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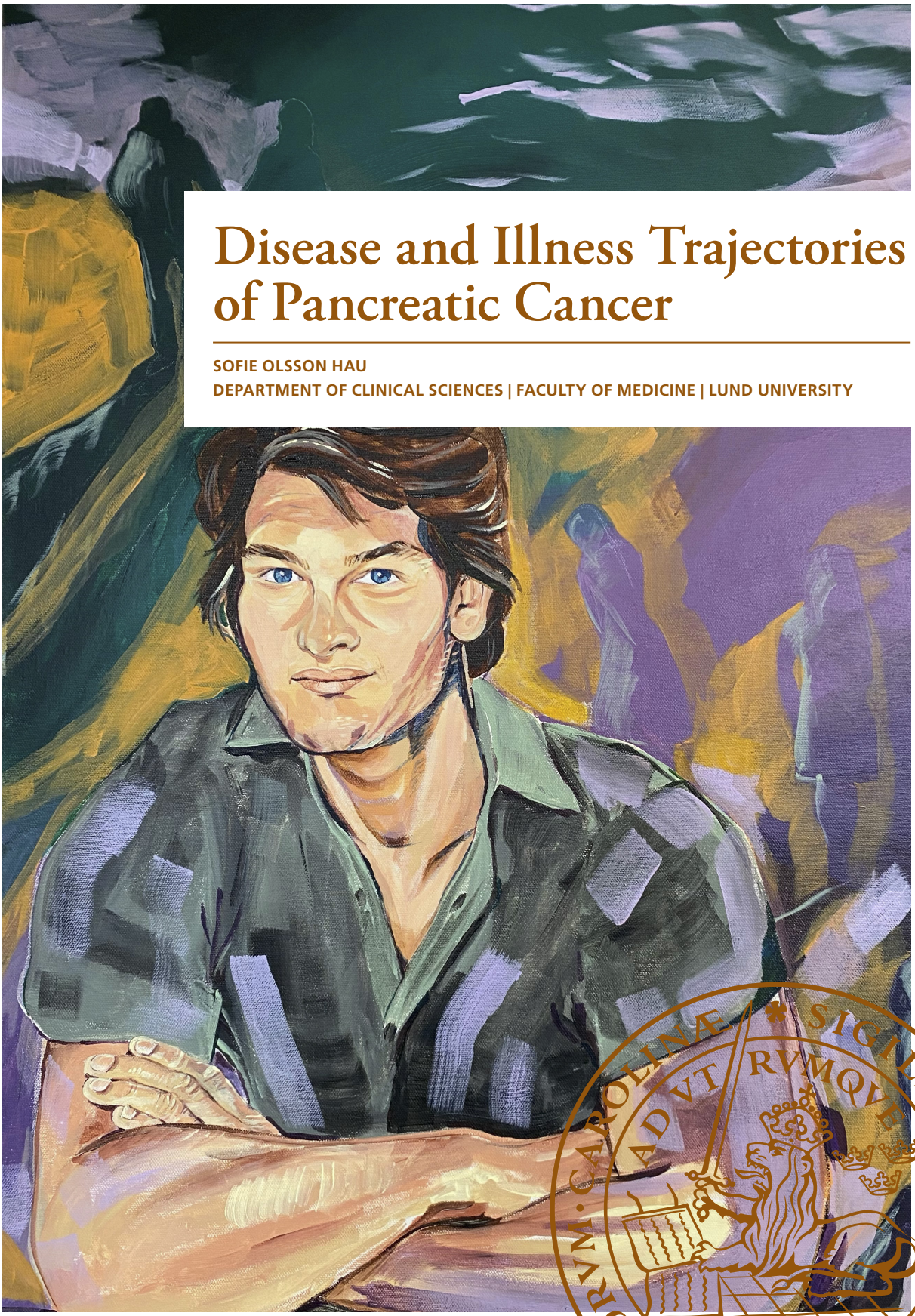

Disease and Illness Trajectories of Pancreatic Cancer

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About the author

I am a medical oncologist, currently subspecialising in palliative medicine in Malmö. I have a particular interest in pancreatic cancer, which is a grievous disease with severe symptomatology. The aim of this thesis was to investigate links between disease and illness in patients with pancreatic cancer, with the goal of improving not only outcome, but quality of life.

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Abstract

Pancreatic cancer is the third leading cause of cancer related death. Despite advances in targeted therapy and immunotherapy for many other cancer types, these have not proven successful for the vast majority of pancreatic cancer patients and surgery followed by adjuvant chemotherapy or palliative chemotherapy is the only treatment option. The aim of this thesis was to explore the biological evolution of pancreatic adenocarcinoma during chemotherapy treatment and the implications this may have on the illness of patients.

In Paper I, genes regulated by and cellular processes associated with the prognostic and predictive candidate biomarker RBM3 are explored. Silencing of RBM3 in human pancreatic cancer cell lines revealed 19 dysregulated genes. The top upregulated gene was *CCND3*, and the top downregulated gene was *PDS5A*. *PRR11* was upregulated and and highly prognostic at the mRNA level in The Cancer Genome Atlas. The relationship between these three genes and their corresponding proteins were further validated *in vitro* and analysed by immunohistochemistry in tumours from 46 patients with resected pancreatic cancer. High protein expression of PRR11 was associated with adverse clinicopathological factors and shorter survival. Neither cyclin D3 or PDS5A were prognostic. This study revealed several dysregulated genes with links to cell cycle progression and chromosome formation within the RBM3-related transcriptome, providing further clues about transcriptional partners that may link RBM3 to chemosensitivity in pancreatic cancer.

Paper II is a protocol describing the Chemotherapy, Host response And Molecular dynamics in Periampullary cancer (CHAMP) study. Enrolment started in October 2018 and to date, 127 patients have been included. All patients with periampullary cancer receiving adjuvant or first line palliative chemotherapy at Skåne University hospital are invited to participate. Serial blood sampling is performed at baseline, after each chemotherapy cycle, and at the end of treatment. HRQoL is measured at three month intervals with the EORTC-QLQ-C30 questionnaire. The aim of the study is to explore the interplay between spatial and temporal tumour evolution and host-related factors, and how this interplay may affect response to chemotherapy.

Paper III explores sex and gender differences at baseline regarding treatment intention, HRQoL, performance status, clinicopathological factors and prognosis in the first 100 patients enrolled in the CHAMP study. The results revealed a significant difference between the sexes regarding treatment intention, with fewer women being offered curative surgery. No differences between the sexes were seen regarding demographics or clinicopathological factors. HRQoL was lower in women than men before the start of treatment, bus not associated with performance status. On the contrary, men with poor performance status had increasingly poorer HRQoL. Since no differences were found between the sexes regarding clinicopathological factors or demographics, we conclude that gender bias may be responsible for the discrepancy between men and women being offered curative surgery.

Paper IV investigates trajectories of inflammatory proteins, cell-free DNA and HRQoL in the first 60 patients enrolled in the CHAMP study. We found that HRQoL was increased at three and six months compared to before the start of treatment, independently of treatment intention. High levels of cfDNA at baseline were associated with a decrease in several HRQoL-factors and a poorer OS. Pain at baseline was also associated with poor OS. High levels of three inflammatory proteins, PD-L1, GZMH and IL-12, correlated with pain at several timepoints. High levels of two inflammatory proteins, MMP7 and TNRSF12A, at baseline and one month were associated with a general decrease in HRQoL. To our knowledge, associations between inflammatory serum proteins and cfDNA with HRQoL have not been previously investigated.

Key words Pancreatic cancer, periampullary cancer, the CHAMP study, quality of life, sex, gender, PRR11, serum proteins, cell-free DNA

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For my patients, who live their lives so bravely

Table of contents

Preface

When I was a week old my grandfather passed away after being diagnosed with colon cancer at 62 years of age, and a few years later my grandmother passed away at 53 due to ovarian cancer. Memories of her suffering and my mother's grief have stayed with me. As a child my imagination would often run astray when someone got ill and I would picture the worst possible scenario (dad's cold would turn out to be lung cancer, mum would become paralysed by her herniated disk, my brother would never wake up from the anaesthesia when he had appendicitis surgery and so on). This dread of illness was accompanied by fascination for the complexity of the human body, it seemed to me a whole other universe, hidden and neatly packaged under the skin. I truly believe that the more you know, the less frightening it becomes, so I became an oncologist and am now subspecialising in palliative medicine. I want to give my patients the knowledge to make informed decisions about the remainder of their lives and I hope they feel an increased sense of control and thereby less fear, just like I did the more I learned.

A few days after I started my residency at the oncology department I met Jakob, my clinical supervisor. His enthusiasm, clinical expertise, and sponsoring is the reason I pursued gastrointestinal oncology. Jakob introduced me to research and to Karin, my main supervisor. I was inspired by the work they were doing and by the dynamic and friendly environment Karin had developed in the lab and gladly enrolled in the PhD-programme. Originally, my thesis was focused on colorectal cancer but after seeing the dreadful symptoms and experiencing the helplessness of trying to treat patients with pancreatic cancer I was drawn to focus on this tumour type instead. Although interested in preclinical research, our focus was shifting towards real world studies, and I was thrilled to be able to be a part of the team responsible for starting the CHAMP study with which I feel we are getting closer to improving the patients' quality of life and being able to offer them a more tailored treatment.

Thesis at a glance

Paper	Research Question	Cohort	Methods	Results & Conclusions
I	How do RBM3-regulated genes and cellular processes influence the properties and clinical course of pancreatic cancer?	Retrospective cohort of 46 patients with pancreatic cancer who had undergone pancreatico-duodenectomy between 2001 and 2011.	siRBM3- transfection of MIAPaCa-2 cells followed by RNASeq. Expression of selected genes and proteins explored in 3 siRBM3-treated PDAC cell lines. IHC of cyclin D3, PDS5A and PRR11 on TMAs. Prognostic significance of cyclin D3, PDS5A and PRR11 explored in PDAC patients in TCGA.	Links found between RBM3 and genes involved in DNA replication, repair and cell cycle progression. PRR11 is a robust prognostic biomarker of shorter OS. PRR11 is predictive of shorter OS in adjuvant patients. PRR11 merits further attention in the context of PI3K signalling and potential targeted treatment options.
II	How does the biology of pancreatic cancer change spatially and temporally during chemotherapy ?	Study protocol describing the CHAMP study.	Constructing a study protocol.	Recently started at the time of publication.
III	Are there any differences between the sexes regarding demographics, clinicopathological factors, treatment intention, PS, HRQoL and OS in patients with pancreatic cancer?	The first 100 patients enrolled in the CHAMP study.	Examination of clinicopathological data, demografphc data, PS, survival data and HRQoL data strafied by sex.	There were no differences in disease biology between the sexes. More men than women had curative surgery. HRQoL was stable in women regardless of PS but decreased in men with poor PS. Gender plays an important role when assessing eligibility for surgery.
IV 	How do inflammatory proteins and cfDNA levels during chemotherapy affect HRQoL in patients with pancreatic cancer?	The first 60 patients enrolled in the CHAMP study.	Plasma and serum samples from BL and after 1, 3 and 6 M treatment were examined for cfDNA and 92 inflammatory proteins. HRQoL data were assessed from BL, 3 and 6 months.	HRQoL was improved after initiation of chemotherapy. High levels of cfDNA were linked to poorer HRQoL at BL High levels of TNFRSF12A and MMP7 at BL and 1 month were associated with decreased HRQoL later on. PD-L1, GZMH and IL-12 at BL were associated with increased pain at 3 months. Recognising signs of poor HRQoL at an early stage could improve treatment of symtoms for pancreatic cancer patients.
Abbreviations:	RBM3: RNA-binding motif protein 3, siRNA: Small interfering RNA, IHC: Immunohistochemistry, PDS5A: PDS cohesion associated factor A, PRR11: Proline rich-11, TMA: Tissue microarray, TCGA: The Cancer Genome Atlas, CHAMP: Chemotherapy, Host response And Molecular dynamics in Periampullary cancer, HRQoL: Health realted Quality of Life, OS: Overall survival, PS: Performance status, TNFRSF12A: Tumour necrosis family receptor superfamily member 12 A, MMP-7: Matrix metalloprotienase-7, PD-L1: Programmed death -ligand 1, GZMH: Granzyme H, IL-12: Interleukin-12			

Patientberättelse

Brett Kemp

Biträdande rektor, Sofielundsskolan

Malmö, 230126

Min första upplevelse var på arbetet och hemma att jag fick väldigt ont i magen i samband med att jag åt. Jag resonerade själv att det säkert var någon slags magkatarr och köpte medicin mot detta.

Så småningom bokade jag in en läkartid på Capio där prover togs men där läkaren också menade på att det säkert var magkatarr och ordinerade 2 Omeprazol om dagen. Jag hörde ingenting om proverna på någon vecka. Under tiden åkte jag bort med arbetet på internat och där kände jag mig mycket sjuk, kunde knappt äta men fortsatte med Omeprazolen. Direkt efter åkte jag på nytt internat med rektorsprogrammet men fick lämna internatet dag 2 – jag hade nu rasat i vikt och en rektorskollega hade påpekat för mig att jag var väldigt gul i ansiktet. Nu blev jag rädd, åkte hem till Malmö och direkt upp till Capio Citykliniken – någonstans förstod jag redan då vad det skulle handla om. Min läkare kunde inte ta emot mig utan en annan läkare kom och skrev remiss till akuten direkt.

Jag cyklade upp till akutmottagning, fick en säng där i väntan på prover och röntgen. Så klart var jag livrädd under den här tiden, fick morfin mot smärtan. Nu ringde jag också sambon för att berätta men hade än så länge inga svar att ge. Prover togs som så klart visade att något var fel och jag förstod att symptomen kunde indikera på gallsten men också cancer. Röntgen togs och svaret återkopplades till mig någon gång vid 2 på natten. Då bad en läkare mig att följa henne in i ett annat rum för att visa röntgenbilden och samtidigt förklarade hon hur ledsen hon var för att behöva ge ett sådant besked: det var som jag misstänkte, en tumör. Jag frågade hur länge jag hade att leva, tänkte på mina anhöriga och var totalförstörd. Det var ju så klart en omöjlig fråga att svara på för läkaren i fråga.

Jag placerades därefter på akutavdelningen då det inte fanns lediga platser på Kirurgen där de ville ha mig eftersom det nu skulle genomföras många olika prover och sättas in en stent. Det kändes otroligt fel att ligga på akuten då jag tog en plats som andra skulle behövt som verkligen var i akuta behov. Jag ifrågasatte detta och fick till svar att de inte ville släppa mig för då skulle jag förlora sängplatsen på kirurgavdelningen så småningom. Det var jättekonstigt att behöva vara där fysiskt i väntan på plats. Det togs konstant prover i armen

och till sist gick det inte längre att sticka i armen. Efter diskussioner släpptes jag hem men fick behålla min sängplats och därmed möjligheten till provtagningarna och stentinsättningen. Detta skedde över flera dagar. Problemet var att jag varje dag skulle komma på fastande mage, jag hade redan tappat otroligt mycket vikt och detta fortsatte då under den här tiden. Jag gick från 81,5kg till 63kg på mycket kort tid. När det var klart fick jag reda på att jag skulle få en kallelse till Lund, till onkologen där. Under väntan fick jag ett visst antal smärtstillande. Jag hade fortfarande otroligt svårt att äta, otroliga smärtor i tarmarna och tappade nästan lusten att leva. Jag kände att jag höll på att dö och ganska fort. Det är svårt att förklara. Det som jag reflekterade över under vistelsen på akuten och kirurgavdelningen var att jag tog en sängplats utan att egentligen behöva någon, just för att jag inte skulle missa proverna och ingreppen – någon sorts nummerlappspatient som la beslag på en plats andra borde fått.

Onkologen i Lund gick igenom vilka tumörer som fanns och metastaser samt vad allt innebar. De berättade att jag skulle tillhöra Malmö och bli kallad till Malmö för att diskutera behandling. Creon skrevs ut, liksom smärtstillande och blodförtunnande sprutor då mitt ben börjat svullna och ådrorna såg inflammerade ut. Här tänker jag att utifrån det usla skicket jag var i så hade kortison tillsammans med blod kunnat ha en stor, omedelbar effekt på mitt mående och ätande. Jag hade fortfarande otroligt svårt för att äta och fortsatte att må sämre.

