Acquired resistance in pancreatic cancer: characterization and exploration of actionable targets of a multifactorial disease

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Chapter 1

General introduction and thesis outline

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As the second-leading cause of death globally, cancer represents a major public health concern [1]. Lung and breast carcinomas are the most diagnosed, however, gastrointestinal (GI) tumors are becoming more and more relevant in terms of mortality despite their lower incidence. Among GI neoplasms, pancreatic cancer is the third cause of cancer-related death in the US and the sixth world-wide [2,3]. The 5-year survival rate of pancreatic cancer has shown an increasing trend in the past years, reaching 13% in the US. Nonetheless, this rate remains among the lowest for cancer patients [3].

Histologically, more than 90% of pancreatic cancers cases are represented by pancreatic ductal adenocarcinoma (PDAC), which originates from the exocrine cells of the pancreatic ducts [4]. The exocrine tissue function is to produce and secrete digestive juices, destined to reach the duodenum through the pancreatic duct, as opposed to the endocrine tissue of the pancreas, where islet cells are responsible for secreting metabolism controlling hormones (e.g. insulin)[5]. The pathogenesis of PDAC proceeds as a progressive dysplasia starting from precancerous lesions in the pancreatic duct. These lesions are classified as pancreatic intraepithelial neoplasia (PanIN), which are the most common but hard to detect because of the small size, or intraductal papillary mucinous neoplasms (IPMNs), which are macrocystic lesions [6]. However, a single type of originating cell has not been identified for PDAC [7], as there is evidence that both acinar and ductal cells can generate this tumor type [4]. The morphological changes observed with PDAC development are accompanied by several key driver somatic mutations, usually starting with an activating mutation in *KRAS*, and followed by a loss of *CDKN2A*, *TP53*, *BRCA2,* and *SMAD4/DPC4* [5](Figure 1).

The reasons for the dismal prognosis of PDAC resides in its biology and are multifaceted. An early diagnosis is hampered by the lack of specific biomarkers or symptoms, which occur often in the advanced stage of the disease. This leads to half of the patients being diagnosed with metastatic disease, which is very difficult to treat. Modifiable risk factors correlating with PDAC are smoking, alcohol consumption, obesity, and diets rich in processed meat and saturated fats. Less than 10% of PDAC cases carry an inheritable genetic variant. When they occur, it is usually in DNA damage repair genes, for example *BRCA1, BRCA2* (BReast CAncer gene 1 and 2) *and ATM* (Ataxia-telangiectasia mutated) [8].

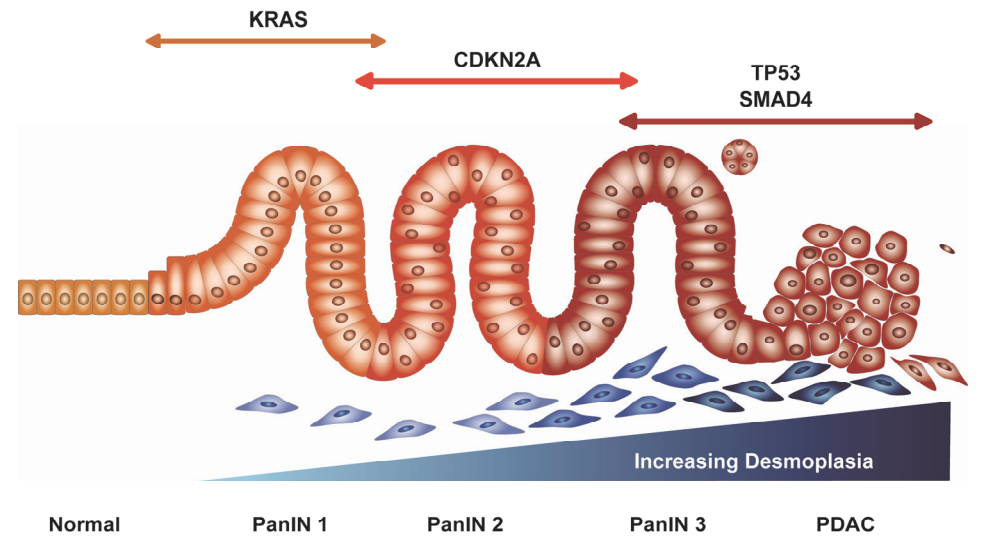

Figure 1: Representative model of carcinogenesis in PDAC. Representation of the sequential grade of lesions observed in pancreatic cells, which ultimately lead to PDAC. With increasing dysplasia, gene mutations are also accumulated, supporting tumor growth. Adapted from Morris et al. 2010[62].

The tumor microenvironment in PDAC

Besides the molecular characteristics of PDAC cells, the tumor microenvironment (TME) plays a major role in the disease progression and therapy resistance, eventually contributing to the bad prognosis. The TME is comprised of cellular and non-cellular elements, with the latter being particularly abundant in PDAC. PDAC cells are immersed in a dense desmoplastic reaction, a dysregulated deposit of extracellular matrix (ECM) proteins, including glycosaminoglycans (GAGs) such as hyaluronan, collagens, fibronectin, and tenascin [9,10], which are organized in a three-dimensional network. Besides an enrichment in ECM, tumor stroma is populated, sustained and remodeled by cellular players of the TME. In PDAC, these include pancreatic stellate cells (PSCs), cancer-associated fibroblasts (CAFs) and immune cells. Activation of PSCs and CAFs can be triggered by PDAC cells and once activated, they contribute to the major part of ECM deposit, which ultimately leads to ~90% of the tumor being represented by stroma. CAFs are a heterogenous population of fibroblasts, and research is ongoing to better characterize their different types [11]. Among CAF mediated processes, stromal remodeling (i.e., cross-linking) leads to increased ECM stiffness. In turn, a stiffer environment can increase tumor aggressive potential and therapy resistance [12]. Moreover, CAFs can support PDAC cell growth and

immune evasion by secreting immune suppressor cytokines (e.g. IL10, TGF-β1 and galectin-1) and C-X-C motif chemokine ligand (CXCL) 10, inhibiting the anti-tumor activity of cytotoxic T-cells and NK cells[9]. Thus, the interplay between the TME and the tumor cells sustains growth and progression of PDAC and its protection from the immune system.

