INTRODUCTION
Chapter 1

Pancreatic cancer, an introductory overview, and outline of the thesis

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Pancreatic cancer, an introductory overview

1.1. General introduction

Pancreatic cancer is the fourth leading cause of cancer death in developed countries [1]. There are approximately 277,000 new cases of pancreatic cancer and 266,000 deaths annually worldwide, indicating a mortality rate of 96% of the cases diagnosed [2-3]. Pancreatic cancer has indeed an extremely poor prognosis, with a 5-year survival rate below 5% [1]. Pancreatic ductal adenocarcinoma (PDAC) includes more than 90% of pancreatic cancers. This thesis focuses mainly on PDAC.

Despite extensive clinical and scientific researches, the extremely poor prognosis of this disease has not improved over the past decades. The main reasons for the lack of effective therapeutic strategies include invasive behavior of PDAC and its intrinsic resistance to most chemotherapy, radiotherapy and immunotherapy regimens. Gemcitabine is the most commonly used chemotherapeutic agent in the treatment of PDAC, however, this therapeutic regimen results in less than 12% response rate in the patients [4]. Thus, there is an urgent need to develop novel anticancer agents that either improve gemcitabine activity, within novel combinatorial regimens, or with a better efficacy than gemcitabine. In particular, novel approaches are warranted to inhibit PDAC invasiveness and its chemoresistance.

Here we hypothesize that lack of drugs that combined synergistically with gemcitabine as well as lack of appropriate models to test the activity of anticancer agents may have, at least in part, hampered clinical progress in the treatment of PDAC. Therefore, the research described in this thesis aimed to (1) investigate the molecular mechanisms underlying the aggressiveness and chemoresistance of PDAC; (2) develop novel in vitro and in vivo PDAC models to evaluate the efficacy of novel anticancer agents; (3) identify new treatment strategies that could be readily translated to the clinic.

The aim of the current chapter is to give an introductory overview of this dismal disease. This introduction will first outline the clinical presentation and management of PDAC, with a brief description of the anatomy of pancreas, as well as of epidemiology and risk factors. We will then provide a brief summary on PDAC treatment, genetic alterations found in PDAC, and preclinical studies carried out in PDAC animal models.

1.2. Pancreas anatomy

Pancreas is a gland organ found in all vertebrates. The pancreas is comprised of two separate functional units, exocrine and endocrine functions, which are involved in two major physiological processes: digestion and glucose metabolism, respectively (Figure 1). The exocrine pancreas consists of acinar and duct cells. The acinar cells produce digestive enzymes, which are secreted into the small intestine to convert food into smaller components (nutrients) that the body can assimilate. The ducts, which add mucous and bicarbonate to the enzyme mixture, form a network of increasing size, culminating in main and accessory pancreatic ducts that empty into the duodenum. The endocrine pancreas is made by four specialized cell types that are organized into compact islets embedded within acinar tissue, and secretes hormones into the blood stream. In particular, α- and β-cells regulate the usage of glucose through the production of glucagon and insulin, respectively. δ-cells and PP cells produce somatostatin and pancreatic polypeptide, respectively, that
modulate the secretory properties of the other pancreatic cell types [5].

1.3. Epidemiology

The incidence of PDAC is approximately 10 per 100,000 people per year in the Western countries. PDAC is more frequently observed in elderly than in younger persons. Diagnosis of PDAC before the age of 40 is rare. Recently, data published by the International Agency for Research on Cancer reported the incidence and mortality from 182 countries in 2008 for 27 different cancers. This study showed that Japan has the highest incidence of PDAC, with a rate of 156 deaths per 1,000,000 inhabitants. Numbers for Italy, France, USA and Russia are 138, 118, 102 and 86, respectively. The rates are 3-4 times higher in countries north or south of the equator (i.e. North America, Europe, Australia, and New Zealand) compared to those near the equator (i.e. South Asia). However, some of these differences can be explained by differences in life expectancy, as the incidence of this disease is highly age related [2,3,6]. In particular, the Japanese have a life expectancy of 82 years, while Russians have a life expectancy of 65 years. Moreover, differences might be related to variation in cancer diagnosis between countries [3].

