy (142), and another study reported an improved survival among patients with stage III cancer receiving adjuvant therapy (143). Most commonly, however, duodenal and ampullary adenocarcinoma with I-type morphology are treated with 5-FU-based chemotherapy, in accordance with the treatment guidelines of colorectal cancer.

Palliative chemotherapy

As previously mentioned, the vast majority of the patients are diagnosed with locally advanced or metastatic disease, and there is currently no possibility of cure for these patients. However, if the patient's performance status allows for it, chemotherapy or chemoradiotherapy (CRT) can be offered with the main intention to prolong the patient's survival. Still, the survival is most often just slightly improved, but the treatment may also to some extent provide relief of symptoms (121). Treatment administered under these circumstances is called palliative treatment, which thus plays the major role in the oncological care of these patients. To initiate palliative chemotherapy in a patient with a non-resectable tumour, a tumour sample is still needed to establish the diagnosis, for example acquired through FNA, core-needle biopsy or surgical biopsy. Most commonly, these cytological or histological samples are sparse and therefore insufficient for determination of tumour origin, however, they may aid in the differentiation between I-type and PB-type adenocarcinoma. The most commonly used regimens of chemotherapy in the palliative setting are 5-FU- or gemcitabine-based therapies, either of which can be chosen as first line treatment, and if feasible, most often followed by the other when the patient shows progress or signs of considerable toxicity (second line treatment) (131, 144, 145).

Radiotherapy

The role of radiotherapy in the treatment of pancreatic cancer is rather limited, and primarily relevant in the palliative setting, however, in rare occasions, radiotherapy may also be considered as pre- and/or postoperative treatment in patients with resectable or borderline resectable tumours.

Neoadjuvant radiotherapy

In the neoadjuvant situation, radiotherapy is seldom used, and in the cases where it is assessed to be beneficial for the patient it is given in addition to chemotherapy, i.e. as CRT. Neoadjuvant CRT is considered in patients with borderline resectable tumours, and the intention is to facilitate a margin-negative tumour resection (146), however, investigations have shown conflicting results, and recently Lutfi et al. (134) reported that there is no survival benefit from CRT compared to chemotherapy alone in patients with resected clinical stage I - II pancreatic cancer.

Adjuvant radiotherapy

In selected patients with non-radically resected tumours, radiotherapy can be considered as adjuvant treatment for local control (147), even though these patients are by definition in a palliative situation. Furthermore, in 2013, a large meta-analysis reported no improved survival among patients who were treated with adjuvant radiotherapy in addition to chemotherapy (148).

Palliative radiotherapy

Accordingly, radiotherapy may be used for palliation in patients with margin-positive resection of the primary tumour to improve survival (147), and, according to the ASCO guidelines from 2016 it should also be considered in patients with local progression without metastasis after induction chemotherapy. Furthermore, in patients with a good response to palliative chemotherapy and stable disease, treatment can be temporarily switched to local radiotherapy of the primary tumour to allow the patient a pause from chemotherapy, particularly when there is difficult toxicity. Moreover, radiotherapy in metastatic pancreatic cancer is mainly applied against metastases to relieve patients of symptoms, such as symptomatic brain or bone metastases (149).

Personalised treatment, targeted therapy and immunotherapy

Due to the heterogeneity of pancreatic cancer and its chemo- and radiotherapy resistant characteristics, increasing attention is drawn to a more personalised treatment approach in these patients. Still, however, the revolutionary breakthrough in the field of targeted therapy and immunotherapy has not yet arrived.

The epidermal growth factor receptor (EGFR) has been suggested as one of the potential attack points for targeted therapy, since it is commonly overexpressed in pancreatic cancer (150, 151). Erlotinib, a tyrosine kinase inhibitor (TKI) which inhibits mutant EGFR (152), administered in combination with gemcitabine, has been reported to improve progression-free survival (PFS) and overall survival (OS), however, only with a minor improvement compared to gemcitabine alone (153).

Poly(ADP-ribose) polymerase (PARP) inhibitors constitute another potential treatment option in pancreatic cancer (154). Particularly, in the treatment of solid tumours, response to PARP inhibitors has been demonstrated in patients harbouring germline "loss-of-function" mutations in the breast cancer gene 1 (*BRCA1*) and/or 2 (*BRCA2*) (155). Mutations in *BRCA* have been reported in approximately 4 % of pancreatic cancers (156). However, there are not yet any large RCTs investigating the effects of PARP-inhibitors on survival in patients with pancreatic cancer.

The role of immunotherapy in pancreatic cancer treatment is still very limited, and trials have mostly failed to provide useful immunotherapeutic drugs. Treatment with checkpoint inhibition targeting for example cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), programmed cell death-ligand 1 (PD-L1) or lymphocyte activation gene 3 (Lag-3) have failed to provide beneficial effects in patients with pancreatic cancer (157). However, periampullary adenocarcinomas with mismatch repair deficiency (dMMR) may respond to PD-L1 receptor (PD-1)/PD-L1 blockade (158), and in 2017, the U.S. Food and Drug Administration (FDA) approved the use of pembrolizumab in patients with solid tumours displaying dMMR, regardless of tumour origin.

Tamoxifen

Tamoxifen is a triphenylene-based, non-steroidal, selective estrogen receptor (ER) modulator (SERM), with a complex variety of agonist- and antagonist-like effects (159). Tamoxifen has been studied in details in breast cancer, and if the ER is present in these tumours, the effect of tamoxifen is primarily anti-estrogenic, through the inhibition of estrogen binding to ER, which decreases the tumour growth (160). Tamoxifen also exerts several other anti-tumoural mechanisms than through ER-signaling. These include for example stimulation of transforming growth factor-α (TGF-α) and inhibition of mitogenic signaling from fibroblasts through inhibition of protein kinase C, mechanisms that result in an anti-angiogenic

effect (161, 162). Furthermore, tamoxifen may decrease endothelial mitosis and exert anti-metastatic effects through reducing the release of metastatic factors (162). Additionally, in postmenopausal breast cancer patients, tumoural expression of the progesterone receptor (PR) has been reported to be a strong predictor of treatment response to tamoxifen (163, 164). Since PR is found downstream of ER, expression of PR has been suggested to mirror an intact ER pathway, thus representing a predictor for endocrine therapy. Moreover, some ER positive/PR negative breast tumours may be resistant to tamoxifen but show response to aromatase inhibitors (165), proposing that other pathways are still functioning.

Some decades ago, several trials reported beneficial effects of tamoxifen treatment in patients with advanced pancreatic cancer (166-168). In particular, the subgroup of older women had an improved survival compared to controls not receiving tamoxifen (169, 170). These trials were conducted as a sequel of the discovery of ER expression in pancreatic tissue and pancreatic cancer of both rats and humans (171, 172), although, remarkably, biomarker analyses were not included in any of these studies, and neither were the potential mechanisms behind the favourable effects of tamoxifen discussed. Moreover, Rosenberg et al. (173) treated patients with tamoxifen plus octreotide, which led to a prolonged survival compared to controls, in both resected and unresected patients. Tamoxifen has also been studied in combination with chemotherapy (174, 175), conferring a plausible survival benefit, but since the trial design did not include any controls, the results should be interpreted with caution. Conflictingly, several trials have failed to confirm the beneficial effects of tamoxifen (176-180). The majority of these studies were however very small and only observational studies. A summary of trials on tamoxifen treatment in pancreatic cancer is shown in Table 3.

Table 3
Summary of trials investigating the effects of tamoxifen tratment in pancreatic cancer

Author, year	Investigated drugs	Treatment effect
Theve et al. 1983 (166)	Nolvadex 20 mg x 2 (n = 14) vs. historical controls (n = 692).	mOS: 8.5 vs. 2.5 months. 3 vs. 0 alive > 21 months.
Tönnesen et al. 1986 (167)	Nolvadex 10 mg x 3 (n = 10) vs. historical controls (n = 14).	mOS: 7 vs. 3 months. 40 % vs. 0 % alive at 14 months.
Crowson et al. 1986 (176)	Tamoxifen 160 loading dose, then 40 mg x 1 (n = 14).	mOS: 5.3 months. No one with partial/complete response.
Keating et al. 1989 (177)	Tamoxifen 20 mg x 2 (n = 37) vs. controls (n = 39).	mOS: 5.3 vs. 3.0 months. Unadjusted p = 0.071, adjusted p = 0.09.
Bakkevold et al. 1990 (169)	Tamoxifen 30 mg x 1 (n = 92) vs. placebo (n = 84).	mOS: 115 vs. 122 days, non-significant. 3 vs. 0 alive at 30 months.
	Women, stage III cancer (n not specified).	mOS: 191 vs. 45 days, p = 0.011.
Scheithauer et al. 1990 (178)	Tamoxifen 20 mg x 2 (n = 19).	No one with objective tumour reponse.
Wong et al. 1993 (170)	Tamoxifen 20 mg x 2 (n = 80) vs. matched controls (n = 80).	mOS: 7 vs. 3 months, p < 0.0001.
	Women, age > 60 years (n = 35 vs. 32).	mOS: 12 vs. 3 months.
Taylor et al. 1993 (180)	Tamoxifen 20 mg x 2 (n = 22) vs. placebo (n = 22).	mOS: 75 vs. 131 days, non-significant. No difference in quality of life.
Swarovsky et al. 1993 (179)	Women only (n = 5). Tamoxifen 30 mg x 1 and buserelin.	No one with stable disease/remission during 6 months of follow-up.
Rosenberg et al. 1995 (173)	Tamoxifen 10 mg x 3 and octreotide (n = 12) vs. historical controls (n = 68).	mOS: 12 vs. 3 months. 1-year survival rate: 59 % vs. 16 %.
	Treated: resected vs. unresected (n = 5 vs. 7).	mOS: 20 vs. 12 months. 1-year survival rate: 80 % vs. 31 %.
	Resected: treated vs. controls (n = 5 vs. 9).	mOS: 20 vs. 12 months. 1-year survival rate: 80 % vs. 44 %.
	Unresected: treated vs. controls (n = 7 vs. 59).	mOS: 12 vs. 2.5 months. 1-year survival rate: 31 % vs. 11 %.
		Treatment and resection independent predictors of OS, p < 0.01. Non-significant interaction.
Horimi et al. 1996 (168)	Tamoxifen 20 mg x 1 and chemo- and immunotherapy (n = 37) vs. chemo- and immunotherapy (n = 28).	1-year survival rate: 65.7 % vs. 22.0 %. 2-year survival rate: 26.3 % vs. 8.8 %. p < 0.01
	Pancreatic head tumour (n = 30 vs. 21).	1-year survival rate: 68.0 % vs. 18.8 %. 2-year survival rate: 24.5 % vs. 0.0 %. p < 0.01
	Pancreatic head tumour, women (n = 13 vs. 7).	1-year survival rate: 53.8 % vs. 21.4 %. 2-year survival rate: 53.8 % vs. 0.0 %. p < 0.01
	Pancreatic head tumour, men (n = 17 vs. 14).	1-year survival rate: 85.6 % vs. 19.1 %. 2-year survival rate: 18.1 % vs. 0.0 %. p < 0.01
	Pancreatic body/tail tumour (n = 7 vs. 7).	1-year survival rate: 57.1 % vs. 28.6 %. 2-year survival rate: 38.1 % vs. 14.3 %. p < 0.05
	Non-curative tumour (n = 15 vs. 19).	1-year survival rate: 57.1 % vs. 18.4 %. 2-year survival rate: 22.9 % vs. 0.0 %. p < 0.05

Eckel et al. 2000 (174)	Tamoxifen 20 mg x 1 and cyclophosphamide and leucovorin and 5-FU (n = 50).	mOS: 8.5 months. Median time to progression: 4.6 months. Partial response: 6 %. Minor response: 4 %. Stable disease > 2 months: 64 %. 1-year survival rate: 28 %.
Tomao et al. 2002 (175)	Tamoxifen 20 mg x 1 and gemcitabine (n = 27).	mOS: 8.0 months. Median time to progression: 4.5 months. Partial response: 11 %. Stable disease for 8 weeks: 48 %. Disease progression: 41 %. 1-year survival rate: 31 %. Clinical benefit: 59 %, median duration: 13 weeks.

Prognosis

In Sweden, the National Board of Health and Welfare reported a relative 5-year OS for patients with pancreatic cancer of 8.3 % and 7.9 % for women and men, respectively, in 2016 (12). These numbers appear rather high, partly because they are relative calculations, i.e. based on a comparison with a sample of healthy individuals with the same sex and age, but also because Swedish healthcare is more advanced than in many other countries. Worldwide, 5-year survival rates of 2 - 9 % have been reported (111). Survival is highly dependent on the limited portion of patients eligible for surgical resection, however, the recurrence rate among surgically treated patients is still very high, approximately 90 % (12).

The mOS for resected patients has been reported to be 17 - 23 months, while the survival for patients with locally advanced or metastatic tumours is significantly shorter, with reported mOS of 8 - 14 and 4 - 6 months, respectively (23).

Investigative biomarkers

Hormone receptors

Estrogen receptors

In the literature, two main types of estrogen receptors are reported, alpha (ERα) and beta (ERβ), which are both members of the nuclear receptor protein family and constitute so called transcription factors. Accordingly, ERs are found mainly in the cell nucleus, but also in the mitochondria or cytoplasm, and together with estrogens they are involved in several physiological mechanisms of the human body. These mechanisms are often rather complex, and encompass regulation of cell growth, reproduction, development and differentiation (181). Estrogen in premenopausal women is primarily synthesised in the ovaries, as 17β-estradiol (E2), which exerts its regulatory effects either locally or systemically in the cells of its target organs. Furthermore, the production of E2 in men and in postmenopausal women is depending on the cytochrome P450 aromatase enzyme, which converts androstenedione and testosterone into E2, a process which takes place in extragonadal tissues, such as the brain, breasts and adipose tissue (182). E2 and ERs do however not only have important roles in normal processes in healthy tissue, but also in many diseases, where abnormal ER signalling is part of the development of, for example; osteoporosis, cardiovascular disease and tumours (183-185).