Despite progress in resolving the diverse composition of the PDAC TME, attempts at modulating the stroma to improve therapy response failed so far. For example, combination strategies using hedgehog signaling inhibitors (IPI-926), pegylated hyaluronidase (PEGPH20), or genetic ablation of αSMA+ myofibroblasts, did not prove superior to chemotherapy alone [13-15]. Strikingly, while the multifaceted tumor promoting effect of the TME is being further unraveled, the stiff ECM-enriched stroma may also restrain PDAC expansion, keeping tumor cells confined in their primary site and preventing metastasis [14,16-18]. Indeed, a recent analysis on resected patients showed that a low amount of stromal collagen in PDAC tumors was associated with poor prognosis and poorly differentiated tumors [19]. These results indicate that the TME can play a dual role in PDAC and highlight the need for a deeper understanding of the interplay of different components of the TME and their influence on PDAC progression and resistance.

Interestingly, a strategy to decrease tissue stiffness without completely removing an element of the ECM could be targeting Rho-GTPases. An *in vivo* PDX study using fasudil (ROCK inhibitor) to prime the tumor before Gemcitabine/nab-paclitaxel treatment showed improved drug exposure and reduced metastatic spread, hence increased efficacy. Collagen production was not affected, but ECM network was less organized, and fibroblast-ECM interactions were still present but shorter and unstable [20]. Priming with a FAK inhibitor is also a viable strategy that is being investigated in a phase Ib/IIa clinical trial [21](NCT05355298).

Current treatment options

PDAC patients often present with non-specific symptoms and a pancreatic mass or abnormality may be discovered on imaging tests made for other reasons. The stage of the tumor and resectability is then defined by computed tomography (CT) or magnetic resonance imaging (MRI) and can be followed by more imaging to investigate the presence of metastasis [22]. Imaging is confirmed by histological examination, and the material should be collected for genetic and molecular testing as well. Before choosing the therapeutic regimen, performance status is also

evaluated (ECOG). In terms of stage, patients receiving the diagnosis of PDAC are divided into 4 broad categories: resectable (10-15% of newly diagnosed patients), borderline resectable (BRPC, 30-35%), locally advanced (LAPC, 30-35%), or metastatic (non-resectable, 50-55%) [8,23]. Stage and performance status parameters will be used to decide the therapeutic strategy. Resectable patients can get the only potentially curative treatment available for PDAC: surgery. Neoadjuvant (pre-operative) chemotherapy can be used to reduce the tumor and make BRPC and LAPC patients better candidates for surgery. Adjuvant chemotherapy takes place after surgery, to reduce the chance of recurrence of the disease [24,25]. After a complete characterization of the tumor (genetic and molecular) and performance status, metastatic patients can be enrolled in a clinical trial, proceed with systemic chemotherapy or palliative care, depending on the clinician indication [22].

Systemic chemotherapy is still the gold standard because only about 25% of PDACs harbor molecular alterations that can be specifically targeted. In a retrospective analysis, patients receiving therapy matched to the molecular alteration had longer overall-survival compared to those receiving unmatched therapy or without molecular alteration [26]. However, new approaches are emerging, which could provide hope for targeted therapy of a bigger proportion of PDAC patients. *KRAS* was considered undruggable, but recently, options for targeting its mutated forms emerged, such as the non-covalent inhibitor MRTX1133 [27] and the protein degrader ASP3082. These molecules target the KRAS G12D mutation and are currently being evaluated in phase I/II and I clinical studies (NCT05737706, NCT05382559), but the pre-clinical activity seems to be promising [27,28]. Moreover, KRAS inhibitors targeting a different mutation (G12C) have been recently approved by FDA for the treatment of non-small cell lung cancer, where that mutation is more common, indicating the potential for this approach [22]. Targeted therapy would represent a promising strategy, but the access to molecular testing and these advanced therapies is still difficult [26] and evidence from G12C-mutated KRAS inhibitors shows that emergence of resistance to these compounds is highly likely [29].

Immunotherapy is also not providing superior efficacy at the moment[30]. Available results of trials with immune checkpoints inhibitors (ICIs) are mostly unfavourable for the treatment of advanced PDAC as monotherapy [31], contrasting their success in other tumor types such as metastatic melanoma, non-small cell lung cancer, colorectal cancer, triple-negative breast cancer and head and neck squamous cell

carcinoma [32]. More promising results are obtained combining them with chemotherapy but clinical trials are still ongoing to evaluate their efficacy. Patients with high tumor mutational burden, specifically with homologous recombination deficiencies (HRD), seem to be good targets for the combination as shown in the preliminary results of the phase II POLAR trial [33](NCT05093231/NCT04666740). Final results of this trial will be shared in 2026. Moreover, the cancer vaccine GVAX, which should have an immunostimulatory effect and was expected to boost immune response against cancer cells, failed to show a benefit compared to FOLFIRINOX, when used in combination with the CTL-4 inhibitor ipilimumab, in metastatic PDAC as maintenance [34]. Finally, the mRNA vaccine cevumeran showed encouraging results in a Phase 1 trial (NCT04161755) where responding patients still have not displayed recurrence, after 3 years follow-up. However, this result was possible in patients with resectable PDAC, and the vaccine is now being investigated in a phase II trial (NCT05968326). Currently, effective immunotherapy in metastatic patients is not available.

