ELSEVIER

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Associate editor: B. Teicher

Pancreatic cancer models for translational research

Diana Behrens^{a,*}, Wolfgang Walther^{b,c}, Iduna Fichtner^a

^a EPO – Experimental Pharmacology and Oncology GmbH – GmbH, Robert-Roessle-Str. 10, 13125 Berlin, Germany

^b Experimental and Clinical Research Center (ECRC), Charité, University Medicine, Berlin

^c Max-Delbrueck-Center for Molecular Medicine, Robert-Roessle-Str. 10, 13125 Berlin, Germany

A R T I C L E I N F O

ABSTRACT

Available online 4 February 2017

Keywords: Translational research Preclinical oncology Mouse models Patient-derived xenografts Pancreatic cancer Pancreatic cancer is a cruel, progressive disease that is highly metastatic and barely treatable, a situation that is devastating for patients, family members, oncologists, clinicians and scientists. Open questions that need to be resolved by research into pancreatic cancer relate to its aggressiveness, the underlying molecular causes, the factors that promote tumor progression, the ways cancer cells interact with their environments, and whether more effective therapeutic options can be developed.

Studies over the last 15 years have provided some partial answers, but in the absence of a real cure the main agenda remains: to identify new therapeutic targets, predictive markers and novel treatment strategies that would help the disease under control. These goals can be advanced by translational research based on clinically relevant and standardized protocols and more reliable disease models. This review gives an overview of the preclinical in vitro and in vivo models for pancreatic cancer that are currently available. The restrictions on applicability, strengths and limitations of various experimental platforms including 3D organoids, syngeneic xenografts and genetically engineered mice are considered with respect to the complexity of pancreating cancer. Patient-derived xenografts (PDX) presently offer the most promise for translational research, so a particular emphasis is placed on key features as preclinical models for pancreatic cancer and their advancement toward precise simulations of clinical problems.

© 2017 Elsevier Inc. All rights reserved.

Contents

| 1. | Introduction | 146 |
|------|--|-----|
| 2. | Translational research (TR) – meaning and claims | 147 |
| 3. | Preclinical in vitro models of pancreatic cancer | 147 |
| 4. | In vivo preclinical models of pancreatic cancer | 148 |
| 5. | Future perspectives for PDX models | 152 |
| 6. | Conclusions | 155 |
| Conf | lict of interest | 155 |
| Ackn | owledgment | 155 |
| Refe | rences | 155 |

1. Introduction

Pancreatic cancer (PC) continues to have one of the worst prognoses for patients due to the late onset of symptoms and the advanced stage that the disease usually reaches before diagnosis. These factors as well as specific anatomical features mean that 80% of patients who cannot be treated by surgery. They often suffer from metastases in the liver and lung, yielding an overall survival rate of less than 20% one year after diagnosis. Pancreatic cancer is the fifth most common cause of





Abbreviations: 2D, two-dimensional; 3D, three-dimensional; CA, cancer; GEMM, genetically engineered mouse model; PC, pancreatic cancer; PDAC, pancreatic ductal adenocarcinoma; PDX, patient-derived xenograft; TR, translational research.

^{*} Corresponding author at: EPO – Experimental Pharmacology and Oncology – GmbH, Robert-Roessle-Str. 10, 13125 Berlin, Germany.

E-mail address: Diana.Behrens@epo-berlin.com (D. Behrens).

cancer deaths in Europe overall, with more than 104,000 victims in 2012 (6% of all cancer related deaths) (Ferlay et al., 2013). In the United States the situation is even worse: pancreatic cancer is the 4th leading cause of cancer-related deaths, with 53,070 new cases and 41,780 fatalities estimated for 2016 (Siegel, Miller, & Jemal, 2016). The development of pancreatic tumors is promoted by a combination of genetic familiar history, environmental and lifestyle factors, and additional causes remain to be identified (Barone, Corrado, Gemignani, & Landi, 2016). 95% of all cases of PC are diagnosed as ductal adenocarcinomas (PDAC) of exocrine origin which exhibit different stages of differentiation. Nearly all tumors harbor a mutation in the Kras oncogene, frequently accompanied by subsequent genetic alterations in the p53, SMAD4 and CDKN2A genes. Pancreatic tumors are characterized by genetic instability, intratumoral heterogeneity and distinct desmoplastic stroma (Hidalgo et al., 2015).