ERα is mainly expressed in the reproductive organs, the ovaries and the uterus, but also in breast tissue, the kidneys, bone, adipose tissue and the liver (181). Additionally, the phenomenon “alternative splicing” of ER-mRNA can result in several isoforms of ERα, and to date, three isoforms have been identified: ERαΔ3, ERα36 and ERα46 (181). Progesterone has been shown to inhibit ERα-activity (186). Similar to ERα, ERβ has been reported to be present in the ovaries and kidneys, but also in cells of the central nervous system (CNS), immune system, blood vessels, heart, lungs, colon, male reproductive organs and prostate. Furthermore, four isoforms of ERβ have been described: ERβ2, ERβ3, ERβ4 and ERβ5 (181).

The presence of ERα and/or ERβ has been demonstrated to be of prognostic and predictive value in several types of cancer, in particular in cancer of the breast, but

also of the endometrium, ovaries, prostate, lung and colon (181, 183). In normal breast as well as in prostate tissue, the expression of ERα is low and ERβ high, respectively, with an inverse relationship in cancer of these tissues (187-189). Furthermore, a decrease or loss of ERβ in breast cancer, and preservation of ERβ in prostate cancer, has been reported to signify an impaired prognosis (190, 191). Ovarian carcinogenesis has been shown to be accompanied by a decreased expression of ERβ, and accumulation of ERβ in the cytoplasm, which has also been reported to correlate with an impaired DFS (192).

Of note, there is a profound issue with research on ERβ, namely the lack of reliable antibodies harbouring high sensitivity and specificity for this receptor (193), and therefore previous studies on ERβ are now being questioned. In the report by Andersson et al., a thorough examination and validation of available anti-ERβ antibodies was performed and only one antibody, the monoclonal PPZ0506, was found to specifically bind to ERβ. Forty-four different normal tissue and 21 different human cancers were exposed to this antibody for protein profiling, and ERβ was only found to be present tumours in the testis, ovary, lymphoid cells, and selected malignant melanoma and thyroid cancer. ERβ was however not expressed in neither normal nor malignant breast tissue. Moreover, these findings were well in line with RNA-sequencing data (193).

Progesterone receptors

The progesterone receptor (PR) is an intracellular nuclear receptor encoded by the progesterone receptor gene (PGR), which requires estrogen and ER for synthesis in normal as well as cancer cells (194). There are three isoforms of PR; PR-A, PR-B and PR-C (195), however, recent studies claim that PR-C is not present in vivo and has no physiological activity in progesterone signaling (196). In normal human tissue PR-A and PR-B are expressed in rather similar proportions (197), and particularly in female reproductive organs such as the breast and uterus, but also in the CNS. Upon binding of its ligand progesterone, PR controls development, differentiation and proliferation of cells in target tissues through regulation of gene networks (198).

The mechanisms of the different PR isoforms in human tissue is not yet fully understood, but PR-A is known to exert antagonistic effects on PR-B, while PR-B upregulates the effects of progesterone (199). Furthermore, in mice, PR-A and PR-B have been shown to have important roles in the development of the uterus and reproductive system (200), and the mammary gland (201), respectively.

Numerous studies have investigated the importance of progesterone in breast cancer, and strong evidence supports that it is the major proliferative hormone in the epithelium of the human breast (202, 203). An increased risk of breast cancer

has been observed in postmenopausal women using HRT (204), and progesterone has been suggested to be the underlying factor for this relationship (205). Furthermore, PR positivity has been reported to correlate with an improved survival, overall and among patients with ER positive tumours treated with endocrine therapy (206, 207).

The androgen receptor

The androgen receptor (AR) is another member of the steroid-hormone receptor family. AR binds androgens, particularly testosterone and dihydrotestosterone (DHT), an action that further controls transcription and thereby expression of specific genes (208). For example, binding of testosterone and DHT to AR regulates sexual development and differentiation in men (209).

The role of the AR in cancer has been most frequently studied in prostate cancer, where stimulation of androgens has been shown to influence both development and progression of the disease. Accordingly, treatment with drugs reducing the exposure of testosterone and DHT to AR expressed by these tumours, or obstructing their possibility of binding, is now widely used in the treatment of prostate cancer (209). Furthermore, the AR has been reported to play an important part in the pathogenesis of breast cancer, in particular triple-negative breast cancer, where 50 % of the tumours have been approximated to be androgen dependent (210). This has given rise to several studies on drugs with AR antagonizing effects or regulatory effects on androgen synthesis.

Estrogen, progesterone and androgen receptors in pancreatic neoplasia

Previous studies investigating the expression of ER in human pancreatic cancer are scarce (172), and more recent studies have had much focus on ERβ (211, 212) and phosphorylated ERβ (pERβ) (213). The number of reports on PR expression in these tumours are even fewer (211). The prognostic impact of ER and PR therefore stand without strong evidence, however, presence of ERβ and pERβ have been suggested to signify inferior prognosis (212, 213).

In MCNs, PR is commonly expressed in the ovarian-type stroma (OTS), a characteristic feature of these potentially premalignant neoplasms (214). Decreased or lost expression of PR in the OTS of theses tumours has been reported to correlate with high-grade dysplasia (HGD)/carcinoma in-situ (CIS) or invasive carcinoma (215).

The presence and potential importance of the AR in pancreatic neoplasms is poorly defined, however a few studies have observed AR expression in pancreatic cancer cell lines (216). In a RCT, Greenway et al. (217) compared treatment with the non-

steroidal antiandrogen flutamide versus placebo for local as well as metastasized pancreatic cancer, demonstrating a mOS of 12 and 5 months, respectively, among those treated for > 6 months. Of note, the pancreatic cancer diagnosis was not histologically confirmed in this study, and several others have failed to confirm this beneficial effect of anti-androgenic therapy in pancreatic cancer (177, 218, 219)

Aims of the thesis

- To examine the potential influence of pre-diagnostic anthropometric measures, smoking and alcohol on risk of pancreatic cancer, with particular reference to sex differences.
- To examine the potential influence of female hormonal and reproductive factors on risk of pancreatic cancer.
- To examine the expression, clinicopathological correlates and potential prognostic significance of estrogen (ER) and progesterone receptor (PR) expression in pancreatic and other periampullary adenocarcinoma.
- To compose a protocol for a single-centre, randomized, double-blind, placebo-controlled, two-arm, phase II clinical trial investigating the potential effects of tamoxifen on survival and quality of life in women with advanced pancreatic cancer.

Material and Methods

Patients

Cohort 1

This cohort consists of all participants in the Malmö Diet and Cancer Study (MDCS), which is a Swedish, population-based, prospective cohort, being part of the European Prospective Investigation into Cancer and Nutrition (EPIC) (220, 221). The MDCS/EPIC encompasses 28 098 individuals (17 035 women and 11 063 men), recruited between 1991 – 1996, and has been described in more detail previously (222, 223). Up until 31 December 2013, a total number of 163 histologically confirmed cases of incident pancreatic cancer was reported among the study participants (102 women and 61 men). A flowchart of the cohort is shown in Figure 2.

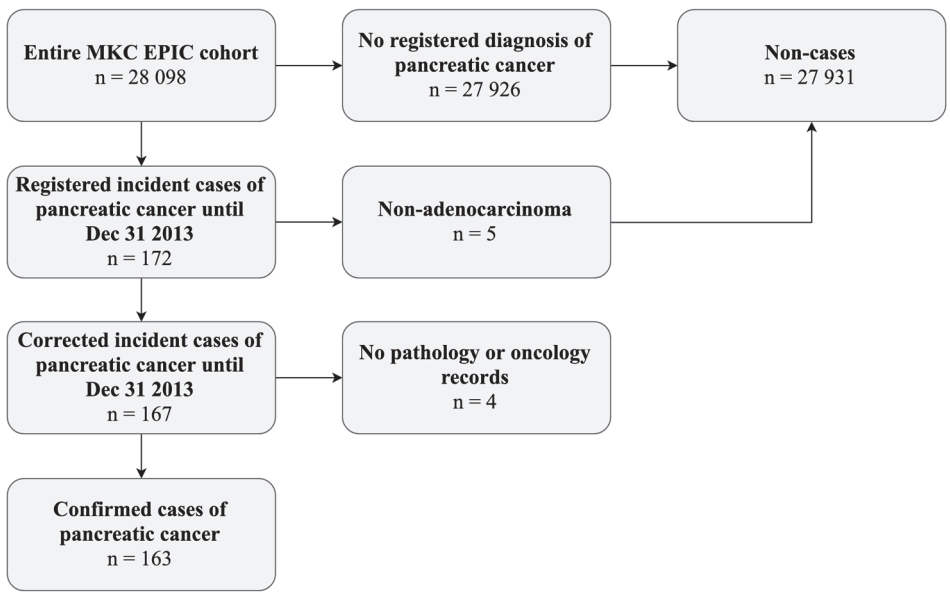

Figure 2
Flowchart of cohort 1.

Cohort 2

This cohort consists of all women in the MDCS/EPIC, with an updated follow-up at 31 December 2015, rendering a total number of 110 histologically confirmed cases of pancreatic cancer.

Cohort 3

This cohort is a retrospective consecutive cohort of 175 patients diagnosed with periampullary adenocarcinoma, who were all resected with pancreaticoduodenectomy in the Malmö and Lund University Hospitals, Sweden, between 1 January 2001 and 31 December 2011. The cohort was assembled by a systematic review of pathology records from this period and re-evaluation of all haematoxylin and eosin stained tumour slides by a board-certified pathologist, blinded to the original reports and patient outcomes (107). Additional patient data, concerning clinicopathological factors, recurrence, neoadjuvant and adjuvant chemotherapy and cause of death, were obtained from medical records, and survival data were provided by the Swedish National Civil Register. Baseline was set at the date of surgery, and the most recent follow-up was made at 31 March 2015 (224).

Two cases in this cohort received neoadjuvant chemotherapy and due to its potential effect on the expression of ER and PR (225), these patients were excluded from all descriptive analyses, as well as from the survival analyses. Additionally, two patients who died within one month from baseline and one patient who emigrated five months after surgery were excluded from the survival analyses.

Investigative factors

Paper I

In line with the aim of the first paper, and based upon previous studies with partially inconclusive results, the following factors were selected for inclusion in the analyses; age at baseline, sex, smoking habits (never/former/occasional/regular), exposure to ETS, alcohol consumption (grams per day), diabetes, and anthropometric data, namely height (centimetre), weight (kilogram), BMI (kilogram per square metre), waist and hip circumference (centimetre), WHR and body fat percentage. ETS was defined as exposure during childhood (no/yes) and exposure at home respectively exposure at work (no/< 10 years/10 - 20 years/> 20 years). Having a history of diabetes was initially recorded as only no or yes in the dataset, however, to distinguish between new-onset diabetes, i.e. diagnosis < 24 months prior to pancreatic cancer diagnosis, and diabetes occurring > 24 moths before detection of pancreatic cancer, this variable was dichotomized accordingly. Moreover, anthropometric measurements were coded as continuous variables as well as divided into tertiles.

Paper II

The following factors, associated with female physiology and reproduction, and potential correlations with risk of pancreatic cancer, were selected for inclusion: age at menarche and menopause, menstrual status at baseline (pre-/peri-/postmenopausal), nulliparity (no/yes), age at first childbirth (years), number of children, total duration of breastfeeding (months), number of reproductive years (years between menarche and menopause), ever use of OC (no/yes), ever use of HRT (no/yes), also specified as estrogen- or gestagen-only, or combination therapy, hysterectomy and/or oophorectomy. Additionally, the impact of age, smoking habits, alcohol consumption, diabetes, BMI and exposure to ETS on risk was re-examined.

Furthermore, age at menarche was recorded as a continuous variable and also as four categories (≤ 11 years/> 11 - ≤ 14 years/> 14 years - ≤ 16 years/> 16 years). Concerning menstrual status, perimenopausal women were classified as postmenopausal for the analyses, resulting in only two categories. Additionally, due to the substantial difference between earlier and modern contraceptives, ever users of OC also were divided according to in which decade they started using OC.

Paper III

The following patient and tumour characteristics were available and selected for inclusion in this study: age at surgery, tumour origin (duodenum/ampulla I-type/ampulla PB-type/distal bile duct/pancreas), tumour size (millimetre), differentiation grade (well - moderate/poor - undifferentiated), T-stage, N-stage, resection margin (R0/R1 - Rx), perineural growth (no/yes), invasion of lymphatic vessels (no/yes) and blood vessels (no/yes), growth in peripancreatic fat (no/yes) and adjuvant chemotherapy (none/5-FU/gemcitabine/GemCap/Oxaliplatin+5-FU/Gemox).

Investigate factors of interest in accordance with the aim of the study were the hormonal receptors ER, PR and AR. The Cancer Genome Atlas (TCGA) reported low mRNA levels of ERα, ERβ, PR and AR in 176 cases with PDAC (average FPKM 0.1, 0.2, 0.4 and 0.9 respectively). However, initial immunohistochemical (IHC) staining of tissue microarrays (TMAs) from the herein investigated cohort revealed no expression of AR, which was therefore not further assessed in this study. Expression of ER and PR was mainly observed in the tumour associated stroma, which will be further discussed later, and was denoted as the number of stromal cells with nuclear staining. Assessment of the staining intensity was not feasible.

Additionally, the mutational status of the investigated tumour samples had been previously analysed by next generation sequencing (NGS). For this procedure, DNA of sufficient quality could be obtained from 102 cases, which were characterised by a panel of 70 selected cancer-associated genes (226). Only mutations being present in > 10 % of the cases were included in the analyses.

Paper IV

At study entry of this trial, the following patient characteristics will be gathered: age at menarche and menopause, parity, ever use of OC and ever use of HRT. Blood samples will be collected at baseline and at every 2-month follow up and analysed for routine blood investigations, as well as for levels of estrogen, progesterone and testosterone, and circulating tumour DNA (ctDNA). Additionally, at two months of follow-up tamoxifen metabolites in blood will be measured.

All previously obtained tumour specimens from the cases will be analysed for expression of ER, PR and Ki-67 by IHC, if tissue quantity and quality is sufficient. Moreover, NGS will be performed using a panel of > 500 genes to examine mutational status in tumour tissue and ctDNA. DNA obtained from buffy coats in baseline blood samples will be genotyped and polymorphisms in genes related to hormonal synthesis and metabolism as well as tamoxifen metabolism will be examined.