1.4. Risk factors

Environmental risk factors are responsible for the majority of PDAC cases (Figure 2). Age is the main risk factor. It has been shown that less than 10% of patients develop PDAC
before the age of 50, and this younger group has a higher proportion of patients with underlying predisposing genetic disorders [3,7]. Chronic pancreatitis represents another important risk factor for the development of PDAC [7]. Several other risk factors have been identified for PDAC, including family history, male sex, substance abuse (e.g., smoking and heavy alcohol), the metabolic syndrome (e.g., diabetes and obesity), and the vitamin D system (i.e., a lower incidence and death rate from PDAC has been registered in people exposed to higher levels of solar UVB light, and with adequate levels of vitamin D; [Ref#8-10]). In particular, smoking can increase the risk of developing PDAC by 2-3 folds. Conversely alcohol consumption does not seem to be a risk factor unless the alcohol abuse results in pancreatitis [8]. Diabetes patients also appear to have a higher chance of developing PDAC [11]. However, the risk increase with diabetes, overweight, and obesity is relatively small with respect to age and chronic pancreatitis [3].

1.5. Clinical presentation, diagnosis and management of PDAC

The symptoms of PDAC are non-specific, with abdominal pain, weight loss, general malaise, diarrhea, anorexia and vomiting as the most commonly reported complaints. Upon progression of the tumor, the pancreatic and bile ducts often become blocked, resulting in reduction of bile and pancreatic juice secretion into duodenum. Abdominal pain becomes more localized mainly in the upper middle of the abdomen, which is initially caused by tumor growth into the celiac and superior mesenteric plexus. Back pain is also reported, when the tumor progresses into the retroperitoneal plexus. Loss of body weight is most likely caused by the decreased food intake and fat uptake by small intestinal epithelium due to the decreased bile secretion. The energy consuming aerobic glycolysis of the tumor cells, known as Warburg effect, also contributes to the weight loss of the patients.

The most common sign of PDAC is painless jaundice (yellow tint to whites of eyes (sclera) or yellowish skin, possibly in combination with darkened urine), when a cancer of the head of the pancreas (75% of cases) obstructs the common bile duct as it runs through the pancreas. This may cause pale-colored stool and steatorrhea. Physical examination of PDAC patients often shows a palpable mass in the upper abdomen, lymphadenopathy, hepatomegaly and ascites. However, most of these signs usually indicate an advanced stage of tumor. Hematological abnormalities can present with non-specific mild anemia and hyperglycemia. Prothrombin time is often increased due to malabsorption of vitamin K, as a
consequence of reduced fat absorption [12].

PDAC can lead to malfunctioning of the β-islets in the pancreas resulting in diabetes mellitus. Since at the time of diagnosis about 80% of PDAC patients have impaired insulin secretion [13], PDAC should be considered in differential diagnosis with respect to type II diabetes. Of note, a dysbalance in plasma glucose levels is often observed in early stages of PDAC, which can be considered a possible marker for diagnosis [14].

Carbohydrate antigen 19-9 (CA 19-9) is the most commonly used marker for therapeutic monitoring and detection of recurrent disease in PDAC [15]. However, CA 19-9 is significantly increased only in larger tumors, which makes it a disappointing tool for screening [16].

Several imaging techniques are being used for diagnosis of PDAC, including ultrasonography (US) in combination with Doppler imaging, Computed Tomography (CT), and/or Positron Emission Tomography (PET) scan. In particular, US provides information on the presence or absence of a pancreatic tumor, bile duct enlargement and liver metastasis. However, it is difficult to accurately identify the stage of a tumor with US [17]. Although US is an appropriate initial imaging technique, thin-slice contrast-enhanced CT should always be used for diagnosis and staging. CT allows the visualization of the tumor in relation to surrounding structures. Finally, PDAC can also be visualized by PET. In the context of the high metabolic rate of tumor cells, this technique allows evaluation of metabolic activity of cells. Serrano and colleagues showed that PET scanning can be a useful tool for diagnosis and staging of PDAC, specifically in the cases where CT scanning fails to identify a discrete mass [18].

PDAC is staged according to the T(umor) N(ode) M(etastasis) classification of the American Joint Committee on Cancer. The TNM classification of PDAC is as follow: T1, tumor size ≤2.0 cm (limited to pancreas); T2, tumor size >2.0 cm (limited to pancreas); T3, tumor extends beyond the pancreas but does not involve the celiac axis or the superior mesenteric artery; T4, tumor has grown into these structures. N0 or N1 illustrate the absence or presence of lymph node metastases, respectively, while the tumor is designed as M0 or M1 when distant metastases are not detectable or detectable, respectively.