Despite these promising targeted therapies, the gold-standard for patients with unresectable PDAC thus remains chemotherapy using either FOLFIRINOX (5-fluorouracil (5-FU), irinotecan, leucovorin, oxaliplatin) or gemcitabine combined with nanoparticle albumin-bound paclitaxel (nab-paclitaxel) [6,35]. Gemcitabine was the first drug approved for the treatment of PDAC patients, and the recent updates to the treatment regimens improved the median overall survival to only 12 months. The choice between the two regimens relies on the performance status of the patients, since FOLFIRINOX is associated with more adverse events. One major reason for the unfavorable prognosis is the occurrence of chemoresistance.

Gemcitabine (2'-2'-difluorodeoxycytidine, dFdC) is a nucleoside analogue that, to achieve its cytotoxic effect, has to first enter the cell through nucleoside transporters (primarily the human equilibrative nucleoside transporter h-ENT1). Subsequently, it has to be activated by three phosphorylations, generating dFdCTP [36]. The triphosphate form is recognized as nucleotide and therefore is incorporated into newly synthesized nucleic acids followed by a subsequent nucleotide, which masks dFdCTP from repair enzymes. Thus, DNA/RNA synthesis is arrested [37,38]. Moreover, gemcitabine inhibits the ribonucleotide reductase (RR)[39]. Eventually, the sum of the intracellular effects of dFdC and its metabolites impairs cell replication and leads to cell death [40,41].

Paclitaxel promotes microtubules polymerization by binding to- and stabilizing β-tubulin heterodimers. Being fundamental for an effective mitosis, interfering with microtubules dynamics causes and arrest of cell division followed by cell death [42,43]. The formulation of paclitaxel as an albumin-bound complex entered the clinical practice when its combination with gemcitabine was demonstrated to improve overall survival and progression-free survival of metastatic PDAC patients, compared to gemcitabine monotreatment, in the MPACT phase III trial (NCT00844649)[44].

Therapy resistance

The unfavourable prognosis of patients that can only be treated with systemic chemotherapy is often caused by the occurrence of chemoresistance, which causes the tumor to cease responding to the treatment. Several mechanisms of resistance have been described, mostly against gemcitabine. They can be directly linked to its pharmacology: downregulation of nucleotide transporters (h-ENTs - reducing the access to the cell) or of the key enzyme starting the sequence of phosphorylation, deoxycytidine kinase (dCK), upregulation of ribonucleotide reductase (RR) subunits RRM1 and RRM2, which support the production of new nucleotides, competing with dFdCTP, and upregulation of cytidine deaminase (CDA), de-aminating dFdC, and therefore preventing its activation. However, clinical trials focusing on these players as biomarkers produced confusing results [45-47]. Other mechanisms of resistance are related to activation of pathways protecting the PDAC cells against drug induced damage. For example, increased activation of the anti-apoptotic PI3K/AKT pathway, constitutive activation of NF-kB or the pro-proliferative MAP kinase pathway have been described [41].

In addition to changes in the PDAC cells themselves, the desmoplastic TME can also drive resistance (Figure 2). For example, ECM components can stimulate protective signals contributing to resistance. CD44 activation by hyaluronan can lead to PI3K/AKT pathway activation, preventing apoptosis [48]. Another study showed how fibronectin (FN) secreted by PSCs can promote chemoresistance in PDAC cell lines, by increasing the phosphorylation of ERK1/2. In this study, no activation of AKT was detected and gemcitabine uptake was not affected. Moreover, PDAC cells grown on FN-coated surfaces displayed higher resistance to gemcitabine compared to cells grown on non-coated surfaces [49]. Some forms of collagen have also been implicated in activation of tumor promoting pathways, such as upregulation of Mcl-1 caused

by collagen type I, which protected PDAC cells from apoptosis when treated with 5-FU [50]. The same integrin is potentially responsible for the membrane type-1 matrix metalloproteinase (MMT-MMP1) increase observed when cells were grown in three-dimensional collagen as compared to plastic substrate [51-53]. In turn, MMT-MMP1 mediated an increased phosphorylation of ERK, increased expression of the chromatin remodeling protein high mobility group A2 (HMGA2), and of histone acetyltransferases, causing resistance to gemcitabine in PDAC cell lines exposed to collagen [51,54].

Lastly, mechanical changes in the TME also contribute to resistance. The desmoplastic reaction around PDAC cells causes PDAC tissues to be stiffer as compared to healthy pancreas [55-57].