Nästa steg var att jag blev kallad till onkologen i Malmö. Det var ett jättefint bemötande där, behandlingen förklarades tydligt och metodiskt, vi gick genom alla mediciner jag skulle ha, vad de var till för, risker mm och så lades en plan upp för allt. Det kändes mycket bra – mötet riktade sig även till min sambo vilket kändes positivt och inkluderande. Det skrevs också ut näringsdrycker mm för att hjälpa mig att gå upp i vikt. Vid den här tiden var det så illa att jag ibland bara kunde dricka en halv sådan åt gången.

Behandlingen kom i gång och kändes ganska tuff i början. Jag var fortfarande kopplad till min vårdcentral som stod för omläggningar och egentligen allt medicinskt utom behandlingarna om inte jag minns fel. Det var svårt att hitta tider för omläggningar och provtagningar, vi (sambon och jag) fick bevaka alla symptom och mediciner själva och det kändes som att vi var lämnade med totalansvar själva i detta. Vi fick själva fråga hur länge vi skulle fortsätta med tex blodförtunnande sprutor och vilka sorter. Det blev ingen helhet, jag mådde inte bättre, hade svårt att börja äta och var extremt orolig för alla andra symptom som kom.

Jag kommer så väl ihåg en fredag kväll strax efter att behandlingen börjat så ringde telefonen. Det var från onkologen – då hade en propp upptäckts i min lunga från första röntgenomgång. Då fick vi ta taxi upp till akuten (som så klart var överbelastad), mitt immunförsvar var nere, för att ta en akutspruta för detta. Akuten ville då ta prover först, jag förklarade att det inte längre gick i armarna men att jag hade en piccline. Detta var de

obekväma med och insisterade på att försöka – det gick inte. Till sist fick jag sprutan jag behövde i alla fall och var hemma någon gång vid 1 på natten (vi kom dit ca kl 20.00).

Jag vill bara lägga till att samtlig personal som jag mött under alla olika vistelser på akuten, onkologen och kirurgavdelningen har haft ett jättefint bemötande och alltid gjort det de kunnat. Jag tänker bara att så fort man visste att detta var cancer, att jag rasat 20 kg och hade extrema tarmsmärtor så kunde man kanske satt in vissa mediciner för att lindra och möjliggöra ätandet.

Min sambo och jag kände att vi befann oss i något slags tomrum där vi pendlade mellan onkologen och vårdcentralen utan att det fanns något sammanhållande i detta. Vi var livrädda för att missa någonting medicinskt samtidigt som vi arbetade med det overkliga, sorgen över det som blivit. Jag hade bestämt mig för att kämpa med allt jag hade för att se till att jag kunde få vara med min familj så mycket som möjligt av den tiden som jag hade kvar att leva.

Ordningen i allting har jag säkert inte kronologiskt perfekt men jag vet att Anna (sambon) fick höra om ASIH och att vi blev rekommenderade att ta kontakt där. Det vi längtade efter var att någon/en instans kunde greppa helhetsbilden och samordnar vården. Vi hade varken kunskaperna eller förmågan till att göra detta. Vi kände oss lämnade, maktlösa och rädda inför allt.

Vi var nervösa inför mötet, i dåliga skick men ändå med förhoppning. Det blev ett jättebra möte där ASIH förklarade att de var beredda att ta emot oss och förklarade vad det innebar. Det är något vi fortfarande pratar mycket om – lättnaden när vi fick beskedet. Jag gråter sällan men då… och min sambo.

Skillnaden sedan dess har varit enorm. Regelbundna uppföljningar i hemmets trygga miljö och helhetsperspektivet har gjort att vi känner oss så otroligt mycket tryggare med allt, att det alltid finns någon att fråga. Samordningen av allting och tillförseln av blod hade en stor effekt på mig (och därmed också familjen), jag kunde börja äta igen, omläggningar och provtagning strukturerades – helvårdsperspektiv infördes vilket har inneburit allt för oss. Jag kunde börja äta igen och nu, flera månader senare, har jag i princip nått ordinarievikt. Livslusten kom tillbaka ganska snabbt när jag/vi upplevde att det fanns någon där med helhetsperspektivet och koll. Nu känner jag att jag är i de bästa av händerna, känner stort tillit och förtroende för de professionella som ger mig vård men som också ett helhetsperspektiv kring vård i det här skedet där omsorg av familjen även finns med. Jag har inga ord egentligen utan en otrolig känsla av tacksamhet att vi inte längre famlar själva utan har ett starkt stöd som vi verkligen behöver.

Generellt:

Jag förstod egentligen när jag fick höra att jag såg gul ut att jag fått cancer. Min tanke när jag kom till vårdcentralen var just ”nu får jag domen”, jag stod i hissen på väg upp när jag tänkte så. Det finns väl inget bra sätt att berätta för någon tänker jag men alla jag mött har varit fina kring det

Det jag tänker på är veckorna innan allt kom igång, hur jag kände att jag höll på att dö ganska fort och orkade knappt gå upp och kämpa emot. Hade helhetssynen funnits då så hade kanske trombosproblemen upptäckts, blodtransfusion kunnat göras och cortison sättas in jag har kanske fel som okunnig men tänker att då hade matlusten kanske ökat och därmed någon form av förbättring skett.

Jag tänker också kring det här med att ha en fysisk säng för att vara inskriven – jag behövde för det mesta inte det utan var nummerlappspatient som väntade på röntgen, ultraljud, provtagning, stent. Jag hade möjlighet att komma dit och behövde egentligen inte sängen. Det blev också ett problem för mig med fastandet över så många dagar när jag rasat som jag gjorde.

All personal jag mött har varit jättefina mot mig och bemött mig med respekt och omsorg och förståelse. Det är jag imponerad av!

ASIH är den största förändringen och skillnaden går inte att beskriva – det har förändrat vårt liv att ha det stödet, omsorgen och samordnande helhetsperspektivet. Utan tvekan är det ASIHs arbete med oss som gör att vi mår så pass bra som vi gör trots att omständigheterna är vad de är.

Populärvetenskaplig sammanfattning

Bukspottkörteln, som kallas pankreas på latin, är en körtel som ligger djupt inne i buken, bakom magsäcken och nära tolvfingertarmen. Den har två funktioner; dels utsöndrar den enzymer som hjälper till att spjälka mat, dels utsöndrar den hormoner, bland annat det viktiga insulinet som reglerar vårt blodsocker. Varje år drabbas ungefär 1400 personer i Sverige av cancer i bukspottkörteln, pankreascancer. Kvinnor och män drabbas i nästan samma utsträckning. Trots att pankreascancer är en relativt ovanlig cancerform, så är det en dödlig sådan. Förra året var det den tredje vanligaste orsaken till cancerdöd, efter de betydligt vanligare cancerformerna tjocktarmscancer och lungcancer. Det förutspås att pankreascancer kommer att vara den näst vanligaste orsaken till cancerdöd inom 10 år. I genomsnitt överlever patienter med pankreascancer cirka sju månader efter att diagnosen ställs. och den enda möjligheten till bot är operation. Tyvärr kan de flesta patienterna inte botas då sjukdomen sällan ger symtom förrän den har spridit sig till stora kärl och omkringliggande organ, vilket omöjliggör operation. Av de patienter som blir opererade får de flesta återfall efter kort tid och endast en fjärdedel av opererade patienter lever efter fem år.

Cellgiftsbehandling är ett viktigt komplement till operation och ges för att slå ut mikroskopisk cancerväxt och därmed minska återfall. Cellgiftsbehandling förlänger dessutom livet och minskar cancersymtom för patienter som inte kan opereras. Cellgift, eller cytostatika som det också kallas, fungerar genom att på olika sätt förstöra DNA hos snabbt delande celler. De senaste decennierna har många nya cancerläkemedel utvecklats, som i stället för att söka sig brett till celler med snabb tillväxt, söker sig till specifika mål i cancerceller (bland annat mutationer). eller hjälper kroppens egna immunförsvar att attackera cancern. Dessa nya läkemedel har påtagligt förbättrat utsikterna för patienter med många olika typer av cancer men har tyvärr inte visat sig effektiva mot pankreascancer.

I den här avhandlingen ville vi undersöka kopplingar mellan de biologiska mekanismerna bakom sjukdomen pankreascancer (disease) och hur de kan påverka patienters mående (illness).

I den första studien i avhandlingen undersöktes hur uttrycket av gener och proteiner ändras när man tystar genen *RBM3* i pankreascancerceller. Högt uttryck av RBM3 har visat sig vara kopplat till förbättrad överlevnad i flera olika cancerformer, medan det motsatta tycks vara fallet i pankreascancer. Mängden RBM3 i tumörceller verkar dessutom kunna förutspå vilka patienter med pankreascancer som gynnas av cellgiftsbehandling efter operation. Vi ville därför närmare undersöka mekanismerna bakom detta.

Det visade sig att 19 gener antingen uppreglerades (ökade) eller nedreglerades (minskade) i pankreascancerceller när *RBM3* tystades. Vi gick vidare med att undersöka gen- och proteinuttryck av den mest uppreglerade genen *CCND3*, som kodar for proteinet cyklin D3, samt den mest nedreglerade genen *PDS5A*, som kodar för ett protein med samma namn. Förutom dessa två gener valdes en annan uppreglerad gen, *PRR11*, ut för vidare analys eftersom vi i den publika databasen The Cancer Genome Atlas fann att överuttryck av denna gen gav sämre överlevnad hos patienter med pankreascancer. Vi fann vidare att överuttryck av proteinet PRR11 var förenligt med mer aggressiva tumörer och sämre överlevnad när vi undersökte tumörer från 46 patienter som opererats för bukspottkörtelcancer i Skåne. PRR11 är en del av en viktig signalväg i cancer som kallas PI3K/AKT och som är aktiverad i cirka 60% av all pankreascancer. Denna signalväg kan i sin tur aktiveras av mutationer i KRAS-genen, som förekommer i nästan alla fall av pankreascancer. Det skulle därför vara av värde att undersöka PRR11 ytterligare, särskilt som mål för riktad cancerbehandling.

Den andra studien är ett studieprotokoll för en klinisk obeservationsstudie som startade hösten 2018. Ett studieprotokoll är en beskrivning av utformning och syfte med en studie. Studien heter "Chemotherapy, Host response And Molecular dynamics in Periampullary cancer" (CHAMP). Alla patienter med pankreascancer som behandlas med cellgifter vid Skånes universitetssjukhus i Malmö och Lund tillfrågas om de vill delta. Studien syftar till att analysera tumörvävnad och markörer i blodprover som tas före varje behandlingscykel. Studiedeltagarna svarar också på livskvalitetsfrågor var tredje månad. Studien är pågående och hittills har 127 patienter inkluderats. CHAMP-studien ligger till grund för de tredje och fjärde delarbetena i den här avhandlingen.

I det tredje delarbetet undersöktes könsskillnader avseende behandling, livskvalitet och läkarens bedömning av funktionsnivå hos de första 100 patienterna som gick med i CHAMP studien. Det var 49 kvinnor och 51 män och av totalt 25 opererade patienter var endast sju kvinnor och övriga män. Det sågs inga skillnader mellan könen avseende tumörstorlek eller andra biologiska faktorer som kan påverka möjligheten att operera bort en tumör. Vi fann att kvinnor generellt hade en sämre livskvalitet än män men att denna inte var beroende av hur låg deras funktionsnivå bedömdes vara av läkaren. För män däremot, som uppgav en generellt bättre livskvalitet än kvinnor, var livskvaliteten lägre ju lägre funktionsnivå de bedömdes ha. Eftersom det inte sågs några biologiska skillnader mellan tumörer hos kvinnor och män konkluderades att genusroller, även kallat socialt kön, kan vara bidragande till vilka patienter som erbjuds kirurgisk behandling. Genus påverkar hur patienter, vårdpersonal och samhället interagerar. I framtiden bör större vikt läggas vid genusfaktorer som kan påverka behandlingsbeslut och kvinnor bör i större utsträckning uppmanas till kirurgi, vilket skulle kunna förbättra överlevnaden för patienter med pankreascancer.

I fjärde delarbetet undersöktes hur inflammatoriska proteiner och cellfritt DNA i blod påverkar livskvalitet och överlevnad. Blodprover och livskvalitetsformulär vid olika tidpunkter analyserades från de första 60 patienterna som inkluderats i CHAMP-studien. Cellfritt DNA utsöndras från alla döende och tillväxande celler i kroppen och även om det inte är tumörspecifikt visar studier att det är ett bra mått på tumörbördan, samt att det kan fungera som biomarkör för överlevnad.

Glädjande nog fann vi att alla patienter angav bättre livskvalitet efter att cellgiftsbehandling påbörjats, samt att detta var oberoende av patienternas kön eller om de behandlades i botande eller tumörbromsande syfte. Vi fann vidare samband mellan smärta före behandlingsstart och sämre överlevnad samt att höga nivåer av två inflammatoriska proteiner, TNFRSF12A och MMP7, vid start av behandling var kopplade till en generell livskvalitetsförsämring vid tre och sex månader. Dessa två proteiner har kopplats till sämre prognos i cancer men detta är första gången de även kunnat kopplas till försämrad livskvalitet. Höga nivåer av tre inflammatoriska proteiner, PD-L1, GZMH och IL-12, före behandlingsstart var kopplade till ökad smärta efter tre månaders behandling. Högt cell-fritt DNA före behandlingsstart var kopplat till sämre överlevnad samt till sämre livskvalitet. Att tidigt kunna förutspå vilka patienter som kommer att drabbas av försämrad livskvalitet längre fram i sjukdomsförloppet kan vara ett viktigt redskap för patientcentrerad vård. Det är väl känt att cancerpatienter som får symptomlindring på ett effektivt och adekvat sätt överlever längre. Eftersom överlevnaden i pankreascancer är fortsatt låg och patienterna ofta lider av svåra fysiska och psykiska symtom är symtomlindring och åtgärder för att förbättra livskvaliteten av största vikt och bör därför vara fokus för fler studier.

List of papers

The thesis is based on the following papers which will be referred to in the text by Roman numerals:

I. Olsson Hau S, Wahlin S, Cervin S, Falk V, Nodin B, Elebro J, Eberhard J, Moran B, Gallagher WM, Karnevi E, Jirström K. PRR11 unveiled as a top candidate biomarker within the RBM3-regulated transcriptome in pancreatic cancer. *J Pathol Clin Res*. 2022 Jan;8(1):65-77

II. Hau SO, Petersson A, Nodin B, Karnevi E, Boman K, Williamsson C, Eberhard J, Leandersson K, Gisselsson D, Heby M, Jirström K. Chemotherapy, host response and molecular dynamics in periampullary cancer: the CHAMP study. *BMC Cancer*. 2020 Apr 15;20(1):308.