Tumors are usually divided into stages I, II, III or IV using the TNM classification. Stage I tumors have no lymph node/distant metastases, either T1 or T2 tumor grade. Stage II tumors either have T3N0M0 or T1/T2/T3 with lymph node involvement. Stage III tumors are T4 with either N0 or N1, while any tumor with M1 is stage IV. Currently, stage III/IV tumors are per definition unresectable [19].

1.6. Treatment

Treatment option for each patient with PDAC differs depending on tumor resectability. Currently, surgical resection remains the only potentially curative treatment for localized tumors that are confined to the pancreas. Patients undergo classical pancreaticoduodenectomy, distal pancreatectomy or total pancreatectomy, depending on the tumor location.

Most patients (~80%) present with advanced disease (i.e., locally-advanced or metastatic) at diagnosis. The primary goals of treatment in this group of patients are survival prolongation and palliation [20]. Gemcitabine is being recommended for therapy as a treatment of choice since 1997, when it was shown to increase overall survival and improve quality of life [21,22].
Chemoradiotherapy has been used in USA since the Gastro-Intestinal Tumor Study Group trial demonstrated longer overall survival in the group treated with chemoradiation [23,24]. However a similar investigation failed in Europe. Chemoradiation therapy is not considered as a standard in treatment of PDAC patients in western countries [25,26], but recent studies have been initiated by the Dutch Pancreatic Cancer Group to evaluate radiochemotherapy versus immediate surgery for resectable and borderline resectable PDAC [27].

Over the last few decades very few of the doublets in combination with gemcitabine have succeeded. Erlotinib-gemcitabine was the only combination that got approved, but on average, this treatment provided only one month of additional life. However, a recent landmark phase III trial demonstrated that the combination of oxaliplatin, irinotecan, fluorouracil, and leucovorin (FOLFIRINOX) is an option for the treatment of metastatic patients with good performance status [28]. The objective response rate was 31.6% in the FOLFIRINOX group versus 9.4% in the gemcitabine group (P<0.001). Moreover, the median overall survival in the FOLFIRINOX group was 11.1 months as compared with 6.8 months in the gemcitabine group (hazard ratio for death, 0.57; 95% confidence interval [CI], 0.45 to 0.73; P<0.001). Similarly, in a recent randomized phase III trial, the regimen of Abraxane plus gemcitabine showed better antitumor activity with respect to gemcitabine alone [29]. However, these therapeutic strategies are associated with increased toxicity and further studies are needed to evaluate novel strategies to improve gemcitabine efficacy against PDAC.

1.7. **Tumor biology**

1.7.1. **Pathogenesis of PDAC**

PDAC develops in a step-wise manner in which non-malignant pre-invasion lesions progress to an invasively growing tumor (Figure 3). Shortly, pancreatic intraepithelial neoplasia (PanIN) lesions are the pre-invasive form of tumor with microscopic dimensions, which is found in smaller pancreatic ducts. These lesions are classified into different stages, PanIN-1, PanIN-2 and PanIN-3, on the basis of cellular and nuclear atypia and architectural changes. PanIN-1 lesions illustrate little cytonuclear atypia and have retained their cellular polarity. This type of lesion is subdivided into two groups: PanIN-1A and PanIN-1B based on whether the cells have a squamous-like fashion (1A) or a micropapillary architecture (1B). PanIN-2 lesions show evident cytonuclear atypia and infrequent mitoses, while PanIN-3 lesions, also referred to as carcinoma in situ, show all the hallmarks of cancer except the invasive growth [30,31]. Other recognized precursor lesions of adenocarcinoma (intraductal papillary mucinous neoplasm [IPMN] and mucinous cystic neoplasm [MCN]) likely harbor a distinct compendium of genetic alterations in their path to invasive cancer. PDAC is also characterized by the presence of a dense stroma of fibroblasts and inflammatory cells, termed desmoplasia. This tumor-associated desmoplasia has been described in recent years as being complicit in PDAC growth. In normal tissues, the stroma provides nutrients and regulatory signals for proper cellular polarity and function. However, following oncogenic transformation, the stromal compartment is conscripted to provide stimulatory signals and protection to tumor cells. Several types of tumor-stroma interactions have been implicated as having the potential to promote PDAC invasion and metastasis. Cancer cell derived growth factors, such as fibroblast growth factors (FGFs), transforming growth factor-betas (TGF-βs), insulin-like growth factor-1 (IGF-1) and platelet-derived
growth factor BB (PDGF-BB), become sequestered within the stroma, which thus acts as a storage site for these factors. The invading cancer cells produce matrix metalloproteinases (MMPs) that release these growth factors [32].