Tissue microarray (TMA)

A TMA is a collection of multiple tumour tissue samples, embedded as cores in a paraffin block. The tissue cores most commonly measure 0.6 - 2 mm in diameter. The final paraffin block containing multiple tissue cores from different donor tissue is then sliced into thin layers measuring 4 - 5 µm which are mounted on glass slides for e.g. immunohistochemistry. The system for TMA construction was first described in 1998 (227), and has become an important and widely used technique for biomarker research (228). Figure 3 illustrates the procedures during TMA construction.

One of the most important advantages with a TMA, compared with full-face tissue sections, is that it allows for simultaneous, reagent-saving analysis of tissue cores from a large number of tumours. Obviously, there is however a risk of losing information on relevant heterogenic features of a tumour with this technique, and therefore, ideally, tissue cores should be sampled from multiple sites within each tumour.

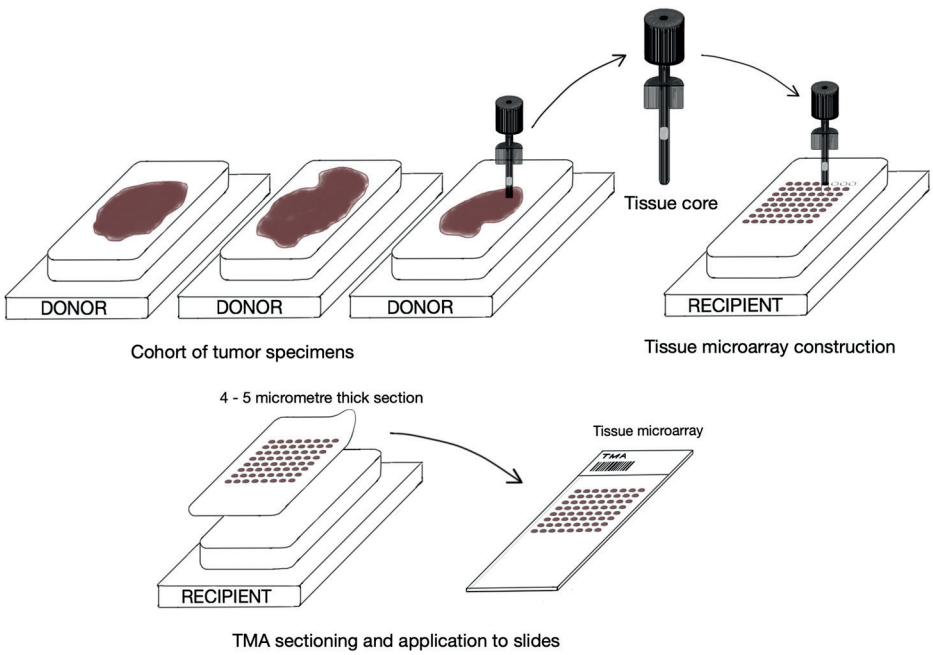

Figure 3
Contruction of a tissue microarray. Multiple cores are collected from each formalin-fixed, paraffin embedded tumour sample/donor tissue specimen, and are further arranged in a recipient paraffin block from which thin sections can be obtained to create TMA slides.

Immunohistochemistry

The technique of immunohistochemistry (IHC) was first described in the early 1940s, and is, simplified, a procedure where either monoclonal or polyclonal antibodies are used to detect specific antigens, most commonly proteins, in a tissue sample (229). Since its invention, IHC has gradually been further refined to provide more sensitive and specific results (228).

A fundamental requirement for IHC to be a reliable technique is that the applied antibodies are well-validated, which can be confirmed by several different methods. First, the specificity of the antibody must be determined, which is often achieved by Western blot (230). This is a procedure using gel electrophoresis to separate proteins based on their different molecular weights. When the gel containing the different proteins are further exposed to an antibody, a single band at the place of the selected antigens molecular weight should appear (231). In many cases however, such a result is not sufficient to consider an antibody to be satisfactorily validated. To further verify the specificity of the antibody, a method using small interfering ribonucleic acids (siRNAs), synthesised from ribonucleic acids (RNA), can be applied. In this procedure, the siRNA is transferred into a cell, where it binds to a complementary mRNA strand which in turn is cleaved. This hinders translation into the protein encoded by the targeted mRNA, also known as knockdown. Consequently, this means that the siRNA transfected cells will serve as negative controls (232). Thus, if an antibody still turns out to bind proteins in this environment, it is considered non-specific. Finally, it is of great importance to verify the reproducibility of the antibody, i.e. that staining of the antibody does not change over time or between different batches (230).

Statistical analysis

In paper I and paper II, all statistical analyses were performed in IBM SPSS Statistics version 22.0 (SPSS Inc., Chicago, IL, USA), whereas version 25.0 was used for the analyses in paper III. Additionally, heat maps visualizing the distribution of mutations in cohort 3 were produced in OncoPrinter, provided by cBioPortal Web (http://www.cbioportal.org/oncoprinter.jsp, access date: 2018-11-05) (233, 234). Power analyses in paper IV were performed in SAS 9.4, SAS Institute Inc., Cary, NC, USA.

Paper I

The distribution of investigative factors between cases and non-cases was analysed by non-parametric and Chi square tests. Cox regression analysis was applied to examine the associations between investigative factors and risk of pancreatic cancer. Analyses were performed as age-adjusted and multivariable, adjusted for sex, age, smoking and alcohol habits, and diabetes. Interactions between sex and investigative factors were calculated in the age-adjusted as well as in the multivariable model. The time variable was defined as pancreatic cancer-free person-years, i.e. the time between baseline and date at diagnosis, migration, death or end of follow-up on 31 December 2013, whichever occurred first. All statistical calculations were two-sided with p-values < 0.05 considered significant.

Paper II

Similarly to paper I, non-parametric and Chi square tests were applied to calculate the distribution of investigative factors among cases and non-cases, and Cox regression analysis was applied to estimate HRs and 95 % confidence intervals (CI) for risk of pancreatic cancer. Calculations were performed as age-adjusted and multivariable, including adjustment for age, smoking habits, alcohol consumption and BMI. These potential confounders were previously suggested or confirmed to be risk factors for pancreatic cancer or to be associated with estrogen metabolism. Again, the primary underlying time scale used in the analyses was time on study, however with the updated end of follow-up on 31 December 2015. Additionally, all analyses were also performed with attained age as time scale, and with and without adjustment for calendar year effects by 5-year intervals (235). The associations of HRT use on risk of pancreatic cancer was analysed in postmenopausal women only. The proportional hazards assumption was tested by Cox regression analysis with time-dependent covariate analysis, as well as log-minus-log plots, and was considered satisfactory for all analyses. All statistical calculations were two-sided with p-values < 0.05 considered significant.

Paper III

As aforementioned, expression of ER and PR was recorded as a continuous variable with the number of stained tumour-associated stromal cells, as well as binary variables (positive vs negative), based on whether there were any stained stromal cells present or not. Spearman's rank correlation test was applied to investigate the relationship between the number of ER positive and PR positive stromal cells, according to sex and morphology. Non-parametric and Chi square tests were used to analyse the distribution of ER and PR expression in relation to patient and tumour characteristics, in the entire cohort and in subgroups according to sex and morphology. Heat maps were used to illustrate the distribution of mutations according to ER and PR expression status, stratified by sex and tumour morphology.

Cox regression analysis was used to calculate HRs and 95 % CIs for long-term risk of death and recurrence, as univariable and multivariable analyses. Time on study was used as time scale and defined by the time between surgery and event, migration or end of follow-up on 31 March 2017, whichever occurred first. Additionally, Cox regression analysis was applied to test for potential interactions between *KRAS* mutation status and expression of ER and PR.

Kaplan-Meier curves and log-tank tests were calculated to illustrate OS and RFS in relation to ER and PR expression status, stratified by sex, tumour morphology and *KRAS* mutation status. All statistical calculations were two-sided with p-values < 0.05 considered significant.

Paper IV

The mOS for patients in the placebo group was estimated to be 2 - 3 months, based on clinical experience and previous studies (236), and the expected benefit from tamoxifen treatment on mOS was 2 - 3 months, in accordance with previous studies on tamoxifen treatment of pancreatic cancer (166, 167, 169, 170, 177). Study sample size was calculated by simulations. For each combination of mOS and total number of participants, 10 000 datasets were simulated. A censor rate of 10 % was assumed for all simulations. Cox regression analysis was applied for the simulated datasets and the reported power represent the proportion of significant HRs achieved with a two-sided significance level of 0.05. To reach a power of > 80 %, approximately 150 participants would be required, 75 in each treatment arm.

Results and Discussion

In this section, the primary findings are summarised and discussed. A more thorough presentation of the results can be found in the original papers. Since paper IV constitutes a protocol for a future clinical trial, there are no preliminary results. Therefore, this section will mainly be a discussion of the method and the future perspectives.

Paper I

In this study, smoking was confirmed to be a strong risk factor for pancreatic cancer, conferring a HR of 2.62 among women and 3.57 among men who were regular smokers, compared to never smokers, in multivariable analysis. Moreover, only women were at a significantly increased risk if they were occasional smokers (HR = 3.29) or exposed to ETS at work for more than 20 years (HR = 2.01), compared to women never smoking and never exposed, respectively. Despite the lack of significant interactions between sex and smoking or passive smoking status in this study, similar results have been reported previously (19, 48), and Silverman et al. (47) reported a significant interaction between sex and duration of smoking. On the other hand, large meta-analyses have not been able to confirm these findings (49, 50). The association between exposure to ETS and risk in relation to sex has however, to the best of our knowledge, not been investigated previously.

Several studies claim an anti-estrogenic effect of smoking (237, 238) which raises questions on whether this is one of the reasons for the levelling incidence of pancreatic cancer between sexes in Sweden. The habit of smoking has been decreasing in the Swedish population, at least since 1980, and more so in men. Thus, the previously higher proportion of male smokers compared to female smokers is no longer apparent (239, 240).

Moreover, the degree of daily alcohol consumption was not associated with risk of pancreatic cancer in any of the sexes in this study, which stands in contrast to the review by Maisonneuve et al. (43), where the evidence of such an association was assessed as strong. However, several prospective cohort studies have not found any significant association between alcohol consumption and pancreatic cancer risk

(241-243). The reliability of subjectively reported alcohol consumption is often limited, which may be one of the reasons for the inconclusive results.

Waist-hip-ratio was the only pre-diagnostic anthropometric factor associated with pancreatic cancer risk, and only in analysis of the entire cohort. Individuals in the highest tertile of WHR had a HR of 2.36 compared with the lowest tertile, including a significant positive trend. Body mass index was not shown to correlate with risk, which is noteworthy, since previous reports claim that the evidence of such an association is strong (28-31). Conclusively, this study does not support any robust associations between anthropometric factors and pancreatic cancer risk. Perhaps the inconclusive results from the existing literature propose that anthropometric measurements may merely represent inferior surrogate markers for other relevant factors such as metabolic imbalance, immunological function, inflammation and degree of physical activity.

Paper II

The results from this study demonstrated that higher age at menarche was positively associated with risk of pancreatic cancer, in age-adjusted as well as in multivariable analysis yielding HRs of 1.17 and 1.17, respectively. This association remained significant after additional adjustment of total years of menstrual activity. Pancreatic cancer is thought to have a long latent phase with the first cancer cells occurring long before a manifest disease is discovered. Therefore, the inverse relationship between age at menarche and pancreatic cancer risk could be interpreted as a sign of estrogen playing a protective role, namely that an early burst of estrogen in life would decrease the risk of pancreatic cancer cells to arise. It is however noteworthy that, except for Lin et al. who reported an increased risk of death by pancreatic cancer with higher age at menarche (244), no other studies appear to confirm this relationship, but rather an increased risk of pancreatic cancer with early menarche (36, 37). Nevertheless, the number of previous studies are limited, and it may therefore be troublesome to draw reliable conclusions from existing results. On the other hand, this association may also just be a result of the high number of analyses performed in our study.

Except for the increased risk of pancreatic cancer in women with a history of hysterectomy and/or oophorectomy observed in crude analysis (HR = 2.00), no other reproductive factors were found to be significantly related to risk. Earlier reports in this area have shown rather scattered results, and thus, there is as of yet no clear indications of any associations of female reproductive factors with pancreatic cancer risk. Nevertheless, the reported decreased risk among women with; low age at first childbirth (39, 41), a high number of children (39, 40), long

duration of breastfeeding (42), intact ovaries (38) and high age at menopause (38), imply that estrogen is a relevant factor, likely conferring a protective effect. Importantly, some reports do however claim contrasting associations between these factors and pancreatic cancer risk, and therefore further studies are warranted.

Moreover, ever use of HRT in postmenopausal women, particularly in terms of estrogen-only regimen, significantly decreased the risk of pancreatic cancer, with age adjusted HRs of 0.47 and 0.21, and multivariable HRs of 0.48 and 0.22, respectively. These results are in line with a few previous studies (63, 67), even though the latter only found significance in hysterectomised women. Furthermore, ever use of gestagen-only regimen did not significantly alter the risk of pancreatic cancer. In addition to HRT, ever use of OC was associated with a decreased risk in crude analysis (HR = 0.54), which did however not reach significance in the adjusted models presented in the paper. As there is a marked difference between earlier versus later regimens of OC regarding the content of estrogen, analysis of pancreatic cancer risk in relation to OC use was also performed based on whether the study participants used the earlier or later regimens of OC. This revealed that in crude analysis, women who started using OC between 1960 - 1970 had a decreased risk of pancreatic cancer (HR = 0.51), compared with never users, while women who started after 1970 did not. Additionally, initiation of OC use 1960 - 1980, but not after 1980, was associated with a significantly reduced risk compared with never use (HR = 0.53). Since these associations did not remain significant in adjusted models, they were not presented in the paper. The evidence of an association between OC use and risk of pancreatic cancer is weak, mostly since the number of studies are few, however, in 2015 Tang et al. reported an increased risk among ever users of OC compared to never users, when limiting their meta-analysis to cohort studies or studies adjusting for smoking, diabetes and BMI (64).

Paper III

The median follow-up in this study was 29.7 months. Expression of ER and PR was primarily observed in tumour-associated stromal cells, with positivity in 31 and 29 % of the cases, respectively. Stromal ER and PR positivity (ER+ and PR+) was significantly correlated in both men and women, overall and in the subgroup of PB-type tumours. ER positivity was evenly distributed between I-type and PB-type tumours, but significantly more common among women in both I- and PB-type tumours. PR positivity was however significantly more common in PB-type than in I-type tumours, overall and in women, although the difference between sexes was non-significant.