III. Olsson Hau S, Williamsson C, Andersson B, Eberhard J, Jirström K. Sex and gender differences in treatment intention, quality of life and performance status in the first 100 patients with periampullary cancer enrolled in the CHAMP study. *Submitted manuscript*

IV. Olsson Hau S, Svensson M, Petersson A, Eberhard J, Jirström K. Trajectories of circulating inflammatory proteins, cell-free DNA and quality of life in patients with pancreatic cancer. *Manuscript*

Related papers not included in the thesis:

Lundgren S, Hau SO, Elebro J, Heby M, Karnevi E, Nodin B, Eberhard J, Holm K, Staaf J, Jönsson GB, Jirström K. Mutational Landscape in Resected Periampullary Adenocarcinoma: Relationship with Morphology and Clinical Outcome. *JCO Precis Oncol*. 2019 Mar 21;3

Petersson A, Andersson N, Hau SO, Eberhard J, Karlsson J, Chattopadhyay S, Valind A, Elebro J, Nodin B, Leandersson K, Gisselsson D, Jirström K. Branching Copy-Number Evolution and Parallel Immune Profiles across the Regional Tumor Space of Resected Pancreatic Cancer. *Mol Cancer Res*. 2022 May 4;20(5):749-761

Abbreviations

AJCCC	American Joint Committee on Cancer
BRAF	Proto-oncogene B-raf
BRCA	Breast cancer susceptibility gene
BSRI	Bem sex role inventory
CA 19-9	Carbohydrate antigen 19-9
CAIX	Carbonic anhydrase IX
CHAMP	Chemotherapy, Host response And Molecular dynamics in Periampullary cancer
CEA	Carcinoembryonic antigen
CDKN2A	Cyclin dependant kinase inhibitor 2A
CDK4/6	Cyclin dependent kinase 4/6
cDNA	complementary DNA
cfDNA	Cell-free DNA
CRP	C-reactive protein
CSF	Colony stimulating factor 1
CT	Computed tomography
ctDNA	circulating tumour DNA
DCN	Decorin
DNA	Deoxyribonucleic acid
EORTC-QLQ-C30	European Organisation for research and treatment of cancer quality of life questionnaire C30
EOT	End of treatment
ERCP	Endoscopic retrograde cholangiopancreatography
ESMO	European Society of Medical Oncology
FOLFIRINOX	Fluorouracil, irinotecan, oxaliplatin, leucovorin
GemCap	Gemcitabine, capecitabine
GZMH	Granzyme H
HRQoL	Health related quality of life
IFN-γ	Interferon gamma
IHC	Immunohistochemistry
IL-12	Interleukin-12
IL-18	Interleukin-18
IPNM	Intraductal papillary mucinous neoplasm
KPS	Karnofsky performance scale
KRAS	Kirsten rat sarcoma virus
LAMP-3	Lysosome associated membrane glycoprotein-3

MCN	Mucinous cystic neoplasm
MMP7	Matrilysin, matrix metalloproteinase-7
MRCP	Magnetic cholangiopancreatography
mRNA	Messenger RNA
MRI	Magnetic resonance imaging
MSI	Microsatellite instability
NALIRIFOX	Nanoliposomal irinotecan, 5FU/leucovorin, oxaliplatin
NGS	Next generation sequencing
NSCLC	Non-small cell lung cancer
NTRK	Neurotrophic tyrosine receptor kinase
OS	Overall survival
PALB2	Breast cancer 2 early onset gene
PanIN	Pancreatic intraepithelial neoplasm
PARP	Poly ADP ribose polymerase
PCR	Polymerase chain reaction
PDAC	Pancreatic ductal adenocarcinoma
PD-1	Programmed cell death protein 1
PD-L1	Programmed death ligand 1
PDS5A	PDS5 cohesin associated factor A
PPPD	Pylorus preserving pancreatoduodenectomy
PRR11	Proline rich 11
PS	Performance status
PTC	Percutaneous transhepatic cholangiography
RBM3	RNA-binding motif protein 3
RBP	RNA-binding protein
RNA	Ribonucleic acid
SMA	Superior mesenteric artery
SMV	Superior mesenteric vein
TCGA	The Cancer Genome Atlas
TMA	Tissue microarray
TNFRSF12A	Tumour necrosis factor receptor superfamily member 12A
TP53	Tumour protein 53
tRNA	Transfer RNA
TWEAKR	Tumour necrosis factor-like wear inducer of apoptosis receptor

Introduction

Historical perspectives

The first written accounts of cancer were found in Egypt in the beginning of the 20th century and date back to 1500-1600 BC. They are possibly written by the physician-architect Imhotep and describe surgical, pharmacological, and magical treatment of tumorous growths. The earliest cancerous growths in humans have been found in Egyptian and Peruvian mummies, also dating back to 1500 BC.

After the decline of Egypt, Roman medicine became leading largely due to the lifelong observations of Hippocrates (460-360 BC) and Galenus (129-216). Hippocrates described diseases that produced masses (onkos) and ulcerating, non-healing lumps (karkinomas), while Galenus classified growths into three categories from benign to malignant [1]. The origin of the word pancreas is not known. “Pan” means whole or complete and “creas” means meat, giving pancreas the meaning “whole meat”, possibly because of its homogenous structure.

Due to its location deep within the abdomen, the first known description of pancreatic cancer understandably comes much later. The first report was given by Giovanni Battista Morgagni in 1761 but it was not until 1858 that Jacob Mendez De La Costa reported the first microscopic diagnosis of pancreatic adenocarcinoma, making pancreatic cancer an entity in its own right.

Pancreatic cancer surgery became possible with the introduction of general anaesthesia at the turn of the 20th century. Although a few successful resections were completed then, it was not until Allen Oldfather Whipple presented his surgical technique of pancreatoduodenectomy at the 1935 annual meeting of the American Surgical Association that broad interest in pancreatic cancer surgery was awakened [2]. After World War II, it became apparent that chemical agents could affect cancer. In the late 1940s, Sidney Farber was the first to give chemotherapy to children with leukaemia, but although initially being successful, the children were not cured with single agents and the introduction of combination chemotherapy was soon to follow [3].

Chemotherapy treatment of patients with pancreatic cancer has been given with little success and in various regimens since the 1980s. Two landmarks leading to significantly increased survival deserve to be mentioned; the introduction of the drug gemcitabine in 1997 [4] and of combination treatment with FOLFIRINOX in 2011 [4, 5].

Periampullary Adenocarcinoma

Periampullary adenocarcinoma is a term referring to the origin of a group of tumours arising in the pancreas or in its near proximity, i.e. the distal bile duct, the ampulla of Vater or the duodenum. Since the majority of patients are inoperable at diagnosis and are diagnosed by radiology and biopsy or cytology alone, the exact location of the tumour's origin often remains unknown. Although the vast majority of periampullary adenocarcinomas originate within the pancreas, chemotherapy regimens differ according to tumour location, making a correct diagnosis of tumour origin of importance whenever possible.

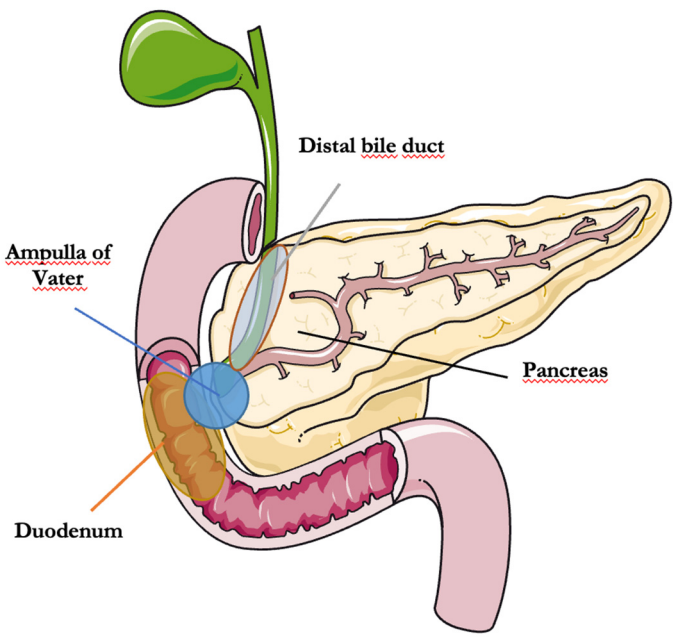

Figure 1. Anatomical location of periampullary tumours.
Courtesy of Alexandra Petersson.

Although the cohorts that form the basis for this thesis encompass the whole spectrum of periampullary tumours, the majority of tumours are located within the pancreas. In the literature, pancreatic cancers make up 80% of periampullary tumours [6] and an even larger amount in the Chemotherapy, Host response And Molecular dynamics in Periampullary cancer (CHAMP) study. The focus of this work will therefore be on pancreatic cancer.

Epidemiology

Pancreatic adenocarcinoma originates in the ducts of the exocrine pancreas and account for 95% of cancers originating in the pancreas. Other pancreatic cancers, such as those arising from the endocrine pancreas, are an entirely different entity and will not further considered in this work. About 65% of pancreatic cancers arise in the pancreatic head, 15% are found in the corpus or cauda with the remaining 20% diffusely involving the gland. Patients with pancreatic cancer in the tail of the pancreas have significantly poorer survival and less chance of receiving surgery, largely due to the fact that they are diagnosed at a later stage than patients with tumours in the pancreatic head [7].

Pancreatic cancer affects 1 in 64 people in their lifetime. It is a deadly disease with a death rate remaining relatively stable over many decades, incidence rates are however rising. In 2022 it was the third leading cause of cancer related death in the United States after colorectal cancer and lung cancer, and it is projected to rise to the second by year 2030 [8]. In Sweden, approximately 1400 people are diagnosed with pancreatic cancer every year. The median five-year survival is only six percent and the median survival time is only between six and eight months. Median age at diagnosis is 73 years and women and men are affected more or less equally. Early detection, making subsequent surgery possible, is key to improving survival, however even with surgery the five year-survival is below 25% [9]. There are significant racial differences in the incidence of pancreatic cancer. In the US, African Americans have higher incidence rates than Caucasians while the lowest incidence is found in Asian-Americans and Pacific Islanders. Racial differences are most likely attributed to modifiable risk factors. A significant difference in incidence between the sexes according to geographic distribution can also be observed. Women in Western Europe, North America, Northern Europe, and Australia/New Zealand have the highest incidence while the highest incidence in men is observed in Central and Eastern Europe followed by Uruguay and Japan [10, 11].

Clinical presentation

The clinical presentation of pancreatic cancer overlaps with the presentation of many other diseases making diagnosis a challenge for primary care physicians. Many patients experience diffuse abdominal discomfort, light anorexia, and early satiety for months before seeking medical attention. When patients present with unexplained weight loss and any of the following symptoms, they should be referred for radiology:

- Back pain
- Abdominal pain
- Nausea or vomiting
- Diarrhoea or steatorrhea
- New-onset diabetes

Jaundice is a common symptom of pancreatic cancer with as many as 75% of patients with tumours in the pancreatic head being afflicted at some point in their disease. For many of these patients, painless jaundice is the first sign of disease [12, 13].

Diagnosis

A diagnosis is made through a computed tomography (CT) scan with intravenous contrast, optimised for visualisation of the pancreas. Apart from being able to visualise a pancreatic tumour, CT is often adequate for visualising spread to important surrounding structures. Spread to the portal vein, the superior mesenteric artery (SMA) and the superior mesenteric vein (SMV) gives important information about operability. Apart from visualising local and locally advanced disease, a CT-scan also gives information about distant metastasis and, since most patients present with inoperable disease, a CT-scan is often adequate for diagnosis together with a biopsy.

In patients with inoperable disease a histopathological diagnosis is required before initiating palliative chemotherapy. This is made by performing a biopsy when possible, either from the primary tumour or from liver metastases. If the location of the tumour is hard to reach safely, a fine needle aspiration is made for cytological diagnosis instead.

In some cases, a magnetic resonance imaging (MRI) or magnetic cholangiopancreatography (MRCP) is necessary. It is superior to CT when it comes to distinguishing between pancreatic cancer and chronic pancreatitis as well as visualising non-regional lymph nodes (MRI). It can also differentiate between benign and malign liver masses [14].

Despite extensive research there is only one robust biomarker in clinical use today. Carbohydrate antigen 19-9 (CA 19-9) was discovered in 1979 and has been in clinical use since 1983. It is a serum biomarker used as an aid in setting a pancreatic cancer diagnosis but more importantly for assessment of treatment response, relapse, or progression. As it is neither very specific (80%) nor very sensitive (80%) in finding pancreatic cancer among periampullary diseases, it is not a suitable screening tool [15]. CA 19-9 can be elevated with benign cholestasis as well as in other types of tumours, and is lacking in up to 10% of Caucasian patients [16].

Carcinoembryonic antigen (CEA) is the second most utilised biomarker in detecting pancreatic cancer. It has a specificity of 79-85% and a sensitivity of 45-54%, making it less accurate than CA 19-9 at detecting malignant disease [15, 17]

Risk factors

Table 1. Risk factors for pancreatic cancer.

Risk factor	Risk
Age	Pancreatic adenocarcinoma risk increases with increasing age
Sex	Globally, more men than women are diagnosed with pancreatic cancer, possibly due to the protective effect of female sex hormones [18, 19]
Cigarette smoking	Current smokers have a 1.7-1.8 times higher risk of developing pancreatic cancer than never smokers [20-22]
Obesity[23]	~ 1.6 times higher risk of developing pancreatic cancer compared to individuals with normal weight [24, 25]
Alcohol	Increased risk (~ 1.6-1.8) mainly with heavy drinking (also risk of chronic pancreatitis [26-29]
Pancreatitis	2-3 times higher risk in patients with chronic pancreatits, 20-30 times higher risk with acute or newly diagnosed pancreatitis [30, 31]
New- onset diabetes	0.3-1% higher risk within 3 years of diabetes diagnosis compared to 0.1% higher risk in the general population [32, 33].
Oral microbiome	Peridonatal disease increases risk by ~1.5-1.7 times[34, 35]
Allergy	Allergies have a protective effect with odds ratios between 0.4-0.7 depending on the allergy and study [23, 36-38]
Family history of pancreatic cancer	Patients with two close relatives with pancreatic cancer have a 6.8 times higher risk than those without [39]

Pancreatitis and diabetes are both risk factors of pancreatic cancer, however they can also be caused by pancreatic cancer itself. It is not uncommon for patients with new-onset diabetes to return to healthy blood sugar levels after pancreatic cancer resection [33].

Genetic predisposition

The occurrence of familial clustering of pancreatic cancer can be attributed to both environmental and hereditary factors. An underlying genetic predisposition is present in ~20-35% of patients with pancreatic cancer. Of these only 20% have an identified causative gene (~ 5% of all cases), and for the remaining 80%, no genes can be identified [29, 40]. Some familial pancreatic cancers have better treatment options and therefore these patients have a prolonged overall survival. Lynch syndrome with subsequent defective mismatch repair genes enable treatment with programmed death ligand 1 (PD-1) blockade and for breast cancer type 1 and 2 susceptibility gene 1 and 2 (BRCA 1 & 2) and breast cancer 2 early onset gene (PALB2) mutations, targeted therapy with poly ADP ribose polymerase (PARP) inhibitors is an option [41-43].

Table 2.Prevalence and cancer risk of hereditary genes and syndromes in pancreatic cancer.

Gene	Prevalence %	Risk	
BRCA 2	1.4-7	3.5-5.8	[43-46]
BRCA 1	0.35-1	2.7-4.1	[47]
PALB 2	0.2-0.4		[47, 48]
ATM	0.5-2.3		[49, 50]
CDKN2A	< 1	12-38	[51]
Lynch syndrome	0.5-1	8.6	[52]
Peuz Jeghers syndrome	< 1	11-32% lifetime risk	[53, 54]

Clinicopathological assessment

Pancreatic cancer is classified according to the American Joint Committee on Cancer (AJCC) tumour, node, metastasis (TNM) classification system (Table 3). Correct TNM-classification is an important tool for prognostication, treatment decision making and a means of exchanging information. The 8th edition of the AJCC TNM classification system for pancreatic adenocarcinoma was presented in 2018 and is the basis for staging (Table 3) [55]. Two major adjustments have been made in the 8th edition compared to the 7th edition. In the previous edition, T3 stage was defined as "tumour extension beyond the pancreas", a definition which lacked prognostic correlation and was interpreted differently by pathologists. T3 stage is now based on tumour size. N1 was in the previous edition defined as "regional metastases" whereas

there is now a subdivision into N1 and N2. According to a recent nationwide Dutch study in an unselected cohort of 750 patients who had undergone pancreatic cancer surgery, a much better stratification of overall survival was seen using the 8th compared to the 7th edition. There was however no difference in overall survival between stage IIA and IIB, probably due to the small number of T3N0 patients [56].

Table 3. Summary of the 8th edition of the AJCC staging system.

TNM	Description	Prognostic stage	TNM by stages
T1	Maximum tumour diameter < 2 cm	Tis	Cancer in situ (includes IPMN and PanIN)
T2	Tumour diameter ≥ 2 cm ≤ 4 cm	I A	T1N0M0
T3	Maximum tumour diameter > 4 cm	I B	T2N0M0
T4	Tumour involves CA or the SMA	II A	T3N0M0
N0	No regional LN metastases	II B	T1-T3N1M0
N1	Metastases in 1-3 regional LNs	III	TanyN2M0, T4NanyM0
N2	Metastases in ≥ 4 regional LNs	IV	TanyNanyM1
M0	No distant metastases		
M1	Distant metastases		
Abbreviations: LN: Lymph node, CA: Celiac artery, SMA: Superior mesenteric artery			

Other clinicopathological factors which are important to consider are tumour invasiveness and degree of differentiation. Tumour invasion into blood vessels, lymph vessels as well as perineural growth are associated with a poorer prognosis. Differentiation is a term used to describe how well a tumour resembles normal tissue. Well-differentiated tumours have many similarities to normal tissue and have a better prognosis, while poorly differentiated or undifferentiated tumours are chaotic in structure and highly invasive, thus leading to poor prognosis [57-60].