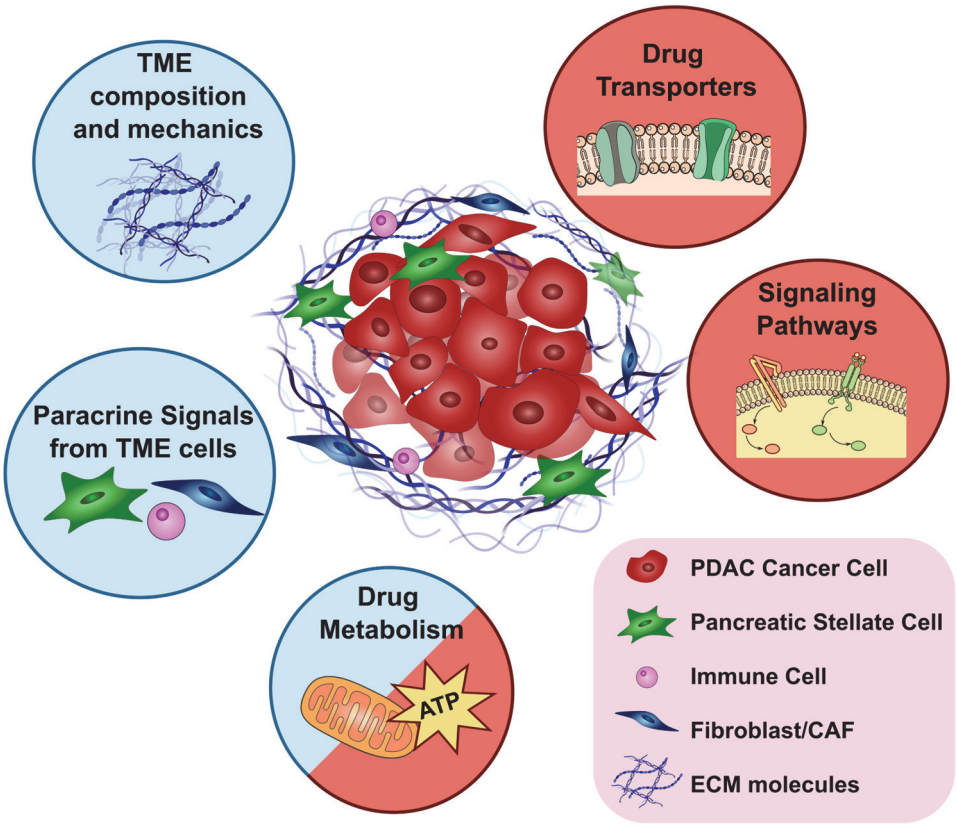

Figure 2. Influence cell intrinsic and cell extrinsic factors on PDAC chemoresistance. Chemoresistance in PDAC is multifaceted, and cell intrinsic (red) as well as extrinsic (blue)

factors from the tumor microenvironment (TME) concur in diminishing treatment efficacy. Tumor cells (red) can undergo changes at multiple levels in the signalling pathways to promote resistance. Moreover, altered expression of drug transporters on the cell surface has been described as possible mechanism of drug resistance. Tumor cells are also surrounded by a dense and dysregulated stroma, consisting of extracellular-matrix (ECM) proteins, fibroblasts, pancreatic stellate cells and immune cells. In addition to the production and remodeling of the ECM, these cells can affect tumor cells drug sensitivity through paracrine signals. Tumor cell metabolism can also be reprogrammed, often in response to altered conditions in the TME.

The term stiffness refers to the elasticity of the tissue, or the resistance of a material to load, and increased stiffness means that the elasticity is reduced. Such stiffness has been shown to contribute to chemoresistance to paclitaxel in PDAC cell lines [58]. The changes in the composition of TME combined with the abnormal growth of the tumour mass in the confined space of the host organ, generates solid stress [59]. In turn, this stress can compress the tumour vasculature and increase the interstitial fluid pressure, generating shear stress and reducing the ability of drugs to reach the tumour [60,61].

Aim and scope of the thesis

In this thesis I aimed to develop multiple *in-vitro* models to study molecular mechanisms of acquired chemoresistance in PDAC cell lines and study the interplay of resistant cells with the mechanical properties of the tumor microenvironment, with the ultimate goal to find alternative therapeutic strategies to overcome this therapeutic challenge. In **chapter 2** we describe the generation of resistant PDAC cell lines and we identify ABCB1 upregulation as a common mechanism of paclitaxel resistance. As the relevance of ABC-transporters as targets for alternative therapies is currently a controversial topic, we discuss in **chapter 3** the evidence in favor or against this idea. In **chapter 4**, we use a similar approach as in chapter 2 to investigate mechanisms driving gemcitabine resistance. Here, we identify reduced dCK activity as a common mechanism, which emerges through different strategies in different cell models. Having investigated the drug-specific mechanisms of chemoresistance, we looked for alternative ways to tackle it, by focusing on the interaction between tumor cells and their microenvironment. **Chapter 5** provides an overview of novel pre-clinical findings and discusses potential opportunities for integrin targeting agents as cancer therapies. In **chapter 6**, we highlight the role of integrin α2β1 as a mechano-sensor and a prognostic factor for gemcitabine resistance in PDAC. **Chapter 7** is a review discussing the plasticity of tumor cells and the effect of

mechanical properties of the tumor microenvironment, and potential therapeutic opportunities to prevent metastasis. This plasticity is found in **chapter 8**, where we observe altered mechanical properties of multiple chemoresistance PDAC cells. Finally, **chapter 9** provides a general discussion and future perspective on the research presented in this thesis. In summary, this thesis describes novel and conserved mechanisms of resistance to commonly use therapeutic agents in pancreatic cancer and highlights new aspects of the role of mechanical cues in the context of chemoresistance.