Further associations of ER and PR expression with patient and tumour characteristics, including tumour mutation status, were rather few and did not follow any particular patterns. Conclusively, the observed associations from these analyses, reported in more detail in the separate papers, may merely be a result of multiple testing.

Stromal ER expression was associated with an improved OS and RFS in the subgroup of women with pancreatic adenocarcinoma in crude, but not in adjusted analysis. Moreover, the interaction between ER and sex was non-significant. No other prognostic associations were observed for stromal ER and PR expression *per se*, neither in the entire cohort nor in any of the sexes separately. However, in multivariable analysis, a significant interaction was observed between PR expression and *KRAS* mutation status in relation to OS as well as RFS, in the entire cohort and among women separately.

The mOS for patients with PR+ versus PR- *KRAS*-mutated tumours was 34.3 versus 20.9 months, and the mOS for patients with PR+ versus PR- *KRAS* wild-type tumours was 28.8 versus 41.8 months. In women, the corresponding mOS was 60.5 versus 16.6 months, and 9.9 versus 59.0 months, respectively.

Furthermore, the median RFS (mRFS) for patients with PR+ versus PR- *KRAS*-mutated tumours was 15.0 versus 8.2 months, and the mRFS for patients with PR+ versus PR- *KRAS* wild-type tumours was 19.3 versus 53.9 months. In women, the corresponding mRFS was 66.0 versus 6.7 months, and 5.1 versus 79.2 months, respectively.

The prognostic value of ER and PR expression did not differ in relation to the other most frequently mutated genes.

The findings of stromal expression of ER and PR in periampullary adenocarcinoma are, to the best of our knowledge, novel. In MCNs however, ER and PR expression is a key trait of the OTS (214), and reduction or loss of PR expression has been shown to correlate with HGD/CIS or true invasion (215). Contrariwise, *KRAS* mutations have been reported to be more frequent in MCNs with HGD and/or invasion (245). These potentially premalignant neoplasms are particularly common in women, which is noteworthy since PR as well as ER expression appears to confer a stronger prognostic value in periampullary adenocarcinoma in women. The prognostic interaction of PR expression and *KRAS* mutation status, being particularly evident in women, may mirror a tumour suppressive effect of PR in *KRAS*-mutated tumours, but a tumour stimulating effect in *KRAS* wild-type tumours.

Paper IV and future perspectives

The results from in particular paper II and paper III imply that female hormonal factors play a significant role in the development as well as progression of pancreatic cancer. Early trials have reported a potentially beneficial effect of tamoxifen in elderly women with advanced pancreatic cancer (169, 170), which, together with these results may contribute to future advancement in treating this disease. Therefore, paper IV was designed as a study protocol for tamoxifen treatment in women with advanced pancreatic cancer, who lack the option of receiving further chemotherapeutic treatment. In paper III, PR expression together with *KRAS* mutation status was the most evident prognostic factor, although some weak associations were also seen between ER expression and outcome in women with tumours of pancreatic origin. Therefore, next-generation sequencing including assessment of *KRAS* mutation status, together with immunohistochemical analysis of ER and PR expression, are apparent biomarkers to be tested as predictors of tamoxifen response. Concordantly, the present trial will include baseline analysis of tumour characteristics such as mutation status, hormone receptor expression, and polymorphisms, as well as on-treatment analysis of ctDNA. The latter may turn out to be particularly important, as one of the greatest issues when studying biomarkers in pancreatic and other periampullary tumours is the limited amount of available tumour tissue from patients who do not have their tumours resected, i.e. the vast majority of the patients. From some patients, only cytological specimens are available, and these are in general not sufficient to determine the anatomical origin of the tumours, and often not even the morphological type.

Despite current advancement in uncovering new efficient treatment options for several types of cancers, the progress for pancreatic cancer runs slow. The issue of treating pancreatic cancer, as well as several other intractable diseases, has led to an increasing number of studies describing the importance of drug-repurposing (246, 247) or drug-reprofiling (248), rather than just concentrating on the development of novel drugs. In other words, we may not need to reinvent the wheel over and over, but instead focus on finding other vehicles or machines where our wheel can be of use. Accordingly, the herein described trial is an attempt to reprofile tamoxifen to also include pancreatic cancer.

Strengths and Limitations

Paper I and paper II are based on the MDCS, which is a large prospective population-based cohort, where all non-cases represent controls. This renders a slightly lower number of cases in comparison to many of the case-control studies referred to herein, however, it also contributes to a higher reliability of the results since it should ideally represent the studied population. This may however still be an issue due to bias caused by the potentially skewed selection of participants in the MDCS, i.e. that they do not represent the Malmö population as a whole. In a study by Manjer et al. (223), the representativity of the MDCS, with 39 % participation rate, was investigated through comparison with the non-participants of the study, as well as with participants of another mailed health survey, The Health Situation in Malmö ’94 (HSM:94), with a 75 % participation rate. Manjer et al. reported some differences concerning the incidence of malignancies and mortality between participants and non-participants in the MDCS prior to, during, and following the recruitment period. Furthermore, participants in the MDCS had a better reported subjective health than participants in the HSM:94. There was also a lower proportion of foreign born in the MDCS. There were however no major differences in, the level of education, type of employment, family situation, smoking and alcohol habits, or weight distribution.

The incidence of pancreatic cancer in the MDCS was approximately 0.6 % among both men and women up until 31 December 2015. This is in line with the expected cumulative incidence below 75 years of age reported by The Board of National Health and Welfare of 0.8 and 0.7 % among men and women, respectively (13).

Most of the variables used in the analyses contain only few missing values, however, some lack a larger proportion of information which limits the trustworthiness of the analyses in which these variables are included. Furthermore, multiple testing always comes with a risk of type 1 errors, i.e. that significant associations occur by coincidence. This can in some situations be circumvented by lowering the significance level or by applying different statistical methods.

The application of pre-diagnostic data may also cause inevitable issues in large prospective cohorts, since there is no possibility of assuring that these variables do not change significantly over time. For most variables however, this is most likely not an issue. The median age at baseline in cohort 1 was 57.8 years, and 56.7 years

in cohort 2, an age where physical and socioeconomic changes have probably "stabilized" for most people.

Cohort 3 is a well-characterized consecutive cohort of all resected periampullary tumours during an 11-year period at Lund and Malmö University hospitals. Still, mutation analysis were only completed in 102 out of the 175 cases due to insufficient tumour cell quantity in the remaining cases. This rendered relatively small subgroups in several of the stratified analyses, which may be one of the reasons for a lack of significant findings in relation to morphological type or anatomical origin. On the other hand, in clinical practice, tumours are generally not as well-characterized, in particular in the unresected cases, where morphological type and anatomical origin can seldom be established with certainty.

The TMA technique also has some potential limitations, e.g. the risk of not providing an accurate portrayal of the spatial tumour heterogeneity and, hence, the distribution of the investigated biomarkers. To partly circumvent this issue, several cores from separate donor blocks have been examined.

The reliability of the antibodies in biomarker studies is fundamental. The antibodies used in paper III to detect ER and PR were well-validated and are also used in routine clinical practice.

Concerning paper IV and the planned clinical trial, there are several difficulties which have to be dealt with. The design of a RCT is proposed to confer the highest level of evidence, compared with e.g. cohort or case-control studies. Ideally, the design of a RCT eliminates the need to adjust for confounding variables, however, the assumed equal distribution of characteristics between the placebo group and tamoxifen group still has to be verified when data is collected.

A limitation to this study may be the estimated survival benefit from tamoxifen treatment, which has been estimated from rather few and small earlier trials. If the potential survival benefit of tamoxifen is lower than what has been expected beforehand, the number of participants will not be sufficient.

Conclusions

Smoking was confirmed to be one of the most important risk factors for pancreatic cancer, with women potentially being more susceptible to its carcinogenic effects.

Neither alcohol habits nor prevalent diabetes, new-onset diabetes excluded, were associated with risk of pancreatic cancer.

A high WHR was the only anthropometric factor that was associated with an increased risk, with no differences between sexes. The previously reported positive association between BMI and pancreatic cancer risk could not be confirmed.

Early menarche and ever use of HRT in postmenopausal women were associated with a decreased risk of pancreatic cancer.

ER and PR are mainly expressed in the tumour-associated stroma of periampullary adenocarcinoma.

PR positivity, in combination with *KRAS* mutation status, provided prognostic information on long-term survival in patients with periampullary adenocarcinoma, and these associations were more evident in women.

The results from the first three papers, together with previous reports on a beneficial effect of tamoxifen treatment in patients with advanced pancreatic cancer, in particular in women, spurred the idea of initiating a trial with tamoxifen treatment and biomarker analysis.

Acknowledgements

This thesis was supported by a great bunch of wonderful people and other little fellows, without whom this thesis would not have existed:

My main supervisor **Karin Jirström**, who was the first person to introduce me to the world of research, six years ago. Karin has a tremendous talent to enthuse her students and colleagues and has been a great source of inspiration ever since we started working together.

Signe Borgquist, my co-supervisor and a great dance partner. Signe has been an amazing source of information and ideas, with her great experience in oncology and research.

Everyone in my research team, both former and present members; Björn Nodin, Margareta Heby, Jacob Elebro, Anna Larsson, Lotta Hedner, Sebastian Lundgren, Jonna Berntsson, Maria Svensson, Sofie Olsson Hau, Bahar Rastegar, Christina Siesing, Karolina Boman, Sara Wahlin, Alexandra Petersson, Emelie Karnevi, Alexander Gaber and Richard Fristedt. You are not only decent colleagues but also wonderful friends, always there when needed.

My co-authors who have not been previously mentioned: Christoffer Wennersten, former classmate, research companion during medical school and an amazing friend, and Per Hall who is offering assistance with the study design and important knowledge for paper IV.

Jakob Eberhard, David Borg, Anders Johnsson and Otilia Leon who supported me and taught me about GI-cancer at my first employment as a doctor, in the department of oncology where I mainly served in the GI-cancer ward.

Bo Baldetorp and Mikael Bodelsson, former and present Heads of the Department of Clinical Sciences Lund, for providing a stimulating research environment.

Magnus Zätterström and Susanne André, for excellent administrative support.

Friends; thank you all for support and fruitful discussions.

Liza, the love of my life, who has supported me every day through the journey of composing this thesis.

My father Lars-Göran, my mother Kristina and my brother and best friend Axel, who have always offered their help and love.

Our beloved and beautiful puppy Freja, who sadly passed away in February, but always comforted me when I was feeling distressed or shattered.

And finally our wonderful little puppy Doris, who brought joy and relaxation through the last days of writing the thesis.

References

1. Becker AE, Hernandez YG, Frucht H, Lucas AL. Pancreatic ductal adenocarcinoma: risk factors, screening, and early detection. World J Gastroenterol. 2014;20(32):11182-98.
2. Grant TJ, Hua K, Singh A. Molecular Pathogenesis of Pancreatic Cancer. Prog Mol Biol Transl Sci. 2016;144:241-75.
3. Bilimoria KY, Bentrem DJ, Ko CY, Ritchey J, Stewart AK, Winchester DP, Talamonti MS. Validation of the 6th edition AJCC Pancreatic Cancer Staging System: report from the National Cancer Database. Cancer. 2007;110(4):738-44.
4. Distler M, Aust D, Weitz J, Pilarsky C, Grutzmann R. Precursor lesions for sporadic pancreatic cancer: PanIN, IPMN, and MCN. Biomed Res Int. 2014;2014:474905.
5. Lairmore TC, Moley JF. Endocrine pancreatic tumors. Scand J Surg. 2004;93(4):311-5.
6. Kimura W, Futakawa N, Zhao B. Neoplastic diseases of the papilla of Vater. J Hepatobiliary Pancreat Surg. 2004;11(4):223-31.
7. Carter JT, Grenert JP, Rubenstein L, Stewart L, Way LW. Tumors of the ampulla of vater: histopathologic classification and predictors of survival. J Am Coll Surg. 2008;207(2):210-8.
8. Westgaard A, Tafjord S, Farstad IN, Cvancarova M, Eide TJ, Mathisen O, Clausen OP, Gladhaug IP. Pancreatobiliary versus intestinal histologic type of differentiation is an independent prognostic factor in resected periampullary adenocarcinoma. BMC Cancer. 2008;8:170.
9. Bronsert P, Kohler I, Werner M, Makowiec F, Kuesters S, Hoeppner J, Hopt UT, Keck T, Bausch D, Wellner UF. Intestinal-type of differentiation predicts favourable overall survival: confirmatory clinicopathological analysis of 198 periampullary adenocarcinomas of pancreatic, biliary, ampullary and duodenal origin. BMC Cancer. 2013;13:428.
10. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424.
11. Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, Znaor A, Soerjomataram I, Bray F. Global Cancer Observatory: Cancer Today: Lyon, France: International Agency for Research on Cancer; 2018 [Available from: https://gco.iarc.fr/today. Accessed: 2019-03-14.