1.7.2. Genetic alterations

The progression from histologically normal epithelium to invasive carcinoma is associated with the accumulation of genetic alterations. Telomere shortening is one of the first events in the progression to a pre-invasive lesion and eventually to PDAC, which results in chromosomal instability and allows accumulation of genetic alterations [33]. Telomeres are repeated sequences at the end of linear chromosomes, which prevent fusion between chromosomes. Short telomeres can result in ring chromosomes and dicentric chromosomes that form a so-called anaphase bridge during mitosis. Breakage of anaphase bridges generates highly recombinogenic free DNA ends, leading to chromosomal rearrangements. This event, which is called “anaphase bridge-breakage-fusion cycle”, is repeated, thereby leading to genetic instability that forms the basis of tumor development.

Figure 3. Pancreatic precursor lesions and genetic events involved in pancreatic adenocarcinoma progression. The model illustrates normal duct epithelium progressing to infiltrating cancer (left to right) through a series of histologically defined precursors (PanINs). The various genetic events are listed and divided into those that predominantly occur early or late in PDAC progression. The point mutations in the KRAS gene and/or overexpression of Her-2/neu occur early, inactivation of the p16 gene occurs at an intermediate stage, and the inactivation of p53, SMAD4 and BRCA2 genes occurs relatively late.
Several mutations have been observed either in oncogenes, e.g., KRAS, or tumor suppressors genes, e.g., CDKN2, TP53, DPC4/Smad4 and BRCA2 (Fig. 3). KRAS, CDKN2, TP53, and DPC4 are commonly referred as PDAC “driver genes”. In particular, KRAS is one of the main players in the development of PDAC. This gene is mutated in the majority (90%) of PDACs. KRAS encodes a member of the RAS family of guanosine triphosphate (GTP)-binding proteins that mediates a range of cellular functions, including proliferation, cell survival, cytoskeletal remodeling, and motility. In addition to its role in PDAC initiation, constitutive RAS signaling appears to be important also for PDAC maintenance.

The most commonly affected tumor suppressor gene in PDAC is CDKN2A, which encodes p16 protein. Inactivation of CDKN2A is observed in more than 95% of patients [34]. TP53 is another important tumor suppressor gene, which is inactivated in 25-60% of cases [35]. The gene encodes p53, a protein involved in cell cycle arrest and apoptosis upon cytotoxic stress. This gene is mainly mutated in PanIN-3 in transition to invasive growth, as a late event in PDAC development [36]. Moreover, the activity of p21 is lost in 40-60% of PDACs. The p21 is activated after the stimulation of p53 that inhibits the activity of cell proliferation-stimulating complex containing the protein cyclin D [37,38]. DPC4 mutations are observed in approximately 55% of patients. These mutations occur in late stages of tumor development, mainly in Pan IN-3 [39]. DPC4 encodes Smad4 that is part of the transforming growth factor (TGF)-beta signaling. TGF-beta is one of the most important signaling pathways involved in cell cycle inhibition, differentiation, apoptosis, and angiogenesis [40]. Several studies have supported both the diagnostic and prognostic values of Smad4 expression in PDAC [41,43].

Another set of genes, which is mutated in 4-10% of PDAC cases, includes mismatch repair genes, which are involved in DNA repair system. Notably, genetic or microsatellites instability may occur and genetic changes can accumulate when these genes are mutated [44,45]. BRCA2 is one of DNA repair genes involved in PDAC. This gene is mutated in 7-10% of sporadic PDACs and 10% of familiar PDACs [46]. Involvement of BRCA2 mutations in breast and ovarian cancer as well as in the cancer-susceptibility disorder Fanconi anemia is well known. Mutations in FANCC and FANCG which are involved in interstrand crosslinking repair and cause Fanconi anemia are also found in PDAC [47].

Recent advances in genome sequencing have revealed a complex picture of genetic interaction in the initiation and progression of PDAC [48]. This study showed frequent genetic alterations in a wide variety of core signaling pathways (e.g., Wnt and TGF-beta), and re-activation of embryonic signaling pathways (e.g., Notch and Hedgehog). Although these core signaling pathways were altered in the majority of analyzed tumors, the role of the many identified mutations and genetic aberrations remains unclear. Further understating of these changes at functional levels, specifically related to genetic interactions between KRAS and other observed mutation, will undoubtedly be important in the identification of new genetic targets for both early diagnosis and more effective treatment of PDAC. Targeting Ras has indeed been a holy grail in the treatment of PDAC for many years; either with single-agent or combination therapies with farnesyl transferase inhibitors, such as in the SWOG 9924 study. However all these approaches showed limited activity.