References:

1. Collaboration, G.B.o.D.C. (2022). Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life Years for 29 Cancer Groups From 2010 to 2019: A Systematic Analysis for the Global Burden of Disease Study 2019. JAMA Oncol 8, 420-444. 10.1001/jamaoncol.2021.6987.
2. Bray, F., Laversanne, M., Sung, H., Ferlay, J., Siegel, R.L., Soerjomataram, I., and Jemal, A. (2024). Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians. 10.3322/caac.21834.
3. Siegel, R.L., Giaquinto, A.N., and Jemal, A. (2024). Cancer statistics, 2024. CA: A Cancer Journal for Clinicians 74. 10.3322/caac.21820.
4. Halbrook, C.J., Lyssiotis, C.A., Pasca di Magliano, M., and Maitra, A. (2023). Pancreatic cancer: Advances and challenges. Cell 18c, 1729-1754. 10.1016/j.cell.2023.02.014.
5. Bardeesy, N., and DePinho, R.A. (2002). Pancreatic cancer biology and genetics. Nature Reviews Cancer 2, 897-909. 10.1038/nrc949.
6. Wood, L.D., Canto, M.I., Jaffee, E.M., and Simeone, D.M. (2022). Pancreatic Cancer: Pathogenesis, Screening, Diagnosis, and Treatment. Gastroenterology 1c3. 10.1053/j.gastro.2022.03.056.
7. Grimont, A., Leach, S.D., and Chandwani, R. (2022). Uncertain Beginnings: Acinar and Ductal Cell Plasticity in the Development of Pancreatic Cancer. Cellular and Molecular Gastroenterology and Hepatology 13, 369-382. https://doi.org/10.1016/j.jcmgh.2021.07.014.
8. Park, W., Chawla, A., and O'Reilly, E.M. (2021). Pancreatic Cancer: A Review. JAMA 32c, 851-862. 10.1001/jama.2021.13027.
9. Mercanti, L., Sindaco, M., Mazzone, M., Di Marcantonio, M.C., Piscione, M., Muraro, R., and Mincione, G. (2023). PDAC, the Influencer Cancer: Cross-Talk with Tumor Microenvironment and Connected Potential Therapy Strategies. Cancers 15. 10.3390/cancers15112923.
10. Liu, X., Iovanna, J., and Santofimia-Castaño, P. (2023). Stroma-targeting strategies in pancreatic cancer: a double-edged sword. Journal of Physiology and Biochemistry 7S, 213-222 10.1007/s13105-022-00941-1.
11. Östman, A., and Augsten, M. (2009). Cancer-associated fibroblasts and tumor growth – bystanders turning into key players. Current Opinion in Genetics C Development 1S, 67-73. https://doi.org/10.1016/j.gde.2009.01.003.
12. MacCurtain, B.M., Quirke, N.P., Thorpe, S.D., and Gallagher, T.K. (2021). Pancreatic Ductal Adenocarcinoma: Relating Biomechanics and Prognosis. Journal of Clinical Medicine 10. 10.3390/jcm10122711.
13. Madden, J.I. (2012). Infinity reports update from phase 2 study of saridegib plus gemcitabine in patients with metastatic pancreatic cancer.

https://www.sec.gov/Achives/edgr/data/1113148/000119312512026049/d290658dex991.htm.

14. Özdemir, B.C., Pentcheva-Hoang, T., Carstens, J.L., Zheng, X., Wu, C.-C., Simpson, T.R., Laklai, H., Sugimoto, H., Kahlert, C., Novitskiy, S.V., et al. (2014). Depletion of carcinoma- associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival. Cancer Cell 25, 719-734.10.1016/j.ccr.2014.04.005.
15. Van Cutsem, E., Tempero, M.A., Sigal, D., Oh, D.-Y., Fazio, N., Macarulla, T., Hitre, E., Hammel, P., Hendifar, A.E., Bates, S.E., et al. (2020). Randomized Phase III Trial of Pegvorhyaluronidase Alfa With Nab-Paclitaxel Plus Gemcitabine for Patients With Hyaluronan-High Metastatic Pancreatic Adenocarcinoma. Journal of Clinical Oncology 38, 3185-3194. 10.1200/JCO.20.00590.
16. Hingorani, S.R., Zheng, L., Bullock, A.J., Seery, T.E., Harris, W.P., Sigal, D.S., Braiteh, F., Ritch, P.S., Zalupski, M.M., Bahary, N., et al. (2017). HALO 202: Randomized Phase II Study of PEGPH20 Plus Nab-Paclitaxel/Gemcitabine Versus Nab-Paclitaxel/Gemcitabine in Patients With Untreated, Metastatic Pancreatic Ductal Adenocarcinoma. Journal of Clinical Oncology 3c, 359-366. 10.1200/JCO.2017.74.9564.
17. Rhim, Andrew D., Oberstein, Paul E., Thomas, Dafydd H., Mirek, Emily T., Palermo, Carmine F., Sastra, Stephen A., Dekleva, Erin N., Saunders, T., Becerra, Claudia P., Tattersall, Ian W., et al. (2014). Stromal Elements Act to Restrain, Rather Than Support, Pancreatic Ductal Adenocarcinoma. Cancer Cell 25, 735-747.https://doi.org/10.1016/j.ccr.2014.04.021.
18. Ramanathan, R.K., McDonough, S.L., Philip, P.A., Hingorani, S.R., Lacy, J., Kortmansky, J.S., Thumar, J., Chiorean, E.G., Shields, A.F., Behl, D., et al. (2019). Phase IB/II Randomized Study of FOLFIRINOX Plus Pegylated Recombinant Human Hyaluronidase Versus FOLFIRINOX Alone in Patients With Metastatic Pancreatic Adenocarcinoma: SWOG S1313. Journal of Clinical Oncology 37, 1062-1069. 10.1200/JCO.18.01295.
19. Ashina, S., Masuda, A., Yamakawa, K., Hamada, T., Tsujimae, M., Tanaka, T., Toyama, H., Sofue, K., Shiomi, H., Sakai, A., et al. (2023). A comprehensive analysis of tumor-stromal collagen in relation to pathological, molecular, and immune characteristics and patient survival in pancreatic ductal adenocarcinoma. Journal of Gastroenterology 58, 1055- 1067. 10.1007/s00535-023-02020-8.
20. Vennin, C., Chin, V.T., Warren, S.C., Lucas, M.C., Herrmann, D., Magenau, A., Melenec, P., Walters, S.N., del Monte-Nieto, G., Conway, J.R.W., et al. (2017). Transient tissue priming via ROCK inhibition uncouples pancreatic cancer progression, sensitivity to chemotherapy, and metastasis. Science Translational Medicine S, eaai8504. 10.1126/scitranslmed.aai8504.