12. The National Board of Health and Welfare. Cancer in numbers 2018: Socialstyrelsen; 2018 [Available from: https://www.socialstyrelsen.se/Lists/Artikelkatalog/Attachments/20976/2018-6-10.pdf. Accessed: 2019-03-14.
13. The National Board of Health and Welfare. Statistics on Cancer Incidence 2017: Socialstyrelsen; 2018 [Available from: http://www.socialstyrelsen.se/SiteCollectionDocuments/2018-12-50-tabeller.xls. Accessed: 2019-03-14.
14. Ghadirian P, Boyle P, Simard A, Baillargeon J, Maisonneuve P, Perret C. Reported family aggregation of pancreatic cancer within a population-based case-control study in the Francophone community in Montreal, Canada. Int J Pancreatol. 1991;10(3-4):183-96.
15. Hemminki K, Li X. Familial and second primary pancreatic cancers: a nationwide epidemiologic study from Sweden. Int J Cancer. 2003;103(4):525-30.
16. Bartsch DK, Kress R, Sina-Frey M, Grutzmann R, Gerdes B, Pilarsky C, Heise JW, Schulte KM, Colombo-Benkmann M, Schleicher C, et al. Prevalence of familial pancreatic cancer in Germany. Int J Cancer. 2004;110(6):902-6.
17. Hruban RH, Canto MI, Goggins M, Schulick R, Klein AP. Update on familial pancreatic cancer. Adv Surg. 2010;44:293-311.
18. Bartsch DK, Gress TM, Langer P. Familial pancreatic cancer--current knowledge. Nat Rev Gastroenterol Hepatol. 2012;9(8):445-53.
19. Larsson SC, Permert J, Hakansson N, Naslund I, Bergkvist L, Wolk A. Overall obesity, abdominal adiposity, diabetes and cigarette smoking in relation to the risk of pancreatic cancer in two Swedish population-based cohorts. Br J Cancer. 2005;93(11):1310-5.
20. Elena JW, Steplowski E, Yu K, Hartge P, Tobias GS, Brotzman MJ, Chanock SJ, Stolzenberg-Solomon RZ, Arslan AA, Bueno-de-Mesquita HB, et al. Diabetes and risk of pancreatic cancer: a pooled analysis from the pancreatic cancer cohort consortium. Cancer Causes Control. 2013;24(1):13-25.
21. Bosetti C, Rosato V, Li D, Silverman D, Petersen GM, Bracci PM, Neale RE, Muscat J, Anderson K, Gallinger S, et al. Diabetes, antidiabetic medications, and pancreatic cancer risk: an analysis from the International Pancreatic Cancer Case-Control Consortium. Ann Oncol. 2014;25(10):2065-72.
22. Yeo TP, Hruban RH, Leach SD, Wilentz RE, Sohn TA, Kern SE, Iacobuzio-Donahue CA, Maitra A, Goggins M, Canto MI, et al. Pancreatic cancer. Curr Probl Cancer. 2002;26(4):176-275.
23. Vincent A, Herman J, Schulick R, Hruban RH, Goggins M. Pancreatic cancer. Lancet. 2011;378(9791):607-20.
24. Li D. Diabetes and pancreatic cancer. Mol Carcinog. 2012;51(1):64-74.
25. Raimondi S, Lowenfels AB, Morselli-Labate AM, Maisonneuve P, Pezzilli R. Pancreatic cancer in chronic pancreatitis; aetiology, incidence, and early detection. Best Pract Res Clin Gastroenterol. 2010;24(3):349-58.

26. Duell EJ, Lucenteforte E, Olson SH, Bracci PM, Li D, Risch HA, Silverman DT, Ji BT, Gallinger S, Holly EA, et al. Pancreatitis and pancreatic cancer risk: a pooled analysis in the International Pancreatic Cancer Case-Control Consortium (PanC4). Ann Oncol. 2012;23(11):2964-70.
27. Bracci PM, Wang F, Hassan MM, Gupta S, Li D, Holly EA. Pancreatitis and pancreatic cancer in two large pooled case-control studies. Cancer Causes Control. 2009;20(9):1723-31.
28. Larsson SC, Orsini N, Wolk A. Body mass index and pancreatic cancer risk: A meta-analysis of prospective studies. Int J Cancer. 2007;120(9):1993-8.
29. Arslan AA, Helzlsouer KJ, Kooperberg C, Shu XO, Steplowski E, Bueno-de-Mesquita HB, Fuchs CS, Gross MD, Jacobs EJ, Lacroix AZ, et al. Anthropometric measures, body mass index, and pancreatic cancer: a pooled analysis from the Pancreatic Cancer Cohort Consortium (PanScan). Arch Intern Med. 2010;170(9):791-802.
30. Jiao L, Berrington de Gonzalez A, Hartge P, Pfeiffer RM, Park Y, Freedman DM, Gail MH, Alavanja MC, Albanes D, Beane Freeman LE, et al. Body mass index, effect modifiers, and risk of pancreatic cancer: a pooled study of seven prospective cohorts. Cancer Causes Control. 2010;21(8):1305-14.
31. Genkinger JM, Spiegelman D, Anderson KE, Bernstein L, van den Brandt PA, Calle EE, English DR, Folsom AR, Freudenheim JL, Fuchs CS, et al. A pooled analysis of 14 cohort studies of anthropometric factors and pancreatic cancer risk. Int J Cancer. 2011;129(7):1708-17.
32. Aune D, Vieira AR, Chan DS, Navarro Rosenblatt DA, Vieira R, Greenwood DC, Cade JE, Burley VJ, Norat T. Height and pancreatic cancer risk: a systematic review and meta-analysis of cohort studies. Cancer Causes Control. 2012;23(8):1213-22.
33. Michaud DS, Giovannucci E, Willett WC, Colditz GA, Stampfer MJ, Fuchs CS. Physical activity, obesity, height, and the risk of pancreatic cancer. JAMA. 2001;286(8):921-9.
34. Rosato V, Tavani A, Bosetti C, Pelucchi C, Talamini R, Polesel J, Serraino D, Negri E, La Vecchia C. Metabolic syndrome and pancreatic cancer risk: a case-control study in Italy and meta-analysis. Metabolism. 2011;60(10):1372-8.
35. Esposito K, Chiodini P, Colao A, Lenzi A, Giugliano D. Metabolic syndrome and risk of cancer: a systematic review and meta-analysis. Diabetes Care. 2012;35(11):2402-11.
36. Bueno de Mesquita HB, Maisonneuve P, Moerman CJ, Walker AM. Anthropometric and reproductive variables and exocrine carcinoma of the pancreas: a population-based case-control study in The Netherlands. Int J Cancer. 1992;52(1):24-9.
37. Fernandez E, La Vecchia C, D'Avanzo B, Negri E. Menstrual and reproductive factors and pancreatic cancer risk in women. Int J Cancer. 1995;62(1):11-4.
38. Prizment AE, Anderson KE, Hong CP, Folsom AR. Pancreatic cancer incidence in relation to female reproductive factors: Iowa Women's Health Study. JOP. 2007;8(1):16-27.
39. Kreiger N, Lacroix J, Sloan M. Hormonal factors and pancreatic cancer in women. Ann Epidemiol. 2001;11(8):563-7.

40. Skinner HG, Michaud DS, Colditz GA, Giovannucci EL, Stampfer MJ, Willett WC, Fuchs CS. Parity, reproductive factors, and the risk of pancreatic cancer in women. Cancer Epidemiol Biomarkers Prev. 2003;12(5):433-8.
41. Zhang Y, Coogan PF, Palmer JR, Strom BL, Rosenberg L. A case-control study of reproductive factors, female hormone use, and risk of pancreatic cancer. Cancer Causes Control. 2010;21(3):473-8.
42. Heuch I, Jacobsen BK, Albrektsen G, Kvale G. Reproductive factors and pancreatic cancer risk: a Norwegian cohort study. Br J Cancer. 2008;98(1):189-93.
43. Maisonneuve P, Lowenfels AB. Risk factors for pancreatic cancer: a summary review of meta-analytical studies. Int J Epidemiol. 2015;44(1):186-98.
44. Lugo A, Peveri G, Bosetti C, Bagnardi V, Crippa A, Orsini N, Rota M, Gallus S. Strong excess risk of pancreatic cancer for low frequency and duration of cigarette smoking: A comprehensive review and meta-analysis. Eur J Cancer. 2018;104:117-26.
45. Zou L, Zhong R, Shen N, Chen W, Zhu B, Ke J, Lu X, Zhang T, Lou J, Wang Z, et al. Non-linear dose-response relationship between cigarette smoking and pancreatic cancer risk: evidence from a meta-analysis of 42 observational studies. Eur J Cancer. 2014;50(1):193-203.
46. Iodice S, Gandini S, Maisonneuve P, Lowenfels AB. Tobacco and the risk of pancreatic cancer: a review and meta-analysis. Langenbecks Arch Surg. 2008;393(4):535-45.
47. Silverman DT, Dunn JA, Hoover RN, Schiffman M, Lillemoe KD, Schoenberg JB, Brown LM, Greenberg RS, Hayes RB, Swanson GM, et al. Cigarette smoking and pancreas cancer: a case-control study based on direct interviews. J Natl Cancer Inst. 1994;86(20):1510-6.
48. Vrieling A, Bueno-de-Mesquita HB, Boshuizen HC, Michaud DS, Severinsen MT, Overvad K, Olsen A, Tjonneland A, Clavel-Chapelon F, Boutron-Ruault MC, et al. Cigarette smoking, environmental tobacco smoke exposure and pancreatic cancer risk in the European Prospective Investigation into Cancer and Nutrition. Int J Cancer. 2010;126(10):2394-403.
49. Lynch SM, Vrieling A, Lubin JH, Kraft P, Mendelsohn JB, Hartge P, Canzian F, Steplowski E, Arslan AA, Gross M, et al. Cigarette smoking and pancreatic cancer: a pooled analysis from the pancreatic cancer cohort consortium. Am J Epidemiol. 2009;170(4):403-13.
50. Bosetti C, Lucenteforte E, Silverman DT, Petersen G, Bracci PM, Ji BT, Negri E, Li D, Risch HA, Olson SH, et al. Cigarette smoking and pancreatic cancer: an analysis from the International Pancreatic Cancer Case-Control Consortium (Panc4). Ann Oncol. 2012;23(7):1880-8.
51. Ye X, Lu G, Huai J, Ding J. Impact of smoking on the risk of pancreatitis: a systematic review and meta-analysis. PLoS One. 2015;10(4):e0124075.
52. Zhou J, Wellenius GA, Michaud DS. Environmental tobacco smoke and the risk of pancreatic cancer among non-smokers: a meta-analysis. Occup Environ Med. 2012;69(12):853-7.

53. Luo J, Ye W, Zendehdel K, Adami J, Adami HO, Boffetta P, Nyren O. Oral use of Swedish moist snuff (snus) and risk for cancer of the mouth, lung, and pancreas in male construction workers: a retrospective cohort study. Lancet. 2007;369(9578):2015-20.
54. Boffetta P, Aagnes B, Weiderpass E, Andersen A. Smokeless tobacco use and risk of cancer of the pancreas and other organs. Int J Cancer. 2005;114(6):992-5.
55. Colilla SA. An epidemiologic review of smokeless tobacco health effects and harm reduction potential. Regul Toxicol Pharmacol. 2010;56(2):197-211.
56. Schuller HM. Mechanisms of smoking-related lung and pancreatic adenocarcinoma development. Nat Rev Cancer. 2002;2(6):455-63.
57. Boffetta P, Hecht S, Gray N, Gupta P, Straif K. Smokeless tobacco and cancer. Lancet Oncol. 2008;9(7):667-75.
58. Samokhvalov AV, Rehm J, Roerecke M. Alcohol Consumption as a Risk Factor for Acute and Chronic Pancreatitis: A Systematic Review and a Series of Meta-analyses. EBioMedicine. 2015;2(12):1996-2002.
59. Genkinger JM, Spiegelman D, Anderson KE, Bergkvist L, Bernstein L, van den Brandt PA, English DR, Freudenheim JL, Fuchs CS, Giles GG, et al. Alcohol intake and pancreatic cancer risk: a pooled analysis of fourteen cohort studies. Cancer Epidemiol Biomarkers Prev. 2009;18(3):765-76.
60. Michaud DS, Vrieling A, Jiao L, Mendelsohn JB, Steplowski E, Lynch SM, Wactawski-Wende J, Arslan AA, Bas Bueno-de-Mesquita H, Fuchs CS, et al. Alcohol intake and pancreatic cancer: a pooled analysis from the pancreatic cancer cohort consortium (PanScan). Cancer Causes Control. 2010;21(8):1213-25.
61. Tramacere I, Scotti L, Jenab M, Bagnardi V, Bellocco R, Rota M, Corrao G, Bravi F, Boffetta P, La Vecchia C. Alcohol drinking and pancreatic cancer risk: a meta-analysis of the dose-risk relation. Int J Cancer. 2010;126(6):1474-86.
62. Lucenteforte E, La Vecchia C, Silverman D, Petersen GM, Bracci PM, Ji BT, Bosetti C, Li D, Gallinger S, Miller AB, et al. Alcohol consumption and pancreatic cancer: a pooled analysis in the International Pancreatic Cancer Case-Control Consortium (PanC4). Ann Oncol. 2012;23(2):374-82.
63. Lee E, Horn-Ross PL, Rull RP, Neuhausen SL, Anton-Culver H, Ursin G, Henderson KD, Bernstein L. Reproductive factors, exogenous hormones, and pancreatic cancer risk in the CTS. Am J Epidemiol. 2013;178(9):1403-13.
64. Tang B, Lv J, Li Y, Yuan S, Wang Z, He S. Relationship between female hormonal and menstrual factors and pancreatic cancer: a meta-analysis of observational studies. Medicine (Baltimore). 2015;94(7):e177.
65. Shuster LT, Gostout BS, Grossardt BR, Rocca WA. Prophylactic oophorectomy in premenopausal women and long-term health. Menopause Int. 2008;14(3):111-6.
66. Chen L, Mishra GD, Dobson AJ, Wilson LF, Jones MA. Protective effect of hormone therapy among women with hysterectomy/oophorectomy. Hum Reprod. 2017;32(4):885-92.