Carcinogenesis

Pancreatic cancer evolves from one of three non-invasive precursor lesions. The majority arises from pancreatic intraepithelial neoplasia (PanIN) and from intraductal papillary mucinous neoplasia (IPMN), or less commonly from mucinous cystic neoplasia (MCN). Classification of these lesions is divided into two tiers, a low/intermediate degree of dysplasia and a high degree of dysplasia. Low or intermediate grade dysplasias are subject to clinical observation while high grade dysplasia should be resected since the risk of malignancy development is substantial. It can be difficult to differentiate IPMN from PanIN, but IPMN is often larger (> 1 cm) compared to PanIN (<0.5 cm) [61]. IPMN is located in the main duct of the pancreas

(~ 20%), in the duct branches (~ 50%) or in a mixture of both. Patients with IPMN in the main-duct or of mixed type have an 11-80% risk of developing pancreatic cancer and should be resected, while branch-duct IPMN can be monitored [62, 63].

Activating *KRAS* mutations are more or less ubiquitous in pancreatic cancer and inactivation of *TP53, SMAD4* and *CDKN2A* occurs in over 50% of cases. In addition to these frequently occurring mutations, mutations in a handful of genes involved in DNA damage repair and chromatin remodelling occur in ~10 % of cases. There is also a large diversity in infrequently mutated genes resulting in a tangible intertumoral heterogeneity. Adding to this complexity, variations of chromosomal structure are common in pancreatic cancer. Gene activation is caused through copy number gains or amplifications. Gene disruption can be caused by rearrangement or deletion of genes and gene fusions facilitate the formation of new oncogenic gene products [64]. Single nucleotide variants have been studied fairly extensively in pancreatic cancer, but less is known about the impact of copy number alterations on the disease, although these are known to contribute to tumour evolution and progression [65]. In a recent publication, Petersson et al. constructed phylogenetic trees of primary tumours and lymph node metastases from nine patients with resected pancreatic cancer and found that copy number heterogeneity (regional gains and losses) was the major contributor factor to the branching architecture of the trees and that complex trees were associated with decreased survival, which has also been shown in other solid tumour types [66-69].

Early detection

The majority of patients with pancreatic cancer are diagnosed when the tumour is inoperable, either locally advanced or has metastasised, leaving palliative chemotherapy treatment the only option. Only around 20% of patients with periampullary malignancies are eligible for surgery [9, 70]. Even for resected early-stage tumours, the rate of relapse is high, and patients with T1a tumours have a 5 year OS of only 40%, with even fewer long-term survivors (25%) [71]. Tumour size is, however, directly linked to prognosis and a substantial improvement in survival can be seen in patients with T1a tumours, especially those smaller than one centimetre. The most substantial gain from early detection would be if patients with non-invasive, precancerous lesions such as IPMN and MCN could be identified. Around 15% of pancreatic cancers arise via these precursor lesions and these patients are often cured by surgery alone [62, 72]

Screening for pancreatic cancer and IPMN in the general population is not feasible because of the low incidence. Since only around 11 in 100 000 people are diagnosed

with pancreatic cancer there would be significant harm to a substantial number of healthy patients. There is also the worry of having a life-threatening disease as well as the cost for society of diagnostic tests and radiology to consider. This would be the case even if we had access to a good surveillance biomarker with high specificity (which is not the case at present). The biomarkers used today, such as CA 19-9, are only useful in symptomatic disease and futile in the surveillance setting [62]. Instead, the focus of today should be on secondary prevention of risk groups such as those with a family history, genetic predisposition, those with chronic or hereditary pancreatitis as well as patients with branch-duct IPMN, where those with main-duct or mixed type should go straight to surgery. In Sweden, these patients are screened with CT-scans or MRIs and in some cases diagnosis can be aided by endoscopic ultrasound with biopsies [73].

Treatment

Pancreatic cancer can be divided into four groups, resectable (~20%), borderline resectable or locally advanced (30-40%), and metastatic (50-60%) [70].

Surgery

Surgery is the only treatment with curative potential. The aim of surgery is resection of the tumour with microscopically tumour free margins (R0-resection) as well as ensuring a post-operative convalescence, which allows for adjuvant chemotherapy treatment. Patients with jaundice or acute cholangitis should, if possible, be offered bile duct drainage with stenting via endoscopic retrograde cholangiopancreatography (ERCP). If the location of the stricture is unreachable by ERCP, percutaneous transhepatic cholangiography (PTC) is an equivalent option. Stents can however cause artefacts, making imaging difficult and, if possible, pre-operative staging should be done prior to these procedures.

Pancreatoduodenectomy

For tumours in the pancreatic head, and other periampullary tumours such as tumours in the distal bile duct, duodenum or the ampulla of Vater, pancreatoduodenectomy is performed either with Whipple procedure or by pylorus preserving pancreatoduodenectomy (PPPD). The Whipple technique entails en bloc removal of the distal part of the stomach, the duodenum, the pancreatic head, the common bile duct and the gall bladder [74]. In PPPD, the stomach is not resected in order to preserve the pyloric sphincter function and gastrin production in the antrum of the stomach.

These techniques seem to be equivalent and do not differ regarding radicality, post-operative complications or delayed gastric emptying [75, 76].

Distal pancreatic resection

For tumours located in the corpus or cauda of the pancreas a distal resection of the pancreas is performed. This is however a small group of patients, since left-sided tumours are often asymptomatic and present at a later stage, making surgery impossible. Leakage and subsequent development of post-operative pancreatic fistulas are problematic for these patients, occurring in ~30% of cases [77, 78].

Total pancreatectomy

In patients with multifocal or very large tumours it is not possible to perform a radical operation with the procedures described above, and a total pancreatectomy is therefore performed. Historically, the post-operative mortality was high for these patients, however a study from 2019 showed mortality numbers comparable to those of pancreas-sparing surgery [79]. There are, however, severe later effects on patients' quality of life and significant morbidity with persistent symptoms such as severe diabetes [80].

Venous and arterial resections

A common problem associated with surgical removal of pancreatic tumours is involvement of the portal vein or SMA. Isolated venous involvement is staged as T3 disease and although technically challenging, veins can be resected and reconstructed without affecting morbidity or mortality [81, 82]. Tumour involvement of the celiac artery or SMA is however rarely operable due to tumour infiltration along the celiac nerve plexus and is regarded as T4 disease [83]. In select cases, however, recent studies have shown a certain survival benefit and increased, but acceptable, post-operative complications [84, 85]. All tumours with arterial involvement and extensive vein involvement are considered locally advanced and should only be considered for surgery after pre-operative chemotherapy [86].

Chemotherapy

Chemotherapy is given in both the neoadjuvant, adjuvant and palliative setting. The main chemotherapy agents in clinical use are shown in Table 4.

Table 4. Common chemotherapy agents and their mechanisms.

Chemotherapy Agent	Administration	Mechanism of action
Fluorouracil (5-FU)	Intravenous	Pyrimidine analogue and anti-metabolite. Inhibits thymidylate synthesis and incorporation of its metabolites into RNA and DNA. Leucovorin is added to enhance the effect of 5-FU [87].
Capecitabine	Oral	Prodrug to 5-FU. Converted to active drug in the liver by thymidine phosphorylase [88].
S1	Oral	Prodrug to 5-FU (tegafur) in combination with a DPD-inhibitor (gimeracil) which prolongs 5-FU concentration, and oteracil which reduces 5-FU in the intestine, decreasing gastrointestinal toxicity [89].
Gemcitabine	Intravenous	Anti-metabolite and cytidine nucleoside analogue. Inhibits nucleotide repair and DNA synthesis [90].
Oxaliplatin	Intravenous	Platinum compound and alkylating agent. Forms crosslinks with DNA, thereby inhibiting replication and transcription [91].
Nab-Paclitaxel	Intravenous	Paclitaxel is a taxane which targets tubulin, thereby blocking mitosis and triggering apoptosis. Taxanes require solvents for delivery but albumin-bound (Nab)-Paclitaxel is solvent-free, thus having a decreased toxicity [92].
Irinotecan	Intravenous	Inhibits the enzyme topoisomerase-1, which causes DNA-breakage and cell death. Can be combined with liposomes to prolong its duration (nal-Iri) [93].

Neoadjuvant and conversion chemotherapy

Neoadjuvant chemotherapy treatment is given preoperatively to patients with resectable tumours. Historically, neoadjuvant chemotherapy for pancreatic cancer has not been used, there are however several potential benefits, and the paradigm is shifting more and more towards a neoadjuvant approach both in pancreatic cancer and in other gastrointestinal tumours (rectal, gastric and oesophageal) [94-96]). Considering that the majority of recurrences in pancreatic cancer are systemic, it is apparent that systemic treatment must target micro-metastatic disease, making neoadjuvant treatment the logical choice. There are several other reasons behind the rationale for giving chemotherapy preoperatively. Firstly, pancreatoduodenectomy is a major surgical procedure, and a long convalescence can sometimes make it difficult to deliver timely, full-course adjuvant chemotherapy [97, 98], whereas patients rarely become unfit for surgery during neoadjuvant chemotherapy treatment [99, 100]. Secondly, neoadjuvant chemotherapy can help determine the optimal regimen. While some might argue that patients who are operable up-front may progress during neoadjuvant chemotherapy, thereby missing their chance for cure, it is more likely that they are, in fact, spared futile surgery. These patients would likely be unresponsive to adjuvant chemotherapy and subsequently experience early recurrent disease [101]. In the phase-II trial SWOG S1505, patients with resectable pancreatic adenocarcinoma were randomised between perioperative modified FOLFIRINOX (fluorouracil, irinotecan, oxaliplatin, leucovorin) or gemcitabine/nab-paclitaxel. The results showed that 30% of the patients did not undergo resection, subsequently avoiding pancreatoduodenectomy which

would not have helped them [102]. In another study by Vreeland et al in 2019 [103], 25 patients with borderline/locally advanced pancreatic cancer switched from FOLFIRINOX to gemcitabine/nab-paclitaxel within the first four months of treatment. Of these patients, 64% switched because of poor response to FOLFIRINOX, 24% switched due to toxicity and 12% due to both. Twenty-one of the 25 patients (84%) had subsequent response to gemcitabine/nab-paclitaxel, 11 of whom were subsequently resected. This is noteworthy, given the general assumption that non-responders to FOLFIRINOX would be unlikely to have significant response to gemcitabine/nab-paclitaxel.

Although randomised, controlled trials to support the wide use of neoadjuvant treatment are lacking, we should not refrain from using this approach in individual cases as the biological rationale is sound. There are a number of ongoing phase II and III trials investigating the efficacy of neoadjuvant chemotherapy, and the oncology department in Skåne University Hospital has participated in the NorPACT-1 trial (NCT02919787), where patients with resectable pancreatic adenocarcinoma are randomised between standard adjuvant treatment or four cycles of neoadjuvant FOLFIRINOX followed by four cycles of adjuvant gemcitabine/capecitabine. ESPAC-5F was a four-armed trial that investigated immediate surgery with chemoradiation, preoperative gemcitabine + gemcitabine/capecitabine or FOLFIRINOX in patients with borderline resectable pancreatic cancer. A clear survival benefit was seen for patients in the neoadjuvant arms, with a one-year survival rate of 77% in the neoadjuvant groups compared to 40% in the up-front surgery arm [104].

Adjuvant chemotherapy

Adjuvant chemotherapy increases overall survival markedly. In the randomised controlled trial CONKO-001, 50 % of the patients in the observation arm (those who had surgery without adjuvant chemotherapy) had recurrent disease or were deceased within six months of surgery [105]. Figure 2 shows a timeline of important trials comparing different regimens of adjuvant chemotherapy.

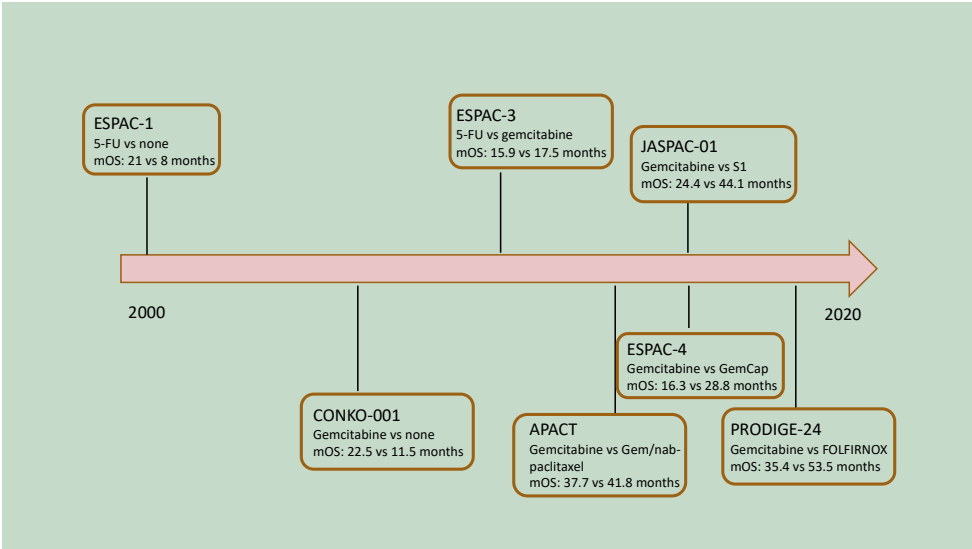

Figure 2. Timeline of randomised controlled trials exploring adjuvant chemotherapy regimens and median OS.

As seen in Figure 2, the ESPAC-1 trial in 2001 and the CONKO-001 trial in 2007 found large improvements in overall survival (OS) with adjuvant chemotherapy compared to no treatment [105-107]. When comparing gemcitabine with 5-FU, the ESPAC-3 trial in 2010 [108] found a small survival benefit favouring gemcitabine, which was also well tolerated. In the APACT trial in 2014, a small survival benefit was found for combination treatment with gemcitabine + *nab*-paclitaxel compared to gemcitabine alone [109]. In 2016, the ESPAC-4 trial studied the potential benefit of combining gemcitabine with capecitabine (GemCap) and found a clear increase in survival for the combination arm, at the cost of increased toxicity. This made GemCap standard of care for fit, adjuvant patients [98]. At the same time the JASPAC 01 trial in Japan found a large survival benefit of S1 compared to gemcitabine in the Asian population, where S1 is now standard of care in the adjuvant setting [89]. Recently, five-year OS has been reported from the PRODIGE-24 trial where gemcitabine was compared with combination treatment with mFOLFIRINOX (5-FU without bolus, irinotecan, oxaliplatin, leucovorin) with an impressive 43.2 months OS for patients receiving combination treatment [97, 110]. Notably, the gemcitabine arm had an OS of 31.4 months which is considerably longer than in previous studies. Since mFOLFIRINOX is known to be associated with toxicity, the patients included in the PRODIGE-24 trial all had a performance status of 0-1, and to ensure that there were no signs of metastatic disease, patients with CA 19-9 over 180 post-operatively were also excluded. Toxicity from mFOLFIRINOX was, unsurprisingly, higher than for

gemcitabine, with 76% of patients experiencing grade 3 or 4 adverse events compared to 53% in the gemcitabine arm.

In conclusion, mFOLFIRINOX is now standard of care as adjuvant treatment for fit patients, although depending on performance status. Standard FOLFIRINOX, gemcitabine + *nab*-paclitaxel, GemCap and single gemcitabine regimens are also used.

Palliative chemotherapy

Palliative chemotherapy is given in order to increase survival by decreasing tumour burden while also upholding a good quality of life [111]. The main randomised controlled trials that are the basis of our arsenal in the clinical setting are shown in Figure 3.