21. Murphy, K.J., Reed, D.A., Vennin, C., Conway, J.R.W., Nobis, M., Yin, J.X., Chambers, C.R., Pereira, B.A., Lee, V., Filipe, E.C., et al. (2021). Intravital imaging technology guides FAK- mediated priming in pancreatic cancer precision medicine according to Merlin status. Science Advances 7, eabh0363. 10.1126/sciadv.abh0363.
22. Szczepanski, J.M., Rudolf, M.A., and Shi, J. (2024). Clinical Evaluation of the Pancreatic Cancer Microenvironment: Opportunities and Challenges. Cancers 1c. 10.3390/cancers16040794.
23. Evans, D.B. (2018). What Makes a Pancreatic Cancer Resectable? American Society of Clinical Oncology Educational Book, 300-305. 10.1200/EDBK_200861.
24. Oba, A., Ho, F., Bao, Q.R., Al-Musawi, M.H., Schulick, R.D., and Del Chiaro, M. (2020). Neoadjuvant Treatment in Pancreatic Cancer. Frontiers in Oncology Volume 10 - 2020.
25. Springfeld, C., Ferrone, C.R., Katz, M.H.G., Philip, P.A., Hong, T.S., Hackert, T., Büchler, M.W., and Neoptolemos, J. (2023). Neoadjuvant therapy for pancreatic cancer. Nature Reviews Clinical Oncology 20, 318-337 10.1038/s41571-023-00746-1.
26. Pishvaian, M.J., Blais, E.M., Brody, J.R., Lyons, E., DeArbeloa, P., Hendifar, A., Mikhail, S., Chung, V., Sahai, V., Sohal, D.P.S., et al. (2020). Overall survival in patients with pancreatic cancer receiving matched therapies following molecular profiling: a retrospective analysis of the Know Your Tumor registry trial. The Lancet Oncology 21, 508-518 10.1016/S1470-2045(20)30074-7.
27. Hallin, J., Bowcut, V., Calinisan, A., Briere, D.M., Hargis, L., Engstrom, L.D., Laguer, J., Medwid, J., Vanderpool, D., Lifset, E., et al. (2022). Anti-tumor efficacy of a potent and selective non-covalent KRASG12D inhibitor. Nature Medicine 28, 2171-2182.10.1038/s41591-022-02007-7.
28. Nagashima, T., Inamura, K., Nishizono, Y., Suzuki, A., Tanaka, H., Yoshinari, T., and Yamanaka, Y. (2022). ASP3082, a First-in-class novel KRAS G12D degrader, exhibits its remarkable anti-tumor activity in KRAS G12D mutated cancer models. European Journal of Cancer 174, S30. 10.1016/S0959-8049(22)00881-4.
29. Chen, Y., Liu, Q.-p., Xie, H., and Ding, J. (2024). From bench to bedside: current development and emerging trend of KRAS-targeted therapy. Acta Pharmacologica Sinica 45, 686-703. 10.1038/s41401-023-01194-4.
30. Lencioni, G., Gregori, A., Toledo, B., Rebelo, R., Immordino, B., Amrutkar, M., Xavier, C.P.R., Kocijančič, A., Pandey, D.P., Perán, M., et al. (2024). Unravelling the complexities of resistance mechanism in pancreatic cancer: Insights from in vitro and ex-vivo model systems. Seminars in Cancer Biology 10c-107, 217-233. https://doi.org/10.1016/j.semcancer.2024.09.002.