More recently, Biankin and collaborators, within the “Pancreatic Cancer Genome Project” [49], performed a whole genome sequencing in combination with copy number analysis of 99 early stage PDACs, which defined 16 significantly top mutated genes. This study reaffirmed the key role of known mutations in KRAS, TP53, CDKN2A, and DPC4, and
unraveled novel mutated genes, such as genes involved in chromatin modification (EPC1 and ARID2), DNA damage repair (ATM) and other mechanisms (ZIM2, MAP2K4, NALCN, SLC16A4 and MAGEA6). Moreover, this study identified frequent and diverse somatic aberrations in genes described traditionally as embryonic regulators of axon guidance, particularly SLIT/ROBO signaling. Loss of SLIT/ROBO signaling (affecting about 25% of patients) promotes stabilization of β-catenin, which decreases E-cadherin complex formation and cell adhesion and augments WNT activity through increased nuclear translocation of β-catenin. In addition, loss of ROBO signaling activity promotes MET signaling and increased the invasive potential. Notably, high expression of the SLIT receptor ROBO2 was associated with a better prognosis while high expression of ROBO3, an inhibitor of ROBO2, showed an inverse relationship, with high levels associated with poor survival.

In terms of reactivation of embryonic signaling pathways, previous studies showed that the Notch pathway is usually inactivated in normal pancreas while it is active in a large proportion of PDACs [50,51]. Activation of the Notch pathway increased the number of PanIN lesions in vivo [52]. The hedgehog pathway is another embryonic signaling pathway, which is often reactivated in PDAC. Importantly, epithelial cells in the tumor do not respond to the pathway and a recent study showed its role in the maintenance of the stromal compartment of the PDAC [53]. Inhibition of the Hedgehog pathway in a transgenic mouse model inhibited the development of tumoral stroma and increased the response to chemotherapy through better drug delivery to cancer cells [54,55].

1.8. Preclinical PDAC models

The most commonly used PDAC models include (1) established cell lines cultivated as monolayers or spheroids, and (2) mouse models. In terms of PDAC cell lines, unfortunately, long term culturing of the cells results in distinct and irreversible loss of important genetic and biological properties. These include complex genomic aberrations affecting critical signaling pathways [48], maintenance of a distinct stem cell population [56,57], and the ability of the PDAC cells to metastasize [58].

Mouse models are an important tool in cancer research. Three major mouse models are available: genetically engineered mouse models (GEMMs), carcinogen-induced mouse models and xenograft implanted mouse models. All these models have their own advantages and disadvantages. In 2003, Hingorani et al., created the first generation of GEMM for PDAC by conditional knock-in of mutant KrasG12D [59]. Mutated Kras expression was limited to the pancreas and embryonic foregut by targeting Pdx1-positive cells. Pdx1 is indeed an important transcription factor expressed in epithelial cells and plays an essential role for pancreatic formation and development. GEMM of PDAC with KRA5 mutation developed PanIN lesions with occasional progression to invasive carcinoma [59]. It has been shown that additional mutations in the GEMM, e.g., p16INK4a/p19Arf [60], p53 [61] or p16INK4a/p53 could result in higher penetrance of malignant progression, earlier onset, progression to distant metastasis and reduced survival [60,61]. Recently, conditional gene knock out of SMAD4 or TGF-betaRII was also induced in mouse. This did not result in pre-invasive lesions or malignant progression, but combination of this mutation with KrasG12D expression resulted in markedly increase in carcinogenesis [62,63].

GEMMs of PDAC provide an essential tool to probe the function of specific genes in tumor initiation and progression. In particular, limiting KrasG12D to the elastase-producing (centro)
acinar cells resulted in mouse-PanIN formation, albeit only with concurrent induction of chronic pancreatitis [64]. GEMM have the advantage to maintain an intact immune system and present tumors histologically similar to human PDACs, including a dense desmoplastic stroma reaction [65]. However, several promising targets identified from these models do not have human counterparts, while several important genes in human PDAC are not expressed in mice [66]. Moreover, their dependence on a few critical genetic lesions may not fully reflect the genetic diversity that characterizes human PDAC.

Recently, primary PDAC xenograft models were successfully established by implanting pieces of human PDACs subcutaneously into mice [67-68]. However, several studies demonstrated that subcutaneously implanted tumors may not optimally recapitulate many of the essential features of tumor growth in patients, such as the ability to metastasize [69]. These findings support further studies for the development of orthotopically implanted tumors as a critical preclinical tool for testing new agents.