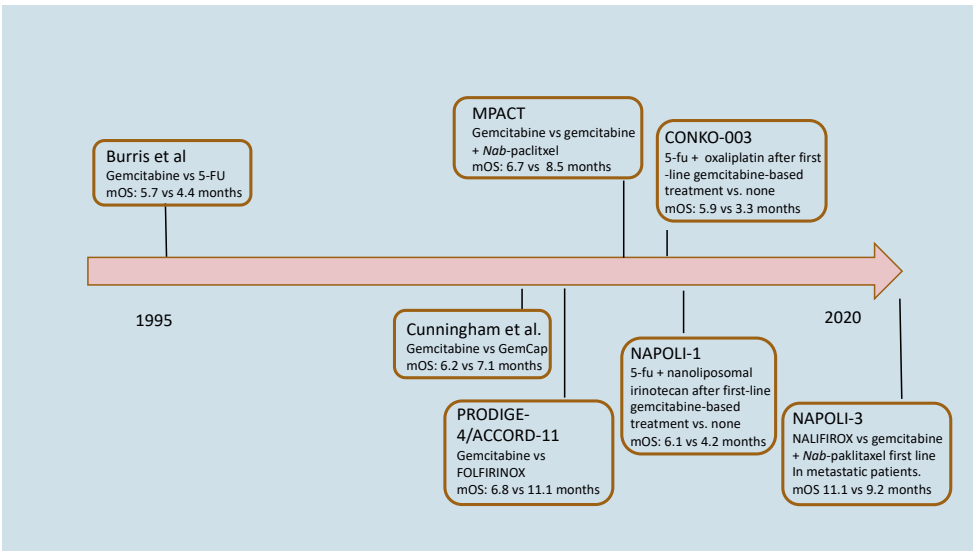

Figure 3. Timeline of randomised controlled trials exploring palliative treatment regimens and median OS.

For many years, single agent gemcitabine was standard of care for palliative pancreatic cancer patients after results showing that gemcitabine was superior to 5-FU in 1997 [4]. Cunningham et al. were the first to investigate combination treatment in the palliative setting in 2009, where a modest survival benefit was seen with the addition of capecitabine to gemcitabine [112]. In 2010, FOLFIRINOX was shown to be superior to gemcitabine alone, and in 2012, the combination of gemcitabine and nab-paclitaxel also showed a survival benefit [5, 113]. A few years later, the first studies examining second-line treatment (CONKO-003 and NAPOLI-1) were published, showing that treatment with 5-FU in combination with nano-liposomal irinotecan or

oxaliplatin after gemcitabine-based treatment led to an increased median survival [114]. In January 2023, data from the NAPOLI-3 trial was presented at the American Society of Clinical Oncology's annual gastro-intestinal cancer meeting. Treatment naïve patients with at least one metastatic lesion were randomised to treatment with gemcitabine in combination with nab-paclitaxel or liposomal irinotecan plus 5-FU/leucovorin and oxaliplatin (NALIRIFOX). Patients in the NALIRIFOX arm had a prolonged median survival of ~two months, but experienced significantly more adverse events compared to patients in the gemcitabine/nab-paclitaxel arm (NCT04083235) [115].

Based on these data, current guidelines in Sweden recommend that all patients who are fit enough should be offered palliative chemotherapy. For patients with a performance status of 0-1, either FOLFIRINOX or mFOLFIRINOX (standard FOLFIRINOX but without the 5-FU bolus and a lower dosage of irinotecan) or gemcitabine + *nab*-paclitaxel should be given, depending on performance status. Frail patients should be offered GemCap or single gemcitabine, but single agent 5-FU is an option when gemcitabine is deemed inappropriate. Regarding second line treatment, patients who are fit enough and have received FOLFIRINOX in first line should be given gemcitabine + *nab*-paclitaxel in second line, and vice versa. For patients with poorer performance status single gemcitabine or 5-FU is a second line option.

Radiation

Many trials have investigated the efficacy of radiation and chemoradiation treatment in both the neoadjuvant and adjuvant setting. There have however been conflicting results, and radiation therapy is not standard of care in Europe. Much of the research has been focused on borderline resectable disease, where chemoradiotherapy has been shown to increase locoregional control and improve surgical outcome, although no survival benefit has been shown [116]. Patients with locally advanced disease often suffer from severe symptoms due to the regional spread of the tumour to adjacent organs and lymph nodes. Results have been conflicting regarding radiotherapy and OS in patients with locally advanced disease. In the randomised, phase III, LAP07 trial, 442 patients with locally advanced pancreatic cancer were given four months induction therapy with either gemcitabine or gemcitabine + erlotinib. After the first randomisation, patients with stable disease either continued with the allocated treatment or received chemoradiation (54 gray + capecitabine). No significant differences in OS were seen between the chemotherapy group and the chemoradiation group or between the gemcitabine and the erlotinib group [117]. Although radiation has shown no clear survival benefit for these patients, local control with fractionated

stereotactic radiation could help decrease morbidity. Fractionated stereotactic radiotherapy in locally advanced disease (33 gray in five fractions) has been tested in a phase II trial after induction with gemcitabine with no significantly improved survival, but the patients experienced a decrease in pain [118]. In the palliative setting, for patients with bone metastases, radiation therapy is useful for pain management [119].

Personalised medicine

While targeted therapy and immunotherapy has had an incredible impact on the treatment and prognosis of many solid tumours in the last decade, little headway has been made in pancreatic cancer. This is in large part due to the low mutational burden and subsequent immune evasion [120]. Until recently, the dense stroma surrounding tumour cells was considered a key contributor to immune evasion and cancer progression. There is however, increasing evidence that the role of the stroma in pancreatic cancer is double-edged. In recent years, a certain subtype of cancer associated fibroblasts (myofibroblasts) have been unveiled as having substantial antitumour properties, and low levels of these have been shown to be associated with shorter OS in patients with pancreatic cancer. These findings have prompted a paradigm shift in how we regard the stroma in pancreatic cancer, indicating that stroma may very well be a friend rather than a foe [121, 122].

For now, personalised medicine is only an option for a small sub-group of pancreatic cancer patients and although many new and exciting trials are underway, one targeted therapy will certainly not fit all, considering the complexity of the disease.

The genomes of cancers with mismatch repair deficiency contain a high number of somatic mutations. These patients have shown sensitivity to checkpoint inhibition with programmed death ligand 1 (PD-1) antibodies [41]. Thus, for patients with microsatellite instability (MSI) due to deficiency in mismatch repair genes, treatment with the PD-1 inhibitor pembrolizumab is recommended as second line treatment after chemotherapy [41]. Since a deficiency in these genes is only found in 1-2% of pancreatic cancer patients, routine MSI-testing is however not yet routinely performed in Sweden.

Although EGFR is frequently overexpressed in pancreatic cancer, *EGFR* mutations are rare. Erlotinib (a tyrosine kinase inhibitor of *EGFR*) combined with gemcitabine has been tested in a randomised phase III trial in patients with locally advanced or metastatic pancreatic cancer, where a modest survival benefit was found, with a one-year-survival of 23% in the erlotinib arm compared to 17 % in the placebo arm [123]. In lung cancer, EGFR mutations rather than overexpression are known to be predictive

of erlotinib response [124]. This could explain the modest benefit of adding erlotinib to gemcitabine considering the low mutation rate of EGFR in pancreatic cancer.

For patients with germline mutations in BRCA1, BRCA 2 and PALB-2, the PARP inhibitor olaparib is an option following first-line, platinum-based chemotherapy, although it is not currently recommended by the Swedish Nya Terapier (NT) council [125]. PARP-inhibition in combination with immunotherapy was tested as maintenance therapy in a recent randomised, phase II trial of patients with advanced pancreatic cancer with stable disease after 16 weeks of platinum-based chemotherapy. Participants received the PARP inhibitor niraparib and immunotherapy with either PD-1 blockade (nivolumab) or CTLA-4 blockade (ipilimumab). An improved six-month progression free survival was observed in 20% of patients in the nivolumab arm and in 60% of patients in the ipilimumab arm. Although 50% of patients in the ipilimumab arm experienced grade III adverse events, the results show the potential for non-cytotoxic maintenance therapy in pancreatic cancer patients [126].

KRAS activation in pancreatic cancer is complemented by loss of the gene cyclin-dependent kinase inhibitor 2A (*CDKN2A*) in up to 80 % of tumours. *CDKN2A* encodes two proteins, one of which inhibits cyclin dependent kinase 4 (CDK4) and cyclin dependent kinase 6 (CDK6). Pharmacological restoration of the lacking protein is possible with CDK4 and CDK6 inhibitors, which are already in clinical use for breast cancer, however they have hitherto not been successful in the treatment of pancreatic cancer. A phase I trial studying sequential chemotherapy and subsequent CDK4/6 inhibition (NTC02501902) has recently been completed. Another phase I trial of CDK4/6 inhibition in combination with inhibition of the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) pathway (NTC03454035) is also underway[127, 128]

Neurotrophic tyrosine receptor kinase *(NTRK)* gene fusions are known oncogenic drivers in <1% of pancreatic cancer patients. For this small subset of patients, targeted treatment with troposine receptor kinase (TRK) inhibitors hold promise[129, 130].

An exciting case study was recently presented by Leidner et al [131]. In this study, a patient with metastatic, treatment refractory pancreatic cancer was treated with a single infusion of autologous T cells engineered to express allogenic T-cell receptors (TCRs) targeting the neoantigen KRAS G12D expressed by the tumour. G12D is the most common allele of KRAS mutation and is present in ~ 50% of tumours [132]. There is an ongoing response and at six months the objective partial response was 72% according to RECIST criteria. Unlike chimeric antigen receptor (CAR) T-cell therapy, which has hitherto proven ineffective in pancreatic cancer [133, 134], TCR therapy is dependent on a patient's specific HLA genotype. Although this limits the current KRAS

G12D specific TCRs used in this study to 10% of patients, other TRCs restricted by different HLA molecules have been identified and the potential of this therapy in pancreatic cancer warrants clinical trials.

Health related quality of life

The Eastern Cooperative Oncology Groups (ECOG) and Karnofsky performance score (KPS) have long been used by physicians to assess patients' physical functioning. However, health related quality of life (HRQoL), describing cancer patients' self-perceived quality of life and functioning, is known to be superior in determining both survival and prognosis. Research has shown that associations between HRQoL and prognosis are not exclusive to composite HRQoL scores alone, but also to individual physical symptoms such as fatigue, and psychological symptoms such as anxiety and depression [135-140]. So, is increased symptom management feasible and does it improve patient outcome? According to a ground-breaking study by Basch et al. in 2016 [141], the answer is yes. In their study, 766 patients with advanced solid tumours undergoing chemotherapy were randomised either to a control arm of standard treatment and visits or to an intervention arm consisting of self-reporting symptoms via an online tool between visits, as well as at visits. Not only did patients in the intervention arm experience a better HRQoL, they also had fewer visits to the emergency department, fewer hospitalisations and tolerated chemotherapy for a longer time. After a median follow-up of seven years, they also had a 5 months longer median OS compared to those in the control arm. These findings underline the importance of optimal symptom detection and control, which can only be achieved through coordination and integration of care. This could be of even greater importance in pancreatic cancer patients, considering the limited efficacy of chemotherapy and the severity of symptoms. In another randomised, controlled trial by Oh et al. [142], 162 patients with various cancer diagnoses were randomised either to standard care or to ten weeks of medical qigong (90 minutes, two times weekly). It was found that patients in the intervention group had significantly better overall HRQoL, less fatigue and mood disturbance, as well as decreased inflammation (lower C-reactive protein in serum). In 2016, Laird et al. compared C-reactive protein (CRP) levels from 2520 patients with locally advanced or metastatic cancers with HRQoL and performance status [143]. They found a significant association between high levels of C-reactive protein (CRP) and decreased global health and functioning, as well as decreased performance status. An increased understanding of inflammation and underlying mechanisms in pancreatic cancer is vital in order to improve symptom control and

management. It would seem that a more holistic approach not only benefits patients' well-being, but could also significantly improve chemotherapy tolerance and patient outcome. Conceptual associations between symptoms and inflammation are shown in Figure 4.

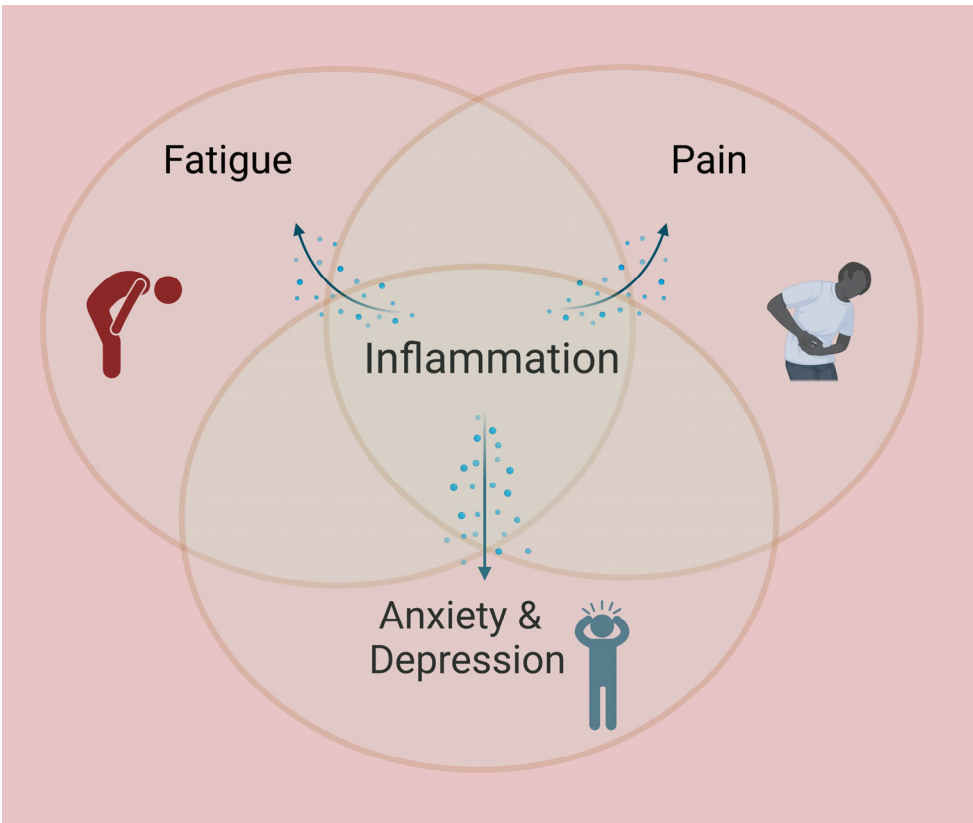

Figure 4. Symptoms and their associations with each other and inflammation.
By the author via BioRender™ with inspiration from Louati et al. [144].

Sex and gender

Sex and gender are important to consider in all health-related research [145]. It is however only in recent years that oncology has opened its eyes to the sex and gender differences regarding both toxicity and efficacy of treatment. Recently, the European Society of Medical Oncology (ESMO) has launched the Gender in Medicine Task Force with the aim to encourage oncologists to consider sex and gender in their

education, practice, research as well as in the development of educational programmes and material for oncologists and society[146]

Sex refers to the biological attributes of an individual. It is primarily physical or physiological, dependent on chromosomes, gene expression, hormone function and reproductive organs, and it is described as either female or male. Gender, on the other hand, is a social construct. Gender identity is fluid and can change over time. It is affected by socially constructed roles, behaviours, and expressions. It influences how people perceive themselves, how they act and interact, and affects distribution of power and resources in society. Gender identities can be classified as woman, man, non-binary and many others. Gender impacts health as well as interactions between patients, caretakers, and physicians [147].

Gender is complex to study, and there is a vast number of instruments in use today, of which the Bem Sex Role Inventory (BSRI) is the most common [148, 149]. BSRI has been in use since 1976 and, despite the name, it questions gender roles rather than sex. This illustrates the fact that many researchers use the terms sex and gender interchangeably, although these are separate entities. Gender affects treatment decision making. A population-based study from the Netherlands in 2018 showed that female patients with advanced gastroesophageal adenocarcinoma received less palliative chemotherapy than men and had a shorter overall survival [150]. A population based study from the Swedish pancreatic cancer registry also showed that women are less likely than men to be offered surgery for periampullary tumours, although it is known that women have a lower post-operative morbidity [151].