31. Smith, C., Zheng, W., Dong, J., Wang, Y., Lai, J., Liu, X., and Yin, F. (2022). Tumor microenvironment in pancreatic ductal adenocarcinoma: Implications in immunotherapy. World J Gastroenterol 28, 3297-3313. 10.3748/wjg.v28.i27.3297.
32. Twomey, J.D., and Zhang, B. (2021). Cancer Immunotherapy Update: FDA-Approved Checkpoint Inhibitors and Companion Diagnostics. The AAPS Journal 23, 39.10.1208/s12248-021-00574-0.
33. Park, W., Connor, C.O., Chou, J., Schwartz, C., Larsen, M., Varghese, A., Yu, K., Balogun, F., Yang, J., Katz, S., et al. (2024). 1504MO Phase II trial of Pembrolizumab and OLApaRib (POLAR) maintenance for select patients (pts) with metastatic pancreatic cancer (mPC) with (A) homologous recombination deficiency (HRD), (B) non-core HRD (ncHRD) and (C) exceptional response to platinum. Annals of Oncology 35, S922. 10.1016/j.annonc.2024.08.1567.
34. Wu, A.A., Bever, K.M., Ho, W.J., Fertig, E.J., Niu, N., Zheng, L., Parkinson, R.M., Durham, J.N., Onners, B., Ferguson, A.K., et al. (2020). A Phase II Study of Allogeneic GM-CSF– Transfected Pancreatic Tumor Vaccine (GVAX) with Ipilimumab as Maintenance Treatment for Metastatic Pancreatic Cancer. Clinical Cancer Research 2c, 5129-5139. 10.1158/1078-0432.CCR-20-1025.
35. Beutel, A.K., and Halbrook, C.J. (2022). Barriers and opportunities for gemcitabine in pancreatic cancer therapy. American Journal of Physiology-Cell Physiology 324, C540- C552. 10.1152/ajpcell.00331.2022.
36. Elnaggar, M., Giovannetti, E., and Peters, G., J. (2012). Molecular Targets of Gemcitabine Action: Rationale for Development of Novel Drugs and Drug Combinations. Current Pharmaceutical Design 18, 2811-2829. http://dx.doi.org/10.2174/138161212800626175.
37. Ruiz van Haperen, V.W.T., Veerman, G., Vermorken, J.B., and Peters, G.J. (1993). 2′,2′- Difluoro-deoxycytidine (gemcitabine) incorporation into RNA and DNA of tumour cell lines. Biochemical Pharmacology 4c, 762-766. https://doi.org/10.1016/0006- 2952(93)90566-F.
38. Bergman, A.M., Pinedo, H.M., and Peters, G.J. (2002). Determinants of resistance to 2′,2′- difluorodeoxycytidine (gemcitabine). Drug Resistance Updates 5. 10.1016/S1368- 7646(02)00002-X.
39. Baker, C.H., Banzon, J., Bollinger, J.M., Stubbe, J., Samano, V., Robins, M.J., Lippert, B., Jarvi, E., and Resvick, R. (1991). 2'-Deoxy-2'-methylenecytidine and 2'-deoxy-2',2'- difluorocytidine 5'-diphosphates: potent mechanism-based inhibitors of ribonucleotide reductase. Journal of Medicinal Chemistry 34, 1879-1884. 10.1021/jm00110a019.
40. de Sousa Cavalcante, L., and Monteiro, G. (2014). Gemcitabine: Metabolism and molecular mechanisms of action, sensitivity and chemoresistance in pancreatic cancer. European Journal of Pharmacology 741, 8-16. https://doi.org/10.1016/j.ejphar.2014.07.041.

41. Koltai, T., Reshkin, S.J., Carvalho, T.M.A., Di Molfetta, D., Greco, M.R., Alfarouk, K.O., and Cardone, R.A. (2022). Resistance to Gemcitabine in Pancreatic Ductal Adenocarcinoma: A Physiopathologic and Pharmacologic Review. Cancers 14, 2486. 10.3390/cancers14102486.
42. Jordan, M.A., and Wilson, L. (2004). Microtubules as a target for anticancer drugs. Nature Reviews Cancer 4, 253-265. 10.1038/nrc1317.
43. Schiff, P.B., and Horwitz, S.B. (1980). Taxol stabilizes microtubules in mouse fibroblast cells. Proceedings of the National Academy of Sciences 77, 1561-1565. 10.1073/pnas.77.3.1561.
44. Von Hoff, D.D., Ervin, T., Arena Francis, P., Chiorean, E.G., Infante, J., Moore, M., Seay, T., Tjulandin Sergei, A., Ma Wen, W., Saleh Mansoor, N., et al. (2013). Increased Survival in Pancreatic Cancer with nab-Paclitaxel plus Gemcitabine. New England Journal of Medicine 3cS, 1691-1703. 10.1056/NEJMoa1304369.
45. Randazzo, O., Papini, F., Mantini, G., Gregori, A., Parrino, B., Liu, D.S.K., Cascioferro, S., Carbone, D., Peters, G.J., Frampton, A.E., et al. (2020). "Open Sesame?": Biomarker Status of the Human Equilibrative Nucleoside Transporter-1 and Molecular Mechanisms Influencing its Expression and Activity in the Uptake and Cytotoxicity of Gemcitabine in Pancreatic Cancer. Cancers 12. 10.3390/cancers12113206.
46. Wei, C.H., Gorgan, T.R., Elashoff, D.A., Hines, O.J., Farrell, J.J., and Donahue, T.R. (2013). A Meta-Analysis of Gemcitabine Biomarkers in Patients With Pancreaticobiliary Cancers. Pancreas 42.
47. Danesi, R., Giuseppe, A., Elisa, G., and and Rosell, R. (2009). Pharmacogenomics of Gemcitabine in Non-Small-Cell Lung Cancer and Other Solid Tumors. Pharmacogenomics 10, 69-80. 10.2217/14622416.10.1.69.
48. Fujita, Y., Kitagawa, M., Nakamura, S., Azuma, K., Ishii, G., Higashi, M., Kishi, H., Hiwasa, T., Koda, K., Nakajima, N., and Harigaya, K. (2002). CD44 signaling through focal adhesion kinase and its anti-apoptotic effect. FEBS Letters 528, 101-108. https://doi.org/10.1016/S0014-5793(02)03262-3.
49. Amrutkar, M., Aasrum, M., Verbeke, C.S., and Gladhaug, I.P. (2019). Secretion of fibronectin by human pancreatic stellate cells promotes chemoresistance to gemcitabine in pancreatic cancer cells. BMC Cancer 1S, 1-16. 10.1186/s12885-019-5803-1.
50. Armstrong, T., Packham, G., Murphy, L.B., Bateman, A.C., Conti, J.A., Fine, D.R., Johnson, C.D., Benyon, R.C., and Iredale, J.P. (2004). Type I Collagen Promotes the Malignant Phenotype of Pancreatic Ductal Adenocarcinoma. Clinical Cancer Research 10, 7427- 7437. 10.1158/1078-0432.CCR-03-0825.
51. Dangi-Garimella, S., Krantz, S.B., Barron, M.R., Shields, M.A., Heiferman, M.J., Grippo, P.J., Bentrem, D.J., and Munshi, H.G. (2011). Three-Dimensional Collagen I Promotes Gemcitabine Resistance in Pancreatic Cancer through MT1-MMP–