67. Lujan-Barroso L, Zhang W, Olson SH, Gao YT, Yu H, Baghurst PA, Bracci PM, Bueno-de-Mesquita HB, Foretova L, Gallinger S, et al. Menstrual and Reproductive Factors, Hormone Use, and Risk of Pancreatic Cancer: Analysis From the International Pancreatic Cancer Case-Control Consortium (PanC4). Pancreas. 2016;45(10):1401-10.
68. Wolpin BM, Chan AT, Hartge P, Chanock SJ, Kraft P, Hunter DJ, Giovannucci EL, Fuchs CS. ABO blood group and the risk of pancreatic cancer. J Natl Cancer Inst. 2009;101(6):424-31.
69. Gandini S, Lowenfels AB, Jaffee EM, Armstrong TD, Maisonneuve P. Allergies and the risk of pancreatic cancer: a meta-analysis with review of epidemiology and biological mechanisms. Cancer Epidemiol Biomarkers Prev. 2005;14(8):1908-16.
70. Behrens G, Jochem C, Schmid D, Keimling M, Ricci C, Leitzmann MF. Physical activity and risk of pancreatic cancer: a systematic review and meta-analysis. Eur J Epidemiol. 2015;30(4):279-98.
71. Zheng J, Guinter MA, Merchant AT, Wirth MD, Zhang J, Stolzenberg-Solomon RZ, Steck SE. Dietary patterns and risk of pancreatic cancer: a systematic review. Nutr Rev. 2017;75(11):883-908.
72. Turati F, Galeone C, Edefonti V, Ferraroni M, Lagiou P, La Vecchia C, Tavani A. A meta-analysis of coffee consumption and pancreatic cancer. Ann Oncol. 2012;23(2):311-8.
73. Guo Y, Liu W, Wu J. Helicobacter pylori infection and pancreatic cancer risk: A meta-analysis. J Cancer Res Ther. 2016;12(Supplement):C229-C32.
74. Liu H, Chen YT, Wang R, Chen XZ. Helicobacter pylori infection, atrophic gastritis, and pancreatic cancer risk: A meta-analysis of prospective epidemiologic studies. Medicine (Baltimore). 2017;96(33):e7811.
75. Wang Y, Zhang FC, Wang YJ. Helicobacter pylori and pancreatic cancer risk: a meta- analysis based on 2,049 cases and 2,861 controls. Asian Pac J Cancer Prev. 2014;15(11):4449-54.
76. Lindkvist B, Johansen D, Borgstrom A, Manjer J. A prospective study of Helicobacter pylori in relation to the risk for pancreatic cancer. BMC Cancer. 2008;8:321.
77. Luo G, Hao NB, Hu CJ, Yong X, Lu MH, Cheng BJ, Zhang Y, Yang SM. HBV infection increases the risk of pancreatic cancer: a meta-analysis. Cancer Causes Control. 2013;24(3):529-37.
78. Hruban RH, Goggins M, Parsons J, Kern SE. Progression model for pancreatic cancer. Clin Cancer Res. 2000;6(8):2969-72.
79. Wood LD, Hruban RH. Pathology and molecular genetics of pancreatic neoplasms. Cancer J. 2012;18(6):492-501.
80. Forbes SA, Beare D, Gunasekaran P, Leung K, Bindal N, Boutselakis H, Ding M, Bamford S, Cole C, Ward S, et al. COSMIC: exploring the world's knowledge of somatic mutations in human cancer. Nucleic Acids Res. 2015;43(Database issue):D805-11.

81. Spaargaren M, Bischoff JR, McCormick F. Signal transduction by Ras-like GTPases: a potential target for anticancer drugs. Gene Expr. 1995;4(6):345-56.
82. di Magliano MP, Logsdon CD. Roles for KRAS in pancreatic tumor development and progression. Gastroenterology. 2013;144(6):1220-9.
83. Weissmueller S, Manchado E, Saborowski M, Morris JPt, Wagenblast E, Davis CA, Moon SH, Pfister NT, Tschaharganeh DF, Kitzing T, et al. Mutant p53 drives pancreatic cancer metastasis through cell-autonomous PDGF receptor beta signaling. Cell. 2014;157(2):382-94.
84. Hruban RH, Offerhaus GJ, Kern SE, Goggins M, Wilentz RE, Yeo CJ. Tumor-suppressor genes in pancreatic cancer. J Hepatobiliary Pancreat Surg. 1998;5(4):383-91.
85. Iacobuzio-Donahue CA, Fu B, Yachida S, Luo M, Abe H, Henderson CM, Vilardell F, Wang Z, Keller JW, Banerjee P, et al. DPC4 gene status of the primary carcinoma correlates with patterns of failure in patients with pancreatic cancer. J Clin Oncol. 2009;27(11):1806-13.
86. Chang DL, Qiu W, Ying H, Zhang Y, Chen CY, Xiao ZX. ARF promotes accumulation of retinoblastoma protein through inhibition of MDM2. Oncogene. 2007;26(32):4627-34.
87. Krech RL, Walsh D. Symptoms of pancreatic cancer. J Pain Symptom Manage. 1991;6(6):360-7.
88. Lee ES, Lee JM. Imaging diagnosis of pancreatic cancer: a state-of-the-art review. World J Gastroenterol. 2014;20(24):7864-77.
89. Puli SR, Bechtold ML, Buxbaum JL, Eloubeidi MA. How good is endoscopic ultrasound-guided fine-needle aspiration in diagnosing the correct etiology for a solid pancreatic mass?: A meta-analysis and systematic review. Pancreas. 2013;42(1):20-6.
90. Tummala P, Junaidi O, Agarwal B. Imaging of pancreatic cancer: An overview. J Gastrointest Oncol. 2011;2(3):168-74.
91. Smith SL, Rajan PS. Imaging of pancreatic adenocarcinoma with emphasis on multidetector CT. Clin Radiol. 2004;59(1):26-38.
92. Adamek HE, Albert J, Breer H, Weitz M, Schilling D, Riemann JF. Pancreatic cancer detection with magnetic resonance cholangiopancreatography and endoscopic retrograde cholangiopancreatography: a prospective controlled study. Lancet. 2000;356(9225):190-3.
93. Ichikawa T, Sou H, Araki T, Arbab AS, Yoshikawa T, Ishigame K, Haradome H, Hachiya J. Duct-penetrating sign at MRCP: usefulness for differentiating inflammatory pancreatic mass from pancreatic carcinomas. Radiology. 2001;221(1):107-16.
94. Goonetilleke KS, Siriwardena AK. Systematic review of carbohydrate antigen (CA 19-9) as a biochemical marker in the diagnosis of pancreatic cancer. Eur J Surg Oncol. 2007;33(3):266-70.
95. Harsha HC, Kandasamy K, Ranganathan P, Rani S, Ramabadran S, Gollapudi S, Balakrishnan L, Dwivedi SB, Telikicherla D, Selvan LD, et al. A compendium of potential biomarkers of pancreatic cancer. PLoS medicine. 2009;6(4):e1000046.

96. Wei CH, Gorgan TR, Elashoff DA, Hines OJ, Farrell JJ, Donahue TR. A meta-analysis of gemcitabine biomarkers in patients with pancreaticobiliary cancers. Pancreas. 2013;42(8):1303-10.
97. Farrell JJ, Elsaleh H, Garcia M, Lai R, Ammar A, Regine WF, Abrams R, Benson AB, Macdonald J, Cass CE, et al. Human equilibrative nucleoside transporter 1 levels predict response to gemcitabine in patients with pancreatic cancer. Gastroenterology. 2009;136(1):187-95.
98. Marechal R, Bachet JB, Mackey JR, Dalban C, Demetter P, Graham K, Couvelard A, Svrcek M, Bardier-Dupas A, Hammel P, et al. Levels of gemcitabine transport and metabolism proteins predict survival times of patients treated with gemcitabine for pancreatic adenocarcinoma. Gastroenterology. 2012;143(3):664-74 e6.
99. Elebro J, Ben Dror L, Heby M, Nodin B, Jirstrom K, Eberhard J. Prognostic effect of hENT1, dCK and HuR expression by morphological type in periampullary adenocarcinoma, including pancreatic cancer. Acta Oncol. 2016;55(3):286-96.
100. Allen PJ, Kuk D, Castillo CF, Basturk O, Wolfgang CL, Cameron JL, Lillemoe KD, Ferrone CR, Morales-Oyarvide V, He J, et al. Multi-institutional Validation Study of the American Joint Commission on Cancer (8th Edition) Changes for T and N Staging in Patients With Pancreatic Adenocarcinoma. Ann Surg. 2017;265(1):185-91.
101. Saka B, Balci S, Basturk O, Bagci P, Postlewait LM, Maithel S, Knight J, El-Rayes B, Kooby D, Sarmiento J, et al. Pancreatic Ductal Adenocarcinoma is Spread to the Peripancreatic Soft Tissue in the Majority of Resected Cases, Rendering the AJCC T-Stage Protocol (7th Edition) Inapplicable and Insignificant: A Size-Based Staging System (pT1: </=2, pT2: >2-</=4, pT3: >4 cm) is More Valid and Clinically Relevant. Ann Surg Oncol. 2016;23(6):2010-8.
102. Basturk O, Saka B, Balci S, Postlewait LM, Knight J, Goodman M, Kooby D, Sarmiento JM, El-Rayes B, Choi H, et al. Substaging of Lymph Node Status in Resected Pancreatic Ductal Adenocarcinoma Has Strong Prognostic Correlations: Proposal for a Revised N Classification for TNM Staging. Ann Surg Oncol. 2015;22 Suppl 3:S1187-95.
103. Strobel O, Hinz U, Gluth A, Hank T, Hackert T, Bergmann F, Werner J, Buchler MW. Pancreatic adenocarcinoma: number of positive nodes allows to distinguish several N categories. Ann Surg. 2015;261(5):961-9.
104. Brierley J, Gospodarowicz M, Wittekind C. TNM Classification of Malignant Tumours, 8th Edition: Oxford: Wiley-Blackwell; 2017.
105. Hruban RH, Fukushima N. Pancreatic adenocarcinoma: update on the surgical pathology of carcinomas of ductal origin and PanINs. Mod Pathol. 2007;20 Suppl 1:S61-70.
106. Garcea G, Dennison AR, Pattenden CJ, Neal CP, Sutton CD, Berry DP. Survival following curative resection for pancreatic ductal adenocarcinoma. A systematic review of the literature. JOP. 2008;9(2):99-132.
107. Elebro J, Jirstrom K. Use of a standardized diagnostic approach improves the prognostic information of histopathologic factors in pancreatic and periampullary adenocarcinoma. Diagn Pathol. 2014;9:80.

108. Jamieson NB, Foulis AK, Oien KA, Dickson EJ, Imrie CW, Carter R, McKay CJ. Peripancreatic fat invasion is an independent predictor of poor outcome following pancreaticoduodenectomy for pancreatic ductal adenocarcinoma. J Gastrointest Surg. 2011;15(3):512-24.
109. Groot VP, van Santvoort HC, Rombouts SJ, Hagendoorn J, Borel Rinkes IH, van Vulpen M, Herman JM, Wolfgang CL, Besselink MG, Molenaar IQ. Systematic review on the treatment of isolated local recurrence of pancreatic cancer after surgery; re-resection, chemoradiotherapy and SBRT. HPB (Oxford). 2017;19(2):83-92.
110. Kim KS, Kwon J, Kim K, Chie EK. Impact of Resection Margin Distance on Survival of Pancreatic Cancer: A Systematic Review and Meta-Analysis. Cancer Res Treat. 2017;49(3):824-33.
111. McGuigan A, Kelly P, Turkington RC, Jones C, Coleman HG, McCain RS. Pancreatic cancer: A review of clinical diagnosis, epidemiology, treatment and outcomes. World J Gastroenterol. 2018;24(43):4846-61.
112. Whipple AO, Parsons WB, Mullins CR. Treatment of Carcinoma of the Ampulla of Vater. Ann Surg. 1935;102(4):763-79.
113. Sun J, Yang Y, Wang X, Yu Z, Zhang T, Song J, Zhao H, Wen J, Du Y, Lau WY, et al. Meta-analysis of the efficacies of extended and standard pancreatoduodenectomy for ductal adenocarcinoma of the head of the pancreas. World J Surg. 2014;38(10):2708-15.
114. Lall CG, Howard TJ, Skandarajah A, DeWitt JM, Aisen AM, Sandrasegaran K. New concepts in staging and treatment of locally advanced pancreatic head cancer. AJR Am J Roentgenol. 2007;189(5):1044-50.
115. Katz MH, Hwang R, Fleming JB, Evans DB. Tumor-node-metastasis staging of pancreatic adenocarcinoma. CA Cancer J Clin. 2008;58(2):111-25.
116. Wong JC, Raman S. Surgical resectability of pancreatic adenocarcinoma: CTA. Abdom Imaging. 2010;35(4):471-80.
117. Katz MH, Marsh R, Herman JM, Shi Q, Collison E, Venook AP, Kindler HL, Alberts SR, Philip P, Lowy AM, et al. Borderline resectable pancreatic cancer: need for standardization and methods for optimal clinical trial design. Ann Surg Oncol. 2013;20(8):2787-95.
118. Crippa S, Bittoni A, Sebastiani E, Partelli S, Zanon S, Lanese A, Andrikou K, Muffatti F, Balzano G, Reni M, et al. Is there a role for surgical resection in patients with pancreatic cancer with liver metastases responding to chemotherapy? Eur J Surg Oncol. 2016;42(10):1533-9.
119. Heinrich S, Lang H. Neoadjuvant Therapy of Pancreatic Cancer: Definitions and Benefits. Int J Mol Sci. 2017;18(8).
120. Kim HW, Lee JC, Lee J, Kim JW, Kim J, Hwang JH. Early versus delayed initiation of adjuvant treatment for pancreatic cancer. PLoS One. 2017;12(3):e0173960.
121. Gupta R, Amanam I, Chung V. Current and future therapies for advanced pancreatic cancer. J Surg Oncol. 2017;116(1):25-34.
122. Longley DB, Harkin DP, Johnston PG. 5-fluorouracil: mechanisms of action and clinical strategies. Nat Rev Cancer. 2003;3(5):330-8.