Sexual dimorphism in cancer is a term that refers to differences in biology between non-sex related cancer arising in men and women. These differences can be attributed to:

1. *Sex hormone signalling*

Pre-menopausal females have a lower incidence of cancer and better overall survival than males and post-menopausal females in many solid non-sex tumours such as colon cancer, melanoma, glioma, squamous-cell head-neck cancer, and NSCLC [152]. This can, at least in part, be explained by the protective effect of female sex hormones, primarily oestrogen [153]. Data from The Cancer Genome Atlas (TCGA) show clear sex differences in tumour mutational burden, immune cell infiltration and expression of immune checkpoints in multiple solid cancers [154].

2. *Tumour biology*

Data from TCGA show sex-biased gene expression signatures in clinically targetable genes and large discrepancies in mutation frequency in several solid cancers [155].

3. Molecular subtypes

Recent advances in molecular profiling have unveiled subtypes with sex disparities for several tumours such as colorectal cancer and gastric cancer. For instance, female patients with colorectal cancer are more likely to have right-sided tumours and BRAF mutations, while females with gastric cancer are more likely to have MSI and poorly differentiated or signet ring cell cancer than male patients [156, 157].

Female patients experience more toxicity, both haematological and non-haematological, than males [145, 158]. According to the ESMO Gender in Medicine Task Force, female and male individuals with non-sex related tumours should be considered biologically distinct groups for whom specific treatment approaches merit consideration in order to improve efficacy and decrease sex disparities [146].

Investigative biomarkers

RNA-binding motif protein 3

Genetic information from DNA (deoxyribonucleic acid) is transferred by RNA (ribonucleic acid) in the process of transcription. RNA information is then translated into proteins in the process of translation. Single stranded messenger RNA (mRNA) is synthesised with DNA as a template in the cell nucleus, and mRNA is the translated form of the DNA code, which the machinery can recognize and use to assemble amino acids for protein construction. mRNA travels from the nucleus to the cytoplasm where the process of translation forms proteins with the help of small transfer RNA (tRNA). The process of protein building takes place in the ribosome, which has two subunits and locks mRNA into place as well as serving as a docking station for tRNA. When protein formation is complete, the ribosome breaks apart. When an amino acid is added to the protein chain, a specific tRNA links to mRNA to make sure the correct amino acid is inserted into the new protein. A translating ribosome is shown in Figure 5. RNA-binding proteins (RBPs) regulate translation and aid the formation of ribosomes. There are hundreds of RBPs, with more being discovered every year [159, 160]. RBPs have many regulatory functions and play a vital role in post-transcriptional control of RNAs, and increasing evidence suggests that RPBs play a vital role in tumour progression, with more than a hundred being dysregulated in cancer [161, 162]

RNA-binding motif protein 3 (RBM3) is an RBP encoded by the *RBM3* gene located on chromosome 11. It is upregulated in response to various types of stress such as hypothermia, hypoxia and oxidative stress, and is required for cell proliferation [163-

166]. Upregulation of RMB3 has been shown to be associated with a better prognosis in many different solid tumour types [167-172] and has also been shown to correlate with a prolonged survival of patients with metastatic colorectal cancer receiving oxaliplatin based chemotherapy [163]. In pancreatic cancer, however, high mRNA levels of *RBM3* were found to be associated with shorter survival, and high protein expression of RBM3 was found to be associated with a prolonged survival in patients with resected periampullary cancer treated with adjuvant chemotherapy [167]. Associations between RBM3 and increased sensitivity to chemotherapy have also been shown in pancreatic and ovarian cancer *in vitro* [167, 173].

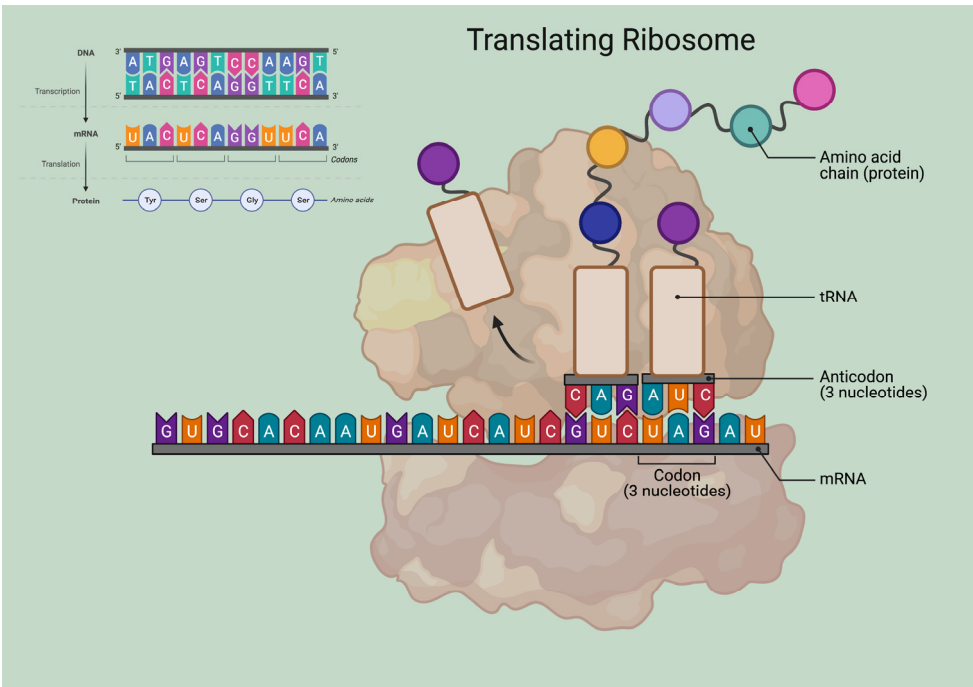

Figure 5. A translating ribosome.
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Aims of the thesis

General aims

The general aim of this thesis was to explore the biological evolution of pancreatic adenocarcinoma during chemotherapy treatment and the implications this may have on the illness of patients.

Specific aims

Paper I

- To explore RBM3-regulated genes and cellular processes that may influence the biological properties and chemosensitivity of pancreatic adenocarcinoma.

Paper II –

a protocol for a prospective, observational study with the following aims:

- To examine the associations between the spatial heterogeneity of cancer cell genotypes and phenotypes with the inflammatory tumour microenvironment and stromal characteristics of resected tumours.
- To examine the associations between spatial genetic heterogeneity and temporal genetic heterogeneity in cases with resected disease.
- To examine the prognostic value of systemic circulating immune cells.
- To examine the associations between characteristics and heterogeneity of the inflammatory microenvironment with host response during treatment.
- To identify patterns of dynamic temporal variations and correlations between genetic alterations and host immune response that impact patient survival and disease progression.

- To examine the associations of circulating concentrations of ctDNA with the spatial and temporal heterogeneity of genetic alterations and the host immune response.
- To examine the prognostic value of circulating levels of ctDNA.

Paper III

- To investigate any differences between males and females regarding demographic and clinicopathological parameters as well as treatment intention, performance status, quality of life and overall survival in the first 100 patients enrolled in the CHAMP study.

Paper IV

- To identify putative biomarkers for improved management and early prevention of disease and treatment-related symptoms in patients with pancreatic or other periampullary cancers by analysing real-world data on how trajectories of circulating inflammatory serum proteins and cfDNA align with HRQoL before start of chemotherapy, and after 3 and 6 months, respectively.

Methodological considerations

Methods in Paper I

Retrospective cohort

The study cohort in Paper I consists of 46 patients with pancreatic cancer included in a consecutive cohort of 175 patients with periampullary adenocarcinoma who underwent pancreatoduodenectomy at Skåne University hospital between 2001 and 2011. Of these 46 included cases, 13 did not receive adjuvant chemotherapy. Last follow-up was at death or 1st March 2017, whichever came first.

Cell lines

Three human pancreatic cancer cell lines (BxPC-3, PANC-1 and MIAPaCa-2), all poorly differentiated and of epithelial morphology, were used in Paper I. BxPC-3 cells were originally isolated from the primary tumour of a 61-year-old female patient in 1986 and are *KRAS*-wildtype. PANC-1 cells were isolated from the primary tumour of a 56-year-old female in 1975 and are *KRAS*-mutated. MIAPaCa-2 cells were derived from a 65-year-old male in 1977, are *KRAS*-mutated and have a higher level of invasiveness and migration than BxPC-3 and PANC-1 cells [174-177]. These three cell lines, which are among the most studied in pancreatic cancer research, were used in the previous paper on RBM3 [167] and therefore also selected for the analyses in paper I.

SiRNA transfection and RNA sequencing

RNA sequencing is a technique which uses next generation sequencing (NGS) to evaluate the presence and quantity of RNA in a sample. In paper I, this was performed after silencing of RBM3 in the three above-mentioned cell lines by transfection with small interfering RNA (siRNA) targeting RBM3. SiRNA is a small double-stranded RNA-sequence that connects to and activates protein complexes which then bind to target messenger RNAs and prevent ribosomes from continuing protein synthesis. Silencing proteins of interest by RNA interference enables us to study events arising in their absence [178].

When NGS became commercially available in 2005, it opened up for endless possibilities of sequencing whole genomes in a short time frame. Although the NGS process is complex and differs from manufacturer to manufacturer, the principles, shown in Figure 6, are as follows: A library is created from a biological sample (in this case RNA) and is processed into shorter segments forming a fragment library. The RNA fragments are then converted to complementary DNA (cDNA) fragments which are more stable than RNA and adapters added in order to enable the cDNA to attach to a solid surface before amplification can begin. In paper I, a paired-end library was created, enabling sequencing from both ends of the cDNA-fragment. The library was then clonally amplified to increase signal detection. After amplification, all the RNA was sequenced at the same time for numerous cycles. The last step is analysis of the data, which is divided into primary analysis of the raw data created in every cycle, secondary analysis with read filtering and quality control, and the most complex, tertiary analysis with interpretation of the results [179, 180].

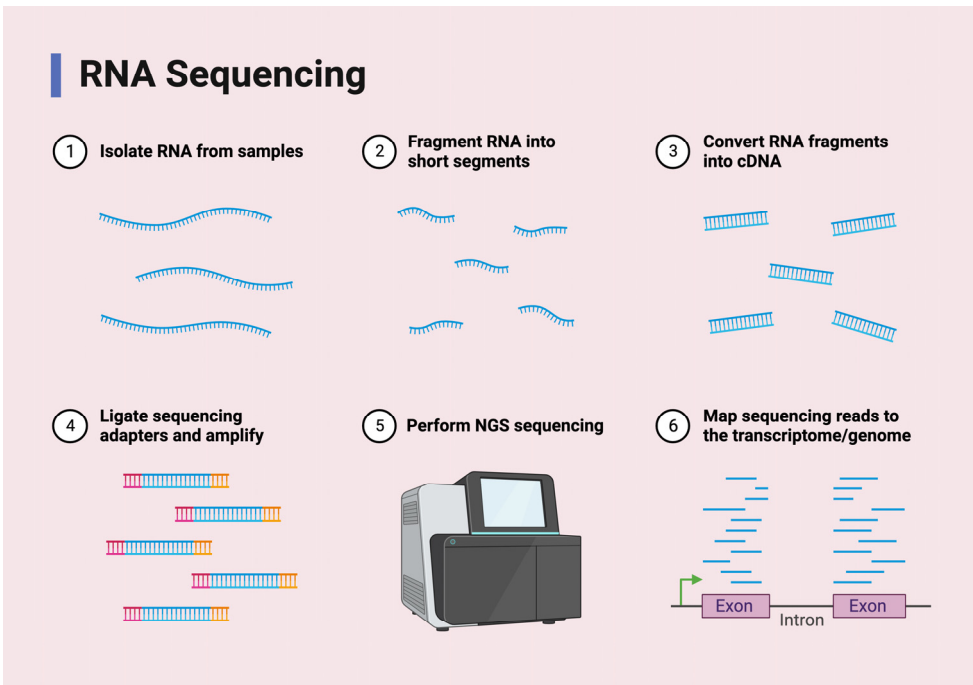

Figure 6. RNA-sequencing using NGS.
Constructed by author with BioRender™.

PCR and western blotting

Polymerase chain reaction (PCR) has been used since the 1980s and is a laboratory technique for rapidly producing millions of copies of a specific DNA segment, which can then be studied in greater detail. DNA fragments called primers select a segment of the genome to be amplified and multiple cycles of DNA synthesis amplify that segment. Since we were interested in the transcriptome in paper 1, reverse transcription polymerase chain reaction was used. This method uses RNA as a template which is converted into cDNA (as in NGS) by the enzyme reverse transcriptase and serves as a template for exponential amplification [181].

Western immunoblotting allows for detection of the presence of a particular protein in a cell culture to determine if proteins are upregulated or downregulated. It is a cost-effective assay and widely used in all biomedical research. Before the procedure is carried out, correct protein extraction and purification is vital. Proteins are then separated by molecular weight using gel electrophoresis and transferred onto a solid membrane before being subjected to immunostaining [182, 183].

The Cancer Genome Atlas

TCGA programme began in 2006 as a joint effort between the National Cancer Institute and the National Human Genome Research Institute. It is a landmark in cancer genomics, matching over 20 000 primary cancers with normal samples in 33 cancer types [184]. The data are publicly available for the research community and a valuable tool in biomarker research. In paper I, the TCGA was used to screen for RBM3-regulated genes that were prognostic at the mRNA level, whereby proline rich 11 (PRR11) was found to be of particular interest and selected for further analysis at the protein level in the in-house cohort.

Tissue microarray

Tissue microarray (TMA) is the process of gathering archival tumour tissue from multiple donor blocks into a recipient paraffin block that can be cut into thin slices and mounted on microscope slides. Although labour intense at construction, it is an efficient and cost-effective way to facilitate the assessment of protein expression via for example immunohistochemistry (IHC) and it is widely used in cancer biomarker studies[185]. The downside of the TMA technique compared to analysis of whole tissue sections is that information on potential intratumoural heterogeneity may be less accurate. This problem can be minimised by sampling many cores from multiple donor blocks, an approach that may actually be better than the use of whole tissue sections, as the latter also merely represent a small part of the entire tumour. As regards PRR11 expression, examined in Paper 1, it was found to be strikingly similar between multiple

tissue cores. Recently PRR11 expression has also been examined in 25 patients from the CHAMP study with the use of "single patient tissue chips" [186] confirming an overall intratumoural homogeneity of PRR11 expression in individual tumours.

Immunohistochemistry

IHC is a widely used, tissue-based method of localising and visualising antigens with antibodies. Most commonly, the antigen is a protein located in the nucleus, cytoplasm, or membrane of the cell. Antibodies are most commonly of IgG class and can be monoclonal or polyclonal. Monoclonal antibodies are developed by one and the same immune cell ex vivo and are highly specific, binding to only one epitope. Polyclonal antibodies, on the other hand, bind to several epitopes on the same antigen and are produced in animals, making their production dependent on the lifespan of the animal [187]. In addition to the primary antibodies, a secondary antibody can also be used for visualisation purposes. It is labelled with chromogenic or fluorescent tags and binds to the primary antibody, thus making them visible in a microscope.

The antibodies used in paper I were all well-validated. Monoclonal antibodies were used for detection of cyclin D3 and polyclonal antibodies for detection of PDS5A and PRR11. Since all three of these proteins were investigative biomarkers not yet used in clinical practice, no standardised method of evaluation was available. For cyclin D3, expression was sparse and, therefore, only the absolute fraction of positive cells was denoted. Expression of PDS5 and PRR11 was more abundant, and both the intensity and fraction (percentage of positive staining) were denoted. The median value of protein expression was used as the prognostic cut-off for all the investigative biomarkers.