Mediated Expression of HMGA2. Cancer Research 71, 1019-1028. 10.1158/0008-5472.can-10-1855.

52. Grafinger, O.R., Gorshtein, G., Stirling, T., Geddes-McAlister, J., and Coppolino, M.G. (2021). Inhibition of β1 integrin induces its association with MT1-MMP and decreases MT1- MMP internalization and cellular invasiveness. Cellular Signalling 83, 109984. https://doi.org/10.1016/j.cellsig.2021.109984.
53. Zigrino, P., Drescher, C., and Mauch, C. (2001). Collagen-induced proMMP-2 activation by MT1-MMP in human dermal fibroblasts and the possible role of α2β1 integrins. European Journal of Cell Biology 80, 68-77. https://doi.org/10.1078/0171-9335-00134.
54. Dangi-Garimella, S., Sahai, V., Ebine, K., Kumar, K., and Munshi, H.G. (2013). Three- Dimensional Collagen I Promotes Gemcitabine Resistance In Vitro in Pancreatic Cancer Cells through HMGA2-Dependent Histone Acetyltransferase Expression. PLoS ONE 8, e64566. 10.1371/journal.pone.0064566.
55. Nabavizadeh, A., Payen, T., Iuga, A.C., Sagalovskiy, I.R., Desrouilleres, D., Saharkhiz, N., Palermo, C.F., Sastra, S.A., Oberstein, P.E., Rosario, V., et al. (2020). Noninvasive Young's modulus visualization of fibrosis progression and delineation of pancreatic ductal adenocarcinoma (PDAC) tumors using Harmonic Motion Elastography (HME) in vivo. Theranostics 10, 4614-4626. 10.7150/thno.37965.
56. Rubiano, A., Delitto, D., Han, S., Gerber, M., Galitz, C., Trevino, J., Thomas, R.M., Hughes, S.J., and Simmons, C.S. (2018). Viscoelastic properties of human pancreatic tumors and in vitro constructs to mimic mechanical properties. Acta Biomaterialia c7, 331-340. https://doi.org/10.1016/j.actbio.2017.11.037.
57. Kim, S.Y., Cho, J.H., Kim, Y.J., Kim, E.J., Park, J.Y., Jeon, T.J., and Kim, Y.S. (2017). Diagnostic efficacy of quantitative endoscopic ultrasound elastography for differentiating pancreatic disease. Journal of Gastroenterology and Hepatology 32, 1115-1122. https://doi.org/10.1111/jgh.13649.
58. Rice, A.J., Cortes, E., Lachowski, D., Cheung, B.C.H., Karim, S.A., Morton, J.P., and Del Río Hernández, A. (2017). Matrix stiffness induces epithelial-mesenchymal transition and promotes chemoresistance in pancreatic cancer cells. Oncogenesis c, e352. 10.1038/oncsis.2017.54.
59. Kalli, M., and Stylianopoulos, T. (2018). Defining the Role of Solid Stress and Matrix Stiffness in Cancer Cell Proliferation and Metastasis. Frontiers in Oncology 8, 55. 10.3389/fonc.2018.00055.
60. Carvalho, T.M.A., Di Molfetta, D., Greco, M.R., Koltai, T., Alfarouk, K.O., Reshkin, S.J., and Cardone, R.A. (2021). Tumor Microenvironment Features and Chemoresistance in Pancreatic Ductal Adenocarcinoma: Insights into Targeting Physicochemical Barriers and Metabolism as Therapeutic Approaches. Cancers 13. 10.3390/cancers13236135.

61. Olive, K.P., Jacobetz, M.A., Davidson, C.J., Gopinathan, A., McIntyre, D., Honess, D., Madhu, B., Goldgraben, M.A., Caldwell, M.E., Allard, D., et al. (2009). Inhibition of Hedgehog Signaling Enhances Delivery of Chemotherapy in a Mouse Model of Pancreatic Cancer. Science 324, 1457-1461. 10.1126/science.1171362.
62. Morris, J.P., Wang, S.C., and Hebrok, M. (2010). KRAS, Hedgehog, Wnt and the twisted developmental biology of pancreatic ductal adenocarcinoma. Nature Reviews Cancer 10, 683-695. 10.1038/nrc2899.