123. Graham J, Mushin M, Kirkpatrick P. Oxaliplatin. Nat Rev Drug Discov. 2004;3(1):11-2.
124. Fujita K, Kubota Y, Ishida H, Sasaki Y. Irinotecan, a key chemotherapeutic drug for metastatic colorectal cancer. World J Gastroenterol. 2015;21(43):12234-48.
125. Kipps E, Young K, Starling N. Liposomal irinotecan in gemcitabine-refractory metastatic pancreatic cancer: efficacy, safety and place in therapy. Ther Adv Med Oncol. 2017;9(3):159-70.
126. Schafer A, Schomacher L, Barreto G, Doderlein G, Niehrs C. Gemcitabine functions epigenetically by inhibiting repair mediated DNA demethylation. PLoS One. 2010;5(11):e14060.
127. Yardley DA. nab-Paclitaxel mechanisms of action and delivery. J Control Release. 2013;170(3):365-72.
128. Zhang D, Yang R, Wang S, Dong Z. Paclitaxel: new uses for an old drug. Drug Des Devel Ther. 2014;8:279-84.
129. Walko CM, Lindley C. Capecitabine: a review. Clin Ther. 2005;27(1):23-44.
130. Malet-Martino M, Martino R. Clinical studies of three oral prodrugs of 5-fluorouracil (capecitabine, UFT, S-1): a review. Oncologist. 2002;7(4):288-323.
131. Conroy T, Desseigne F, Ychou M, Bouche O, Guimbaud R, Becouarn Y, Adenis A, Raoul JL, Gourgou-Bourgade S, de la Fouchardiere C, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. N Engl J Med. 2011;364(19):1817-25.
132. Tang K, Lu W, Qin W, Wu Y. Neoadjuvant therapy for patients with borderline resectable pancreatic cancer: A systematic review and meta-analysis of response and resection percentages. Pancreatology. 2016;16(1):28-37.
133. Ferrone CR, Marchegiani G, Hong TS, Ryan DP, Deshpande V, McDonnell EI, Sabbatino F, Santos DD, Allen JN, Blaszkowsky LS, et al. Radiological and surgical implications of neoadjuvant treatment with FOLFIRINOX for locally advanced and borderline resectable pancreatic cancer. Ann Surg. 2015;261(1):12-7.
134. Lutfi W, Talamonti MS, Kantor O, Wang CH, Liederbach E, Stocker SJ, Bentrem DJ, Roggin KK, Winchester DJ, Marsh R, et al. Perioperative chemotherapy is associated with a survival advantage in early stage adenocarcinoma of the pancreatic head. Surgery. 2016;160(3):714-24.
135. Neoptolemos JP, Palmer DH, Ghaneh P, Psarelli EE, Valle JW, Halloran CM, Faluyi O, O'Reilly DA, Cunningham D, Wadsley J, et al. Comparison of adjuvant gemcitabine and capecitabine with gemcitabine monotherapy in patients with resected pancreatic cancer (ESPAC-4): a multicentre, open-label, randomised, phase 3 trial. Lancet. 2017;389(10073):1011-24.
136. Conroy T, Hammel P, Hebbar M, Ben Abdelghani M, Wei AC, Raoul JL, Chone L, Francois E, Artru P, Biagi JJ, et al. FOLFIRINOX or Gemcitabine as Adjuvant Therapy for Pancreatic Cancer. N Engl J Med. 2018;379(25):2395-406.
137. Doherty MK, Knox JJ. Adjuvant therapy for resected biliary tract cancer: a review. Chin Clin Oncol. 2016;5(5):64.

138. Ghosn M, Kourie HR, El Rassy E, Haddad FG, Hanna C, El Karak F, Nasr D. Where does chemotherapy stands in the treatment of ampullary carcinoma? A review of literature. World J Gastrointest Oncol. 2016;8(10):745-50.
139. Neoptolemos JP, Moore MJ, Cox TF, Valle JW, Palmer DH, McDonald AC, Carter R, Tebbutt NC, Dervenis C, Smith D, et al. Effect of adjuvant chemotherapy with fluorouracil plus folinic acid or gemcitabine vs observation on survival in patients with resected periampullary adenocarcinoma: the ESPAC-3 periampullary cancer randomized trial. JAMA. 2012;308(2):147-56.
140. Schiergens TS, Reu S, Neumann J, Renz BW, Niess H, Boeck S, Heinemann V, Bruns CJ, Jauch KW, Kleespies A. Histomorphologic and molecular phenotypes predict gemcitabine response and overall survival in adenocarcinoma of the ampulla of Vater. Surgery. 2015;158(1):151-61.
141. Shroff RT, Kennedy EB, Bachini M, Bekaii-Saab T, Crane C, Edeline J, El-Khoueiry A, Feng M, Katz MHG, Primrose J, et al. Adjuvant Therapy for Resected Biliary Tract Cancer: ASCO Clinical Practice Guideline. J Clin Oncol. 2019:JCO1802178.
142. Overman MJ, Kopetz S, Lin E, Abbruzzese JL, Wolff RA. Is there a role for adjuvant therapy in resected adenocarcinoma of the small intestine. Acta Oncol. 2010;49(4):474-9.
143. Ecker BL, McMillan MT, Datta J, Mamtani R, Giantonio BJ, Dempsey DT, Fraker DL, Drebin JA, Karakousis GC, Roses RE. Efficacy of adjuvant chemotherapy for small bowel adenocarcinoma: A propensity score-matched analysis. Cancer. 2016;122(5):693-701.
144. Von Hoff DD, Ervin T, Arena FP, Chiorean EG, Infante J, Moore M, Seay T, Tjulandin SA, Ma WW, Saleh MN, et al. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. N Engl J Med. 2013;369(18):1691-703.
145. Wang-Gillam A, Li CP, Bodoky G, Dean A, Shan YS, Jameson G, Macarulla T, Lee KH, Cunningham D, Blanc JF, et al. Nanoliposomal irinotecan with fluorouracil and folinic acid in metastatic pancreatic cancer after previous gemcitabine-based therapy (NAPOLI-1): a global, randomised, open-label, phase 3 trial. Lancet. 2016;387(10018):545-57.
146. Takahashi H, Ohigashi H, Gotoh K, Marubashi S, Yamada T, Murata M, Ioka T, Uehara H, Yano M, Ishikawa O. Preoperative gemcitabine-based chemoradiation therapy for resectable and borderline resectable pancreatic cancer. Ann Surg. 2013;258(6):1040-50.
147. Rutter CE, Park HS, Corso CD, Lester-Coll NH, Mancini BR, Yeboa DN, Johung KL. Addition of radiotherapy to adjuvant chemotherapy is associated with improved overall survival in resected pancreatic adenocarcinoma: An analysis of the National Cancer Data Base. Cancer. 2015;121(23):4141-9.
148. Liao WC, Chien KL, Lin YL, Wu MS, Lin JT, Wang HP, Tu YK. Adjuvant treatments for resected pancreatic adenocarcinoma: a systematic review and network meta-analysis. Lancet Oncol. 2013;14(11):1095-103.
149. Habermehl D, Brecht IC, Debus J, Combs SE. Palliative radiation therapy in patients with metastasized pancreatic cancer - description of a rare patient group. Eur J Med Res. 2014;19:24.

150. Barton CM, Hall PA, Hughes CM, Gullick WJ, Lemoine NR. Transforming growth factor alpha and epidermal growth factor in human pancreatic cancer. J Pathol. 1991;163(2):111-6.
151. Oliveira-Cunha M, Newman WG, Siriwardena AK. Epidermal growth factor receptor in pancreatic cancer. Cancers (Basel). 2011;3(2):1513-26.
152. Chan BA, Hughes BG. Targeted therapy for non-small cell lung cancer: current standards and the promise of the future. Transl Lung Cancer Res. 2015;4(1):36-54.
153. Moore MJ, Goldstein D, Hamm J, Figer A, Hecht JR, Gallinger S, Au HJ, Murawa P, Walde D, Wolff RA, et al. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol. 2007;25(15):1960-6.
154. Yarchoan M, Myzak MC, Johnson BA, 3rd, De Jesus-Acosta A, Le DT, Jaffee EM, Azad NS, Donehower RC, Zheng L, Oberstein PE, et al. Olaparib in combination with irinotecan, cisplatin, and mitomycin C in patients with advanced pancreatic cancer. Oncotarget. 2017;8(27):44073-81.
155. Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, Mortimer P, Swaisland H, Lau A, O'Connor MJ, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. N Engl J Med. 2009;361(2):123-34.
156. Waddell N, Pajic M, Patch AM, Chang DK, Kassahn KS, Bailey P, Johns AL, Miller D, Nones K, Quek K, et al. Whole genomes redefine the mutational landscape of pancreatic cancer. Nature. 2015;518(7540):495-501.
157. Skelton RA, Javed A, Zheng L, He J. Overcoming the resistance of pancreatic cancer to immune checkpoint inhibitors. J Surg Oncol. 2017;116(1):55-62.
158. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, Lu S, Kemberling H, Wilt C, Luber BS, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science. 2017;357(6349):409-13.
159. Mirkin S, Pickar JH. Selective estrogen receptor modulators (SERMs): a review of clinical data. Maturitas. 2015;80(1):52-7.
160. MacGregor JI, Jordan VC. Basic guide to the mechanisms of antiestrogen action. Pharmacol Rev. 1998;50(2):151-96.
161. Gagliardi A, Collins DC. Inhibition of angiogenesis by antiestrogens. Cancer Res. 1993;53(3):533-5.
162. Johnson KE, Forward JA, Tippy MD, Ceglowski JR, El-Husayni S, Kulenthirarajan R, Machlus KR, Mayer EL, Italiano JE, Jr., Battinelli EM. Tamoxifen Directly Inhibits Platelet Angiogenic Potential and Platelet-Mediated Metastasis. Arterioscler Thromb Vasc Biol. 2017;37(4):664-74.
163. Stendahl M, Ryden L, Nordenskjold B, Jonsson PE, Landberg G, Jirstrom K. High progesterone receptor expression correlates to the effect of adjuvant tamoxifen in premenopausal breast cancer patients. Clin Cancer Res. 2006;12(15):4614-8.
164. Nordenskjold A, Fohlin H, Fornander T, Lofdahl B, Skoog L, Stal O. Progesterone receptor positivity is a predictor of long-term benefit from adjuvant tamoxifen treatment of estrogen receptor positive breast cancer. Breast Cancer Res Treat. 2016;160(2):313-22.

165. Dowsett M, Cuzick J, Wale C, Howell T, Houghton J, Baum M. Retrospective analysis of time to recurrence in the ATAC trial according to hormone receptor status: an hypothesis-generating study. J Clin Oncol. 2005;23(30):7512-7.
166. Theve NO, Pousette A, Carlstrom K. Adenocarcinoma of the pancreas--a hormone sensitive tumor? A preliminary report on Nolvadex treatment. Clin Oncol. 1983;9(3):193-7.
167. Tonnesen K, Kamp-Jensen M. Antiestrogen therapy in pancreatic carcinoma: a preliminary report. Eur J Surg Oncol. 1986;12(1):69-70.
168. Horimi T, Takasaki M, Toki A, Nishimura W, Morita S. The beneficial effect of tamoxifen therapy in patients with resected adenocarcinoma of the pancreas. Hepatogastroenterology. 1996;43(11):1225-9.
169. Bakkevold KE, Pettersen A, Arnesjo B, Espehaug B. Tamoxifen therapy in unresectable adenocarcinoma of the pancreas and the papilla of Vater. Br J Surg. 1990;77(7):725-30.
170. Wong A, Chan A. Survival benefit of tamoxifen therapy in adenocarcinoma of pancreas. A case-control study. Cancer. 1993;71(7):2200-3.
171. Molteni A, Bahu RM, Battifora HA, Fors EM, Reddy JK, Rao MS, Scarpelli DG. Estradiol receptor assays in normal and neoplastic tissues. A possible diagnostic acid for tumor differentiation. Ann Clin Lab Sci. 1979;9(2):103-8.
172. Greenway B, Iqbal MJ, Johnson PJ, Williams R. Oestrogen receptor proteins in malignant and fetal pancreas. Br Med J (Clin Res Ed). 1981;283(6294):751-3.
173. Rosenberg L, Barkun AN, Denis MH, Pollak M. Low dose octreotide and tamoxifen in the treatment of adenocarcinoma of the pancreas. Cancer. 1995;75(1):23-8.
174. Eckel F, Lersch C, Lippl F, Assmann G, Schulte-Frohlinde E. Phase II trial of cyclophosphamide, leucovorin, 5-fluorouracil 24-hour infusion and tamoxifen in pancreatic cancer. J Exp Clin Cancer Res. 2000;19(3):295-300.
175. Tomao S, Romiti A, Massidda B, Ionta MT, Farris A, Zullo A, Brescia A, Santuari L, Frati L. A phase II study of gemcitabine and tamoxifen in advanced pancreatic cancer. Anticancer Res. 2002;22(4):2361-4.
176. Crowson MC, Dorrell A, Rolfe EB, Fielding JW. A phase II study to evaluate tamoxifen in pancreatic adenocarcinoma. Eur J Surg Oncol. 1986;12(4):335-6.
177. Keating JJ, Johnson PJ, Cochrane AM, Gazzard BG, Krasner N, Smith PM, Trewby PN, Wheeler P, Wilkinson SP, Williams R. A prospective randomised controlled trial of tamoxifen and cyproterone acetate in pancreatic carcinoma. Br J Cancer. 1989;60(5):789-92.
178. Scheithauer W, Kornek G, Haider K, Depisch D. Unresponsiveness of pancreatic adenocarcinoma to antioestrogen therapy. Eur J Cancer. 1990;26(7):851-2.
179. Swarovsky B, Wolf M, Havemann K, Arnold R. Tamoxifen or cyproterone acetate in combination with buserelin are ineffective in patients with pancreatic adenocarcinoma. Oncology. 1993;50(4):226-9.
180. Taylor OM, Benson EA, McMahon MJ. Clinical trial of tamoxifen in patients with irresectable pancreatic adenocarcinoma. The Yorkshire Gastrointestinal Tumour Group. Br J Surg. 1993;80(3):384-6.