There are a few important factors to consider when assessing IHC expression. If possible, controls should be used [188]. Since all biomarkers in this translational study were investigatory, no controls were available. However, pancreatic cancer cell lines with knockdown of RBM3 showed a consistent increase in PRR11 and cyclin D3 expression and decrease in PDS5A. Thus, these served as controls since the same antibodies were used to stain formalin-fixed paraffin embedded cell pellets and the TMAs. Analysis of staining across at least two tissue cores from the same tumour is recommended [189], and this approach was applied in Paper I, often with three evaluable tissue cores from each invasive tumour. The staining was evaluated in a blinded fashion, as knowledge of patient outcome can lead to bias. An experienced pathologist will however never be blinded to e.g. tumour differentiation. Moreover, when evaluating tumour specimens from a large cohort, a "diagnostic drift" may occur, wherein a gradual change in the assessment of IHC expression occurs over time. To

minimise this risk, at least two separate observers assessed all of the samples in paper I [189].

The CHAMP study

The Chemotherapy, Host response And Molecular dynamics in Periampullary cancer (CHAMP) study was initiated in November 2018 and is registered in clinicaltrials.org (NCT03724994)[186]. It is an ongoing, prospective, observational trial in which all patients with periampullary cancer undergoing neoadjuvant, adjuvant or first line palliative chemotherapy at Skåne University Hospital Lund/Malmö are invited to participate. Exclusion criteria are patients having another concomitant life-threatening disease and patients unable to receive chemotherapy treatment. To date, 127 patients have been included (January 2023). Clinical data are compiled at the start of the study, and all resected tumours and biopsies are re-evaluated by an experienced pathologist. Serial blood sampling is performed before the start of treatment (baseline), before every chemotherapy cycle and at the end of treatment (EOT). In paper III, we investigated potential sex and gender differences the first 100 patients, 75 of whom had completed HRQoL questionnaires at baseline. In paper IV, patients included up until Dec 31st 2020 (n= 60) were selected to allow for a longer follow-up and assessment of the prognostic value of the investigative factors as well as changes in levels of inflammatory proteins, cell-free DNA (cfDNA) and HRQoL over time. Thirtynine patients had completed HRQoL questionnaires at baseline, 16 at three months and 14 at six months.

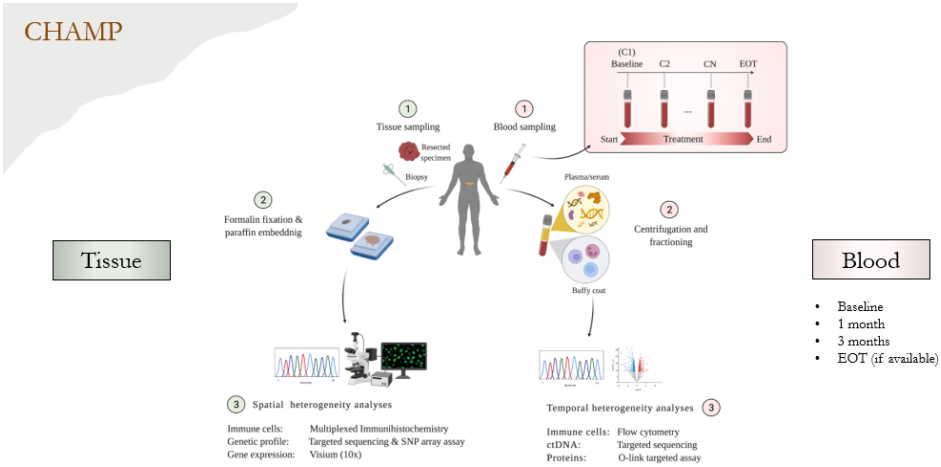

Figure 7.Schematic overview of the CHAMP study.
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Methods in Paper III & IV

EORTC-QLQ-C30 questionnaire

For paper III and IV we used data generated by The European Organisation for Research and Treatment of Cancer quality of life questionnaire C30 (EORTC-QLQ-C30). The questionnaire was answered by patients enrolled in the CHAMP study at baseline as well as after three months and at EOT. EORTC-QLQ-C30 questions patients about their functioning as well as emotional and physical well-being during the past week [190]. The questionnaire is standardised, gender neutral and widely used to assess HRQoL of cancer patients [190-192]. When initiating the CHAMP study, we planned to use a questionnaire specific to pancreatic cancer patients (EORTC-QLQ Pan26), but decided that a questionnaire that allows for comparisons between patients with different tumour types might be more useful, and therefore selected the broader EORTC-QLQ-C30 questionnaire. The questionnaire comprises of 30 questions, of which some are grouped together and some remain as single items. The raw scores of the 15 items are transformed to a scale ranging from 1-100. A high score in functional scales and global health indicates a high level of functioning, while a high score in symptom scales indicates increased severity of symptoms. The items and the questions on which the different scores are based are shown in Table 5.

Table 5.EORTC-QLQ-C30 items and questions.

	Item	Answered questions
Functioning scales High score = High functioning	Global Health	How would you rate your overall health during the past week? How would you rate your overall quality of life during the past week?
	Physical functioning	Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase? Do you have any trouble taking a long walk? Do you have any trouble taking a short walk outside of the house? Do you need to stay in bed or a chair during the day? Do you need help with eating, dressing, washing yourself or using the toilet?
	Role functioning	Were you limited in doing either your work or other daily activities? Were you limited in pursuing your hobbies or other leisure time activities?
	Emotional functioning	Did you feel tense? Did you worry? Did you feel irritable? Did you feel depressed?
	Cognitive functioning	Have you had difficulty in concentrating on things, like reading a newspaper or watching television? Have you had difficulty remembering things?
	Social functioning	Has your physical condition or medical treatment interfered with your family life? Has your physical condition or medical treatment interfered with your social activities?
Symptom scales High score = severe symptoms	Fatigue	Did you need to rest? Have you felt weak? Were you tired?
	Nausea and vomiting	Have you felt nauseated? Have you vomited?
	Pain	Have you had pain? Did pain interfere with your daily activities?
	Dyspnoea	Were you short of breath?
	Insomnia	Have you had trouble sleeping?
	Loss of appetite	Have you lacked appetite?
	Constipation	Have you been constipated?
	Diarrhoea	Have you had diarrhea?
	Financial difficulties	Has your physical condition or medical treatment caused you financial difficulties?

Proximity extension assay

In paper IV, inflammatory protein levels in serum samples from the first 60 CHAMP-patients were analysed with the Olink® Target 96 Immuno-oncology panel. This panel encompasses many important markers of inflammation in cancer and some that have also been associated with symptoms such as pain and fatigue. The panel is analysed by proximity extension assay, whereby protein specific antibodies are linked to DNA-coded tags. When the antibodies bind to their antigens, two strands of DNA hybridise to form a piece of DNA-barcode which is then amplified by PCR ready for quantitative real-time PCR reading. This is a scalable, sensitive and highly specific method which enables quantification of multiple proteins simultaneously [193].

Quantification of cell-free DNA

Quantification of cell-free DNA (cfDNA) in plasma in paper IV was performed using a fluorometer, subsequent to cfDNA isolation. The Qiagen QiaAMP circulating nucleic acid kit was used for cfDNA extraction. In short, fluorometry uses fluorescent dyes to determine the quantity of nucleic acids in a sample. These dyes exhibit very little fluorescence until bound to their target molecule, but upon binding to DNA they become intensely fluorescent in a manner which is directly proportional to the concentration of DNA in the sample [194]. In paper IV, this technique was chosen as a cost-efficient and simple way of measuring cfDNA in plasma. CfDNA is shed by all dying and proliferating cells, and while measuring the fraction of circulating tumour DNA (ctDNA) is more tumour-specific, it requires sensitive assays and costly sequencing techniques. Although less specific, cfDNA could have great potential as a biomarker of treatment response and progression, similarly to ctDNA, but at a fraction of the cost. However, further validation in larger cohorts is needed.

Statistical analyses

In paper I, Student's *t*-test was applied for changes in mRNA levels after siRNA transfection of pancreatic cancer cells. In paper IV, 92 proteins were examined, and Welch's *t*-test (an adaptation of the Student's *t*-test) was applied to investigate changes in protein levels over time, after which correction for multiple testing with the Benjamini-Hochberg method was performed. Nonparametric tests (Chi-square for categorical variables, Mann-Whitney *U* for continuous variables, Kruskal-Wallis for more than two groups and Wilcoxon signed-rank for paired groups) were used to investigate differences in biomarker distribution between primary tumours and lymph node metastases, and in relation to clinicopathological parameters. Spearman's correlation coefficient was applied to evaluate intercorrelations between investigative biomarkers, cfDNA and HRQoL factors. Kaplan-Meier analyses and log-rank tests were applied to evaluate associations between biomarkers, cfDNA, HRQoL-factors and survival. Hazard ratios for death were evaluated by Cox regression proportional hazards modelling, both without taking distribution of other factors into account (univariable analysis) and with adjustment for other factors of relevance (multivariable analysis). In paper III, univariable and multivariable logistic regression was applied to calculate odds ratios for treatment allocation, sex, age and tumour location. In paper IV, linear regression was applied for each investigatory protein and overall survival at each timepoint.

Ethical considerations

In paper I, a retrospective study cohort of periampullary tumours from 175 patients was used. The study received approval from the Regional Ethical Review Board, reference number 2007/445 with amendments 2008/35 and 2014/748. The committee concluded that no necessity for informed consent was required other than the option to withdraw.

For papers III and IV, data from the ongoing CHAMP study were used. An ethical application was written before the start of the study and approval from the Regional Ethical Review Board was granted in January 2018, reference number 2018/13, and two amendments have been approved by the Swedish Ethical Review Authority with reference numbers 2021-00166 and 2021-06065. The study complies with the Helsinki declaration.

Prior to enrolment in the CHAMP study, patients give their written consent after receiving study information by their oncologist or research nurse. Blood samples are taken by a study nurse before each chemotherapy cycle and at the end of treatment. The patients complete EORTC-QLQ-C30 questionnaires before the start of treatment, at three months and at six months. The impact of participating in the study is often perceived as small by patients and many enjoy the time spent with the research nurses. The study does not entail any additional tissue sampling. The only tissue analysed is from archival standard diagnostic biopsies or resected specimens. At the end of treatment a blood sample is taken, usually in conjunction with the standard doctor´s appointment, and therefore does not require an extra hospital visit. Since the patients have central venous catheters (PICC-lines or Porth-a-caths), there is no pain or discomfort associated with drawing blood. Some patients may find the quality-of-life questionnaires tiresome or difficult to fill out.

My experience of treating terminally ill patients is that the vast majority of patients with incurable disease wish to participate in studies as it may help patients with similar diseases in the future, understanding that they themselves have nothing to gain from participating.

The present investigation

Summary of results and discussion

"Come on! Show me the data!"

Karin Jirström

The results are presented in detail in the original papers and therefore only briefly summarised here. Paper II is a study protocol for an ongoing study so the discussion will therefore be an overview of the study and its participants so far.

Paper I

Herein, RBM3-related genes and proteins were investigated and associations of selected biomarkers with prognosis and treatment response were explored. In MIAPaCa-2 cells with downregulated RMB3, 12 genes were up-regulated and 7 genes were down-regulated. *CCND3*, encoding for the protein cyclin D3, was the top up-regulated gene and *PDS5A,* encoding for a protein of the same name, was the top down-regulated gene. Further analysis of the 19 differentially expressed genes in pancreatic cancer (n=145) in the TCGA revealed three genes to be highly prognostic at the mRNA level. High levels of *EPB41L*1 and *PRR11* were associated with shorter OS and high levels of *SLC25A44* were associated with longer OS. Proline rich 11 (*PRR11*), a gene encoding for a protein with the same name, is known to promote cell cycle progression and oncogenesis, and was therefore selected for further study, together with *PDS5A* and *CCND3*.

In vitro, knockdown of RBM3 in MIAPaCa-2 cells led to reduced levels of PDS5A and increased levels of cyclin D3 and PRR11, both at the mRNA and protein levels. Evaluation of protein expression with IHC could be performed on primary tumours from 46 cases in the in-house cohort, 33 of which had paired lymph node metastases. The in-house cohort differs from TCGA cohort in that all patients in the in-house cohort had undergone surgery (stage I disease), whereas in TCGA, only 21 of the 145 specimens were from surgically treated patients and the remainder from biopsies in

patients with stage II-IV disease [184]. Expression of PDS5A and cyclin D3 did not differ between lymph node metastases and primary tumours, but expression of PRR11 was significantly lower in lymph node metastases than in primary tumours. Expression of PDS5A and cyclin D3 did not show any prognostic value, but high expression of PRR11 was associated with a shorter OS. The prognostic value of PRR11 was however only significant in univariable analysis, not after adjustment for established prognostic factors. High PRR11 expression in the study cohort was also associated with a shorter OS in patients who had received adjuvant chemotherapy but not in untreated patients, but there was no significant treatment interaction.

Despite associations between cyclin D3 and RBM3 in pancreatic cancer cells *in vitro,* these were not seen at the protein level in human tumours. In normal cells, cyclin D3 is an important driver of cell cycle progression, pushing cells from the G0/G1 phase to the S-phase. In cancer, including pancreatic cancer, cyclin D3 is often overexpressed due to inactivation of the tumour suppressor P16 [195], and overexpression of cyclin D3 has been associated with poor prognosis in several solid tumour types [196].

The top down-regulated gene was *PDS5A*. PDS5A is one of two cohesion-associated factors (PDS5A and PDS5B) and when PDS5A is absent, binding time of cohesion to chromatin increases, thus slowing down DNA-replication. In cancer, PDS5A has been shown to be overexpressed in several tumour types compared to normal tissue. PDS5A has also been liked to tumour progression *in vitro,* however the underlying mechanisms are hitherto unknown [197, 198]. PDS5A and PDS5B could have potential as therapeutic targets since their absence leads to severe cell defects or death [199]. To our knowledge, this is the first report on PDS5A expression in pancreatic adenocarcinoma.

PRR11 expression was associated with a shorter OS both at the gene expression and protein levels, which is in line with previous studies on other solid tumours [200-203]. As shown in Figure 8, PRR11 promotes oncogenesis and cell cycle progression by interacting with the p85 regulatory subunit of PI3K, thereby reducing homodimerization of p85 and amplifying PI3K-signaling [204]. The PI3K/AKT pathway is activated in ~60% of pancreatic adenocarcinomas, and is, in turn, activated by the KRAS pathway, which is activated in the vast majority of pancreatic adenocarcinomas. Apelisib, an inhibitor of the p110 subunit of PI3K has recently been approved by the US Food and Drug Administration for PI3KCA-mutated, oestrogen receptor positive, HER2 negative breast cancer [205]. We believe that further examination of PRR11 and its associations with the PI3K/AKT pathway could be of interest in pancreatic cancer, given the severe therapeutic resistance of this disease. Our findings of PRR11 expression being associated with poorer prognosis in patients treated

with chemotherapy are in contrast to the findings regarding RBM3 [167] and warrant further validation in a larger cohort of pancreatic cancers.

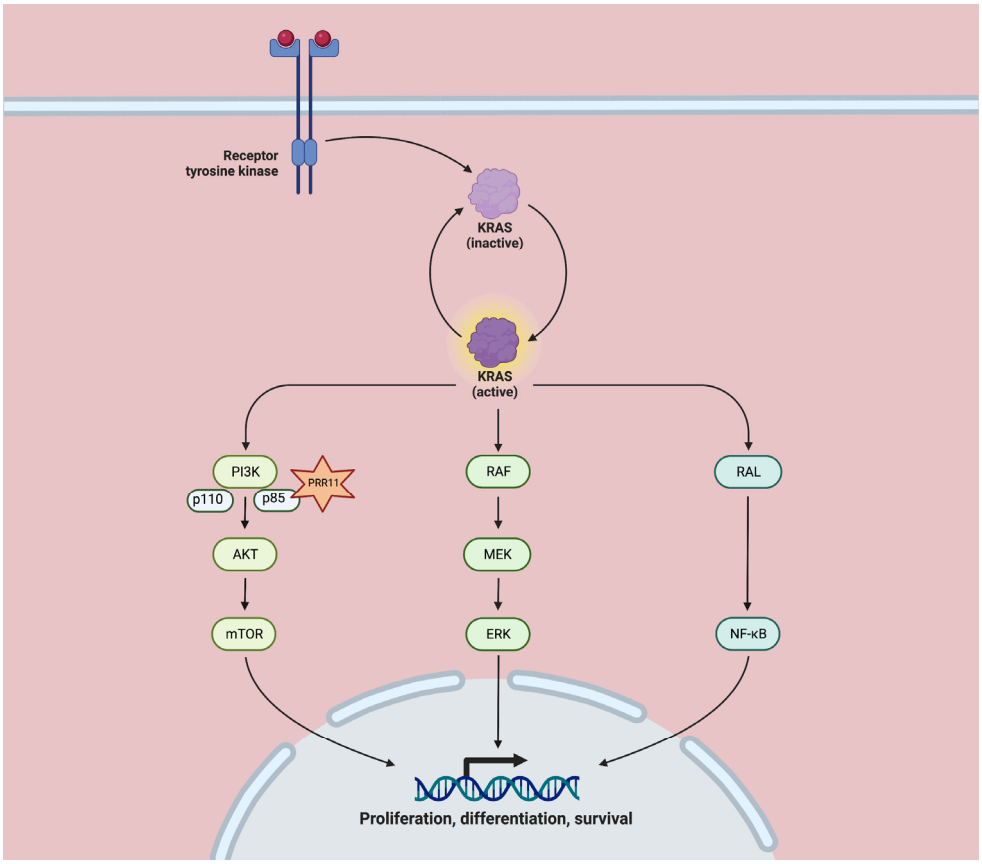

Figure 8. Overview of important KRAS-dependant pathways in pancreatic cancer.
Created by the author with BioRender™.