1.9. Conclusions and future prospects

Extensive research in the last two decades has resulted in a solid understanding of pancreas carcinogenesis. Unfortunately these findings have not led to major advances in diagnostics or treatment, and PDAC is still the most lethal among solid tumors. Moreover, most drugs that pass preclinical tests failed in these patients.

The emphasis of future research should be on (1) screening methods and identification of markers for diagnosis at early stages of the disease, or markers of treatment response, which can improve PDAC clinical management; (2) establishing new preclinical models to test novel anticancer strategies; and (3) development of new (targeted) treatment strategies that increase survival and disease recurrence rates according to the genetic characteristics of each patient (i.e., personalized medicine).
1.10. Outline of the thesis

In recent years, several advances have been made in the understanding of the molecular biology of as well as in the diagnosis of PDAC. However, minimal progress has been achieved in the treatment of this disease, which remains a major unsolved health problem.

The research described in this thesis had three main aims:

- To investigate genetics and epigenetics factors involved in PDAC
- To develop new in vitro and in vivo models of PDAC for drug testing
- To identify new treatment strategies that could be readily translated into the clinic

In chapter 2, we describe the prognostic role of XPD-Lys751Gln polymorphism in patients treated with gemcitabine-cisplatin-based polychemotherapy. The XPD protein is involved in DNA unwinding during nucleotide excision repair, which is the principal repair pathway for removing platinum-DNA adducts. Therefore, we also tested whether the XPD-Gln751Gln genotype was associated with a more efficient DNA repair after cisplatin exposure. For this purpose we evaluated the DNA damage capacity in lymphocytes harboring different XPD-Lys751Gln genotypes.

In chapter 3, we describe the prognostic value of the key epigenetic factor EZH2, by analyses of expression and candidate polymorphisms in locally-advanced or metastatic PDAC patients.

In chapter 4, we discuss the prognostic role of the expression of key determinants of gemcitabine activity (hENT1, RRM1, RRM2 and ERCC1) in resected PDAC patients undergoing gemcitabine-based adjuvant treatment.

In chapter 5, we describe the implication of miR-211 activity in human diseases and neoplasms, including PDAC.

In chapter 6, we evaluate whether comprehensive miRNA expression profiling correlated with overall survival in resected PDAC patients. MiR-211 expression emerged as the most predictive biomarker for treatment outcome in these patients and in a validation cohort treated with the same gemcitabine-based adjuvant therapy. Therefore, we investigated the role of miR-211 in the modulation of RRM2 and gemcitabine cytotoxicity in primary PDAC cultures as well as in PDAC cell lines.

In chapter 7, we investigate EZH2 expression in PDAC primary cells and originator tissues. Moreover, we evaluate the molecular mechanisms underlying the antiproliferative activity of the EZH2 inhibitor DZNeP in combination with gemcitabine in primary PDAC cultures as well as in PDAC cell lines.

In chapter 8, we investigate the role of the Akt signaling pathways in PDAC chemoresistance as well as the therapeutic potential of the novel Akt inhibitor perifosine in combination with gemcitabine in PDAC cells.

In chapter 9, we describe the critical role of the HGF/c-Met signalling pathway in cancer, as well as the preclinical and clinical investigations on c-Met inhibitors in solid tumors, with particular emphasis on recent findings with small-molecule inhibitors of c-Met in gastrointestinal cancers.

Using the knowledge gained from the previous chapter, the c-Met/ALK inhibitor crizotinib
was tested alone and in combination with gemcitabine in PDAC cells as well as in a clone of a PDAC cell line resistant to gemcitabine, as described in chapter 10.

In chapter 11, we describe the development and characterization of four new patient-derived orthotopic mouse PDAC models, genetically engineered to express Firefly- and Gaussia-luciferase. Furthermore, in order to show the feasibility of our PDAC models for drug discovery, we evaluated the effect of crizotinib in a model selected for c-Met overexpression.

In chapter 12, we evaluated whether the mRNAs and/or proteins encoded by the genes in the 18q22.3 cytoband were associated with the outcome of radically resected and metastatic PDAC patients. We also explored the impact of CYB5A expression on primary tumor growth and metastatic spread in a novel orthotopic PDAC mouse model.

In chapter 13, we discuss the results presented in this thesis. Moreover, we provide our view on the future perspectives in PDAC research.

Finally, chapter 14 contains a summary in English, Dutch and Farsi.
1.11. References

Chapter 1