181. Jia M, Dahlman-Wright K, Gustafsson JA. Estrogen receptor alpha and beta in health and disease. Best Pract Res Clin Endocrinol Metab. 2015;29(4):557-68.
182. Vrtacnik P, Ostanek B, Mencej-Bedrac S, Marc J. The many faces of estrogen signaling. Biochem Med (Zagreb). 2014;24(3):329-42.
183. Liang J, Shang Y. Estrogen and cancer. Annu Rev Physiol. 2013;75:225-40.
184. Brown C. Osteoporosis: Staying strong. Nature. 2017;550(7674):S15-S7.
185. Iorga A, Cunningham CM, Moazeni S, Ruffenach G, Umar S, Eghbali M. The protective role of estrogen and estrogen receptors in cardiovascular disease and the controversial use of estrogen therapy. Biol Sex Differ. 2017;8(1):33.
186. Zheng ZY, Bay BH, Aw SE, Lin VC. A novel antiestrogenic mechanism in progesterone receptor-transfected breast cancer cells. J Biol Chem. 2005;280(17):17480-7.
187. Nojima D, Li LC, Dharia A, Perinchery G, Ribeiro-Filho L, Yen TS, Dahiya R. CpG hypermethylation of the promoter region inactivates the estrogen receptor-beta gene in patients with prostate carcinoma. Cancer. 2001;92(8):2076-83.
188. Zhao C, Lam EW, Sunters A, Enmark E, De Bella MT, Coombes RC, Gustafsson JA, Dahlman-Wright K. Expression of estrogen receptor beta isoforms in normal breast epithelial cells and breast cancer: regulation by methylation. Oncogene. 2003;22(48):7600-6.
189. Huang B, Omoto Y, Iwase H, Yamashita H, Toyama T, Coombes RC, Filipovic A, Warner M, Gustafsson JA. Differential expression of estrogen receptor alpha, beta1, and beta2 in lobular and ductal breast cancer. Proc Natl Acad Sci U S A. 2014;111(5):1933-8.
190. Horvath LG, Henshall SM, Lee CS, Head DR, Quinn DI, Makela S, Delprado W, Golovsky D, Brenner PC, O'Neill G, et al. Frequent loss of estrogen receptor-beta expression in prostate cancer. Cancer Res. 2001;61(14):5331-5.
191. Omoto Y, Inoue S, Ogawa S, Toyama T, Yamashita H, Muramatsu M, Kobayashi S, Iwase H. Clinical value of the wild-type estrogen receptor beta expression in breast cancer. Cancer Lett. 2001;163(2):207-12.
192. De Stefano I, Zannoni GF, Prisco MG, Fagotti A, Tortorella L, Vizzielli G, Mencaglia L, Scambia G, Gallo D. Cytoplasmic expression of estrogen receptor beta (ERbeta) predicts poor clinical outcome in advanced serous ovarian cancer. Gynecol Oncol. 2011;122(3):573-9.
193. Andersson S, Sundberg M, Pristovsek N, Ibrahim A, Jonsson P, Katona B, Clausson CM, Zieba A, Ramstrom M, Soderberg O, et al. Insufficient antibody validation challenges oestrogen receptor beta research. Nat Commun. 2017;8:15840.
194. Cui X, Schiff R, Arpino G, Osborne CK, Lee AV. Biology of progesterone receptor loss in breast cancer and its implications for endocrine therapy. J Clin Oncol. 2005;23(30):7721-35.
195. Scarpin KM, Graham JD, Mote PA, Clarke CL. Progesterone action in human tissues: regulation by progesterone receptor (PR) isoform expression, nuclear positioning and coregulator expression. Nucl Recept Signal. 2009;7:e009.

196. Samalecos A, Gellersen B. Systematic expression analysis and antibody screening do not support the existence of naturally occurring progesterone receptor (PR)-C, PR-M, or other truncated PR isoforms. Endocrinology. 2008;149(11):5872-87.
197. Jacobsen BM, Horwitz KB. Progesterone receptors, their isoforms and progesterone regulated transcription. Mol Cell Endocrinol. 2012;357(1-2):18-29.
198. Grimm SL, Hartig SM, Edwards DP. Progesterone Receptor Signaling Mechanisms. J Mol Biol. 2016;428(19):3831-49.
199. Falcone T, W. Hurd W. Clinical Reproductive Medicine and Surgery: A Practical Guide. 2nd ed. 2013 edition: Springer Science & Business Media; 22 May 2013. p. 39.
200. Mulac-Jericevic B, Mullinax RA, DeMayo FJ, Lydon JP, Conneely OM. Subgroup of reproductive functions of progesterone mediated by progesterone receptor-B isoform. Science. 2000;289(5485):1751-4.
201. Mulac-Jericevic B, Lydon JP, DeMayo FJ, Conneely OM. Defective mammary gland morphogenesis in mice lacking the progesterone receptor B isoform. Proc Natl Acad Sci U S A. 2003;100(17):9744-9.
202. Brisken C. Progesterone signalling in breast cancer: a neglected hormone coming into the limelight. Nat Rev Cancer. 2013;13(6):385-96.
203. Hilton HN, Graham JD, Clarke CL. Minireview: Progesterone Regulation of Proliferation in the Normal Human Breast and in Breast Cancer: A Tale of Two Scenarios? Mol Endocrinol. 2015;29(9):1230-42.
204. Beral V, Million Women Study C. Breast cancer and hormone-replacement therapy in the Million Women Study. Lancet. 2003;362(9382):419-27.
205. Diep CH, Daniel AR, Mauro LJ, Knutson TP, Lange CA. Progesterone action in breast, uterine, and ovarian cancers. J Mol Endocrinol. 2015;54(2):R31-53.
206. Liu S, Chia SK, Mehl E, Leung S, Rajput A, Cheang MC, Nielsen TO. Progesterone receptor is a significant factor associated with clinical outcomes and effect of adjuvant tamoxifen therapy in breast cancer patients. Breast Cancer Res Treat. 2010;119(1):53-61.
207. Purdie CA, Quinlan P, Jordan LB, Ashfield A, Ogston S, Dewar JA, Thompson AM. Progesterone receptor expression is an independent prognostic variable in early breast cancer: a population-based study. Br J Cancer. 2014;110(3):565-72.
208. Basile D, Cinausero M, Iacono D, Pelizzari G, Bonotto M, Vitale MG, Gerratana L, Puglisi F. Androgen receptor in estrogen receptor positive breast cancer: Beyond expression. Cancer Treat Rev. 2017;61:15-22.
209. Tan MH, Li J, Xu HE, Melcher K, Yong EL. Androgen receptor: structure, role in prostate cancer and drug discovery. Acta Pharmacol Sin. 2015;36(1):3-23.
210. Gucalp A, Traina TA. The Androgen Receptor: Is It a Promising Target? Ann Surg Oncol. 2017;24(10):2876-80.
211. Georgiadou D, Sergentanis TN, Sakellariou S, Vlachodimitropoulos D, Psaltopoulou T, Lazaris AC, Gounaris A, Zografos GC. Prognostic role of sex steroid receptors in pancreatic adenocarcinoma. Pathol Res Pract. 2016;212(1):38-43.

212. Seeliger H, Pozios I, Assmann G, Zhao Y, Muller MH, Knosel T, Kreis ME, Bruns CJ. Expression of estrogen receptor beta correlates with adverse prognosis in resected pancreatic adenocarcinoma. BMC Cancer. 2018;18(1):1049.
213. Pozios I, Knosel T, Zhao Y, Assmann G, Pozios I, Muller MH, Bruns CJ, Kreis ME, Seeliger H. Expression of phosphorylated estrogen receptor beta is an independent negative prognostic factor for pancreatic ductal adenocarcinoma. J Cancer Res Clin Oncol. 2018;144(10):1887-97.
214. Ishida K, Sasano H, Moriya T, Takahashi Y, Sugimoto R, Mue Y, Murakami K, Fujishima F, Nakamura Y, Morikawa T, et al. Immunohistochemical analysis of steroidogenic enzymes in ovarian-type stroma of pancreatic mucinous cystic neoplasms: Comparative study of subepithelial stromal cells in intraductal papillary mucinous neoplasms of the pancreas. Pathol Int. 2016;66(5):281-7.
215. Jang KT, Park SM, Basturk O, Bagci P, Bandyopadhyay S, Stelow EB, Walters DM, Choi DW, Choi SH, Heo JS, et al. Clinicopathologic characteristics of 29 invasive carcinomas arising in 178 pancreatic mucinous cystic neoplasms with ovarian-type stroma: implications for management and prognosis. Am J Surg Pathol. 2015;39(2):179-87.
216. Corbishley TP, Iqbal MJ, Wilkinson ML, Williams R. Androgen receptor in human normal and malignant pancreatic tissue and cell lines. Cancer. 1986;57(10):1992-5.
217. Greenway BA. Effect of flutamide on survival in patients with pancreatic cancer: results of a prospective, randomised, double blind, placebo controlled trial. BMJ. 1998;316(7149):1935-8.
218. Corrie P, Mayer A, Shaw J, D'Ath S, Blagden S, Blesing C, Price P, Warner N. Phase II study to evaluate combining gemcitabine with flutamide in advanced pancreatic cancer patients. Br J Cancer. 2002;87(7):716-9.
219. Negi SS, Agarwal A, Chaudhary A. Flutamide in unresectable pancreatic adenocarcinoma: a randomized, double-blind, placebo-controlled trial. Invest New Drugs. 2006;24(3):189-94.
220. Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, Charrondiere UR, Hemon B, Casagrande C, Vignat J, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr. 2002;5(6B):1113-24.
221. Slimani N, Kaaks R, Ferrari P, Casagrande C, Clavel-Chapelon F, Lotze G, Kroke A, Trichopoulos D, Trichopoulou A, Lauria C, et al. European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study: rationale, design and population characteristics. Public Health Nutr. 2002;5(6B):1125-45.
222. Berglund G, Elmstahl S, Janzon L, Larsson SA. The Malmo Diet and Cancer Study. Design and feasibility. J Intern Med. 1993;233(1):45-51.
223. Manjer J, Carlsson S, Elmstahl S, Gullberg B, Janzon L, Lindstrom M, Mattisson I, Berglund G. The Malmo Diet and Cancer Study: representativity, cancer incidence and mortality in participants and non-participants. Eur J Cancer Prev. 2001;10(6):489-99.

224. Heby M, Lundgren S, Nodin B, Elebro J, Eberhard J, Jirstrom K. Relationship between mismatch repair immunophenotype and long-term survival in patients with resected periampullary adenocarcinoma. J Transl Med. 2018;16(1):66.
225. Hariri N, Roma AA, Hasteh F, Walavalkar V, Fadare O. Phenotypic alterations in breast cancer associated with neoadjuvant chemotherapy: A comparison with baseline rates of change. Ann Diagn Pathol. 2017;31:14-9.
226. Lundgren S, Olsson Hau S, Elebro J, Heby M, Karnevi E, Nodin B, Eberhard J, Holm K, Staaf J, Jönsson G, et al. Mutational Landscape in Resected Periampullary Adenocarcinoma: Relationship With Morphology and Clinical Outcome. JCO Precision Oncology. 2019(3):1-8.
227. Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton S, Torhorst J, Mihatsch MJ, Sauter G, Kallioniemi OP. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med. 1998;4(7):844-7.
228. Matos LL, Trufelli DC, de Matos MG, da Silva Pinhal MA. Immunohistochemistry as an important tool in biomarkers detection and clinical practice. Biomark Insights. 2010;5:9-20.
229. Coons AH, Kaplan MH. Localization of antigen in tissue cells; improvements in a method for the detection of antigen by means of fluorescent antibody. J Exp Med. 1950;91(1):1-13.
230. Bordeaux J, Welsh A, Agarwal S, Killiam E, Baquero M, Hanna J, Anagnostou V, Rimm D. Antibody validation. Biotechniques. 2010;48(3):197-209.
231. Mahmood T, Yang PC. Western blot: technique, theory, and trouble shooting. N Am J Med Sci. 2012;4(9):429-34.
232. Olds W, Li J. siRNA knockdown validation 101: Incorporating negative controls in antibody research. F1000Res. 2016;5:308.
233. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012;2(5):401-4.
234. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal. 2013;6(269):pl1.
235. Korn EL, Graubard BI, Midthune D. Time-to-event analysis of longitudinal follow-up of a survey: choice of the time-scale. Am J Epidemiol. 1997;145(1):72-80.
236. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin. 2016;66(1):7-30.
237. Key TJ, Pike MC, Brown JB, Hermon C, Allen DS, Wang DY. Cigarette smoking and urinary oestrogen excretion in premenopausal and post-menopausal women. Br J Cancer. 1996;74(8):1313-6.
238. Gu F, Caporaso NE, Schairer C, Fortner RT, Xu X, Hankinson SE, Eliassen AH, Ziegler RG. Urinary concentrations of estrogens and estrogen metabolites and smoking in caucasian women. Cancer Epidemiol Biomarkers Prev. 2013;22(1):58-68.

239. Statistics Sweden Unit of Social Welfare. Use of alcohol and tobacco 2007 [Available from: https://www.scb.se/statistik/_publikationer/LE0101_2004I05_BR_LE114SA0701.pdf. Accessed: 2019-04-05.
240. Public Health Agency of Sweden. Living habits, daily tobacco smoking 2018 [updated 2019-03-14. Available from: https://www.folkhalsomyndigheten.se/folkhalsorapportering-statistik/folkhalsans-utveckling/levnadsvanor/tobaksrokning-daglig/. Accessed: 2019-04-07.
241. Michaud DS, Giovannucci E, Willett WC, Colditz GA, Fuchs CS. Coffee and alcohol consumption and the risk of pancreatic cancer in two prospective United States cohorts. Cancer Epidemiol Biomarkers Prev. 2001;10(5):429-37.
242. Stolzenberg-Solomon RZ, Pietinen P, Barrett MJ, Taylor PR, Virtamo J, Albanes D. Dietary and other methyl-group availability factors and pancreatic cancer risk in a cohort of male smokers. Am J Epidemiol. 2001;153(7):680-7.
243. Stevens RJ, Roddam AW, Spencer EA, Pirie KL, Reeves GK, Green J, Beral V, Million Women Study C. Factors associated with incident and fatal pancreatic cancer in a cohort of middle-aged women. Int J Cancer. 2009;124(10):2400-5.
244. Lin Y, Kikuchi S, Tamakoshi A, Kawamura T, Inaba Y, Kurosawa M, Motohashi Y, Yagyu K, Obata Y, Ishibashi T, et al. Association of menstrual and reproductive factors with pancreatic cancer risk in women: findings of the Japan Collaborative Cohort Study for Evaluation of Cancer Risk. J Gastroenterol. 2006;41(9):878-83.
245. Jimenez RE, Warshaw AL, Z'Graggen K, Hartwig W, Taylor DZ, Compton CC, Fernandez-del Castillo C. Sequential accumulation of K-ras mutations and p53 overexpression in the progression of pancreatic mucinous cystic neoplasms to malignancy. Ann Surg. 1999;230(4):501-9; discussion 9-11.
246. Sleire L, Forde HE, Netland IA, Leiss L, Skeie BS, Enger PO. Drug repurposing in cancer. Pharmacol Res. 2017;124:74-91.
247. Cha Y, Erez T, Reynolds IJ, Kumar D, Ross J, Koytiger G, Kusko R, Zeskind B, Risso S, Kagan E, et al. Drug repurposing from the perspective of pharmaceutical companies. Br J Pharmacol. 2018;175(2):168-80.
248. Dilly SJ, Morris GS. Pimping up Drugs Recovered, Superannuated and Under Exploited Drugs - An Introduction to the Basics of Drug Reprofiling. Curr Drug Discov Technol. 2017;14(2):121-6.

GUSTAV ANDERSSON

Printed by Media-Tryck, Lund 2019 NORDIC SWAN ECOLABEL 3041 0903

LUND UNIVERSITY

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2019:47
ISBN 978-91-7619-776-9
ISSN 1652-8220