Paper II

The CHAMP study started in 2018 and has thus far (January 15st, 2023) enrolled 127 patients. Two patients are currently receiving neoadjuvant treatment, and the final treatment intention remains to be determined. Two additional patients have been included in the study when receiving first-line palliative treatment for recurrent disease after previous surgery and adjuvant chemotherapy. A schematic overview of patients with a primary diagnosis and known treatment intention (n=123) is shown in Figure 9. Of these patients, 23% have undergone surgery with curative intent (28/123), and, of note, there is still a clear discrepancy in treatment intention when stratifying by sex,

with only 13 % (8/61) of women having had surgery with curative intent compared to 32 % (20/62) of men (p=0.017). The age at diagnosis ranges from 38 to 83 years of age, with a median age of 68. Two patients have Lynch syndrome and, subsequently, mismatch repair deficiency. One patient has a germline mutation in *BRCA2* and one patient has a germline mutation in *CDKN2A*. One palliative patient has a tumour located in the distal bile duct, and one adjuvant patient has a tumour located in the ampulla. All other cases have tumours with histopathologically confirmed or radiologically estimated origin in the pancreas.

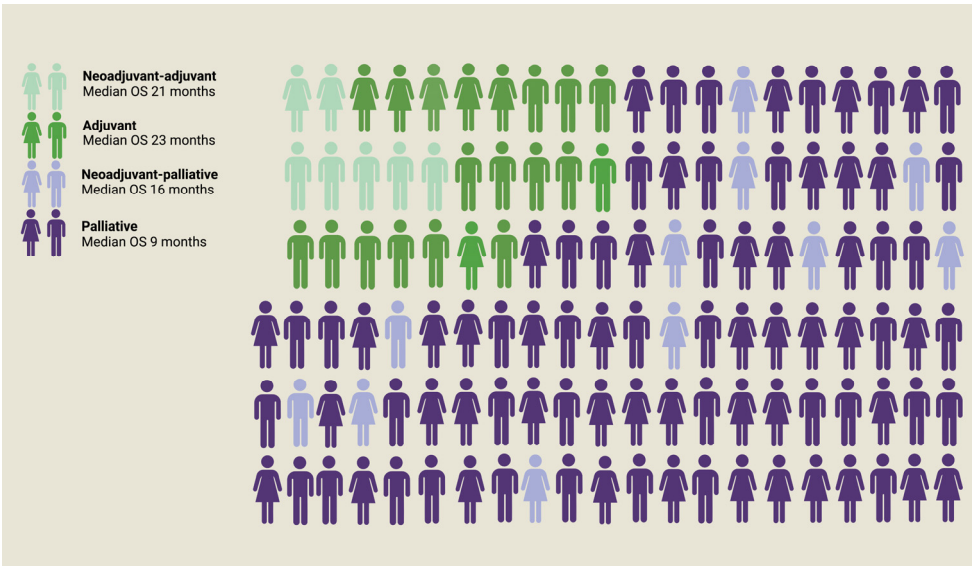

Figure 9. Overview of 123 patients with a primary diagnosis and known treatment intention enrolled in the CHAMP study on January 15th 2023.
Created by Karin Jirström and adapted by the author with BioRender™.

Paper III

Sex and gender differences in treatment intention, performance status, HRQoL and survival were investigated in the first 100 patients enrolled in the CHAMP study. Forty-nine patients were women, and 51 men. A total of 25 patients underwent surgery with curative intent and received neoadjuvant or adjuvant chemotherapy. Of these 25 patients, seven (28%) were women and 18 men (72%). Two women were offered surgery with curative intent but declined. In adjuvant patients, significantly more women had a history of previous malignancies compared to men. None of the women who underwent surgery with curative intent had a performance status of more than one, whereas 28% (5/18) of the men had a performance status of two at the start of

chemotherapy. This difference did however not reach statistical significance. In palliative patients, women had a significantly lower body mass index than men, whereas men had significantly more cardiac comorbidities. Regardless of treatment intention, there were no differences between the sexes regarding age, diabetes, smoking, marital status, neoadjuvant treatment, treatment backbone or tumour location.

Seventyfive patients, 33 women and 42 men, had completed EORTC-QLQ-C30 questionnaires at baseline. There were no significant demographic differences between patients with and without completed questionnaires or when stratifying patients with completed questionnaires by treatment intention. Female patients experienced significantly poorer cognitive and emotional functioning than male patients and had higher levels of fatigue, nausea, loss of appetite and insomnia at baseline. Stratification by treatment intention alone revealed better global health and a better overall functioning, as well as lower levels of fatigue, nausea, pain, and a better appetite in adjuvant patients. Associations between performance status and HRQoL in both sexes were further investigated. In female patients, HRQoL did not decrease in patients with decreased performance status. In male patients however, a poorer performance status correlated significantly with poorer emotional functioning, social functioning, and physical functioning, as well as a higher level of fatigue.

The results revealed no statistical differences between the sexes regarding clinicopathological factors, age or comorbidities. Thus, it is reasonable to assume that gender plays a role in the discrepancy regarding selection for surgery between the sexes. The finding that two women were offered surgery with curative intent but declined is in line with previous studies showing that patients who decline pancreatic cancer surgery generally are of female sex, older age and/or suffer from comorbidities [206-208]. Women have a poorer HRQoL than men, which is true for women with cancer as well as for women in the general population [190, 209]. A limitation of this study is that HRQoL in the reference population was not considered, a fact that may lead to bias when interpreting the results. In a large study of long-term cancer survivors and HRQoL, where adjustment for the reference population was performed, a significant and unexpected impact on male patients was revealed [210]. A further limitation of this study is that it is unknown whether the patients enrolled in the CHAMP study reflect the reference population of patients receiving chemotherapy for pancreatic cancer. However, the percentage of women (49%) and men (51%) included is representative of the known sex distribution of pancreatic cancer in Sweden [9]. As shown above, there has been no increase in the number of women undergoing surgery with curative intent with increased enrolment in the CHAMP study.

The finding that HRQoL in women remained constant independently of performance status while men with decreased performance status experienced poorer functioning

and more fatigue is novel. Gender differences could also be responsible for this finding, which warrants further investigation. Women and men are believed to not only perceive, but also to report symptoms differently due to discrepancies in early socialisation, social position and gender roles between the sexes [211].

Paper IV

Associations between levels of inflammatory proteins, cf DNA, prognosis and HRQoL over time were investigated in the first 60 patients enrolled in the CHAMP study. Of the 92 explored proteins at baseline, the top up-regulated proteins were interferon-γ (IFN-γ), interleukin-15 (IL-15), decorin (DCN) and colony stimulating factor 1 (CSF-1), while no proteins were down-regulated. At three months, the top up-regulated proteins compared to baseline were carbonic anhydrase IX (CAIX), lysosome-associated membrane glycoprotein 3 (LAMP-3), interleukin-18 (IL-18), DCN and CSF-1. The top down-regulated proteins at three months compared to baseline were matrix metalloprotease 12 (MMP12) and tumour necrosis family super member 14 (TNFRSF14). At six months, IL-15 was the only up-regulated protein compared to baseline. IFN-γ, which was the most up-regulated protein at baseline, is a cytokine produced by many cells. It is known to have cytotoxic effects as well as a protective effect on normal cells. It can, however, also contribute to tumour progression as the protective effect on normal cells may lead to an immunosuppressive environment and tumour evasion [212]. CAIX, the most up-regulated protein at three months compared to baseline, is up-regulated in response to hypoxia [213]. It has a key role in cancer development and has drawn interest as a potential therapeutic target, although with little success so far [214]. CAIX-inhibitors may also have potential as chemotherapy sensitisers [215].

A general improvement in HRQoL was seen from baseline to three months, with a large improvement in global health and appetite, moderately decreased pain, as well as less constipation. No significant differences were seen in HRQoL reported at three and six months. The fact that patients experienced an improvement in HRQoL is line with previous findings [216, 217]. Of note, this was true independently of treatment intention. Since patients receiving adjuvant chemotherapy have a minimal tumour burden, this is a clear indication that symptom management improves HRQoL.

High levels of MMP7 at baseline were significantly inversely associated with global health and functioning, and positively associated with fatigue at either or both three and six months. High levels of tumour necrosis receptor superfamily member 12A (TNFRS12A) were also inversely associated with global health at both timepoints as well as with emotional, physical, and social functioning at three months. TNFRS12A

at baseline was positively associated with fatigue at both timepoints. High levels of granzyme H (GZMH), PD-L1 and interleukin-12 (IL-12) at baseline were significantly associated with increased pain at three months. GZMH and IL-12 at one month also correlated with increased pain at three months and IL-12 at three months correlated with increased pain at six months. There were significant correlations between various proteins and other symptoms (insomnia, dyspnoea and constipation), however patients generally had a low score in these symptoms, rendering these correlations less clinically relevant.

Although MMP7 and TNRSF12A are both known biomarkers of adverse prognosis in cancer, their associations with decreased HRQoL have hitherto not been studied. MMP7 has also shown potential as an independent, predictive biomarker in patients with prostate and NSCLC treated with docetaxel and platinum-based chemotherapy, respectively [218, 219] and may also have value as a therapeutic target [220]. TNFRSF12A is also known as tumour necrosis factor-like wear inducer of apoptosis receptor (TWEAKR). The TWEAK/TWEAKR pathway is linked to tumour progression in a number of cancers and TWEAK is abundantly expressed in pancreatic tumour tissue [221]. The proteins, GZMH, IL-12 and PD-L1 at baseline were associated with increased pain at three months. GZMH and IL-12 are important drivers of inflammation [222, 223], although this is, to our knowledge, the first study to report their association with pain. PD-L1 inhibits immune response by binding to T-cells and hindering their function. It is also known that PD-L1 expression on T-cells inhibits pain by suppressing nociceptive neuron activity via the PD-1 receptor [224]. Although the mechanism behind soluble PD-L1 being associated with pain is unknown, it has been postulated that high levels of soluble PD-1 result in excessive binding with PD-L1, thereby blocking and reducing the analgesic effect of the endogenous PD-1/PD-L1 pathway [225].

When stratifying levels of cfDNA by treatment intention, no significant differences could be seen between the three timepoints. There was, however, a significant difference between adjuvant and palliative patients at all timepoints, with the former having consistently lower levels, except for at six months. High cfDNA levels at baseline were inversely correlated with global health, physical, role and emotional functioning, and positively correlated with fatigue, dyspnoea, insomnia, nausea, and loss of appetite at baseline. High cfDNA levels at baseline were also inversely correlated with cognitive functioning at three months. To our knowledge, this is the first study reporting associations of cfDNA with decreased HRQoL.

The findings in this study further support the utility of cfDNA as a prognostic biomarker, as high cfDNA levels at baseline and one month were significantly associated with a shorter OS. The finding that palliative patients had higher levels of

cfDNA than adjuvant patients at all timepoints, except for at six months, could be indicative of early relapse in adjuvant patients, but could also be due to the small sample size for both adjuvant and palliative patients at six months.

High emotional and cognitive functioning at baseline were significantly associated with a prolonged OS in univariable analysis, but not when adjusting for treatment intention, age, sex, and performance status. The same was true for physical functioning at three months. A high score of pain at baseline was significantly associated with decreased OS in both univariable and multivariable analyses. Cancer antigen 125 (CA-125) was the most prognostic protein at all timepoints, being significantly associated with a decrease in OS in both univariable and multivariable Cox regression analyses. High levels of cfDNA at baseline and one month were prognostic of a shorter OS in both univariable and multivariable Cox regression analyses.

The predictive value of cfDNA was less evident than for inflammatory proteins regarding adverse symptoms at a later stage of treatment. This indicates that cfDNA levels represent the disease rather than the illness that patients experience, and that the latter is better reflected by inflammatory protein levels.

Conclusions

"What is it you don't understand?!"

Karin Jirström

PRR11 has been unveiled as an adverse prognostic biomarker in pancreatic cancer and is also predictive of poor outcome in patients treated with chemotherapy. Since PRR11 is a vital part of the PI3K/AKT pathway, it would be of interest to explore further in the context of targeted treatment.

Women with pancreatic cancer experience a worse overall HRQoL compared to men. But although men with pancreatic have a better overall HRQoL than women, it is decreased with decreasing performance status, while HRQoL in women remains constant.

Gender matters. Despite no differences being found regarding clinicopathological factors or demographics, fewer women underwent pancreatic cancer surgery than men. Gender influences how patients and health care providers interact, which in turn affects the outcome for patients with pancreatic cancer. Gender dimensions should be considered when selecting patients for surgery. Particular attention should be given to women and encouragement to undergo surgery may well lead to improved survival rates.

In the future, gender dimensionality should be given additional consideration in order to decrease gender disparities. A heightened awareness both in the clinical setting and in research is necessary to understand how gender may impact the biological outcome for both sexes.

HRQoL is improved in pancreatic cancer patients during chemotherapy treatment compared to before the start of treatment, independently of treatment intention.

Pain before the start of treatment is associated with a poorer OS.

High levels of MMP7 and TNFRSF12A are associated with a generally decreased HRQoL.

High levels of PD-LI, GZMH and IL-12 are associated with increased pain.

High levels of cfDNA are associated with decreased HRQoL and shorter OS.

Given that patients with pancreatic cancer have a poor prognosis with significant physical and emotional suffering, it is vital to optimise symptom management, which may also contribute to a better outcome for these patients.

Future perspectives

"I need paper and a pen"

Jakob Eberhard

Protein expression of PRR11 and RBM3 has been analysed in tumour tissue from 15 adjuvant patients and 10 palliative patients enrolled in the CHAMP study, and has so far not proven to be prognostic. We aim to perform IHC analyses on tumour tissue from future CHAMP patients as enrolment continues, in order to validate the findings from Paper I as well as previous RMB3 studies.

After the CHAMP study had been enrolling patients for a year or so and we began discussing exactly how we were going to analyse the generated data, it became apparent that one piece of the puzzle was missing. Previous tissue-based research has mainly focused on patients with resected tumours, i.e. a minority, and what happens in the tumour after termination of treatment? To answer this question, we initiated an addition to the CHAMP study in the autumn of 2021. In this study, select patients undergo autopsies in order to enable in-depth studies of the terminal tumour burden. To date, 13 patients have been included and nine patients have undergone targeted autopsies. Of these nine patients, some have had response to chemotherapy and a stable disease for many months, while others have presented with aggressive disease, with little or no response to chemotherapy. Although we are just getting started, we believe that analysis of post-mortem tumour tissue is vital to gain an increased understanding of the evolutionary dynamics of pancreatic cancer and that these extended analyses will give us important tools for better patient stratification and adaptive treatment strategies in the future.

In the near future, we hope to initiate a new study based on the results of the CHAMP study, wherein patients will be stratified to adaptive treatment based on the evolvability of the tumours, together with other relevant disease and illness-related factors.

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