Despite the knowledge that has been gained regarding the biology and mechanisms that underlie PDAC tumorigenicity over the last 15 years, there remains a need for better diagnostic and prognostic markers and particularly improved therapeutic strategies (Falasca, Kim, & Casari, 2016). Only moderate progress has been made in the latter are, in contrast to other solid malignancies, despite numerous preclinical investigations and clinical trials. In 1997, gemcitabine emerged as an alternative to 5-Fluorouracil as a first-line therapy that was applied in some cases but ultimately improved overall survival by only a few weeks. The next clinical milestone was the introduction of the FOLFIRINOX treatment scheme (5-fluoruracil, leucovorin, oxaliplatin and irinotecan) for patients with an advanced stage of the disease, which contributed to a small improvement in survival on the one hand but strong side effects on the other. Nab-paclitaxel (Abraxane) was approved for standard of care in 2013 and, in combination with gemcitabine, remains the most effective and tolerated drug. The poor clinical situation and remaining challenges can be summarized by noting that only three improvements have been introduced over the last 20 years, leaving the 5-year overall survival rate below 8%.

Recently the American Society of Clinical Oncology (ASCO) published guidelines for the handling of potentially curable (respectable), locally advanced (non-respectable) and metastatic PDAC (2016; www.asco.org/guidelineswiki). After considering relevant literature from the years 2004 to 2015, their recommendations for therapeutic interventions included FOLFORINOX, radiation and/or gemcitabine in combination with nab-paclitaxel. In addition to these measures and the approval of second line liposomal irinotecan, there is still a desperate need for novel drugs, improved radiation protocols and more avenues for second- and third-line therapies (Strobel & Büchler, 2016), with a push to enroll more patients in clinical trials. A search of the "Pubmed" database for pancreatic cancer yields more than 82,000 entries, which documents the engagement of scientists all over the world in efforts to understand the disease and find new approaches. Currently, this mission is making use of preclinical tools that are multifaceted, specific and tailored to particular, research-orientated applications. This article considers a range of in vitro and in vivo models of pancreatic cancer that are being implemented in current preclinical research and have been designed for translational purposes. Here we provide an overview of platforms for basic and advanced experimental research, with a focus on those devoted to the biology of pancreatic cancer and improving therapeutic options for patients.

2. Translational research (TR) - meaning and claims

TR aims to derive discoveries from basic and advanced laboratory research and apply them to studies of human beings. The NIH divides these efforts into two stages, from basic to clinical research (T1) and from clinical research to applicable practice settings for patients (T2), with the goals of improving public health and reducing cancer incidence, morbidity and mortality (Rubio et al., 2010).

Translational research in oncology combines the perspectives of scientists working in basic research, drug developers in pharmaceutical companies and oncologists in clinics - all of whom share the aim of matching the right patient (or model) to the right drug. For both the later stages of cancer drug development and the management of cancer patients, an essential step is to identify biomarkers that accurately distinguish populations of patients in terms of those likely to be responders vs. non-responders for particular therapeutic intervention strategies (Kelloff & Sigman, 2012). Ideally, such bio-indicators will predict not only the efficacy of a drug, but also give an indication of the stages of the disease's progression. In a recent discussion of the need for predictive and therapeutic biomarkers in handling pancreatic cancer (Karandish & Mallik, 2016; Le et al., 2016), the authors recommended an intensification of efforts to choose model and patient cohorts and to translate preclinical data into the clinic. The major challenge is to develop patient-specific models that reflect the histologic and genetic characteristics of the donor tumor that can be used to provide a platform for the validation of experimental targets, the testing of drugs on individuals, and predictions of their responses.

While this translational concept is crucial to progress, there are numerous roadblocks. Kroetz (2016) outlines deficiencies in the reproducibility, accuracy and transparency of experiments and emphasizes a general need for more training and better access to detailed methods and data. The biology of the disease itself imposes barriers, as do technical limitations. Clinical translations of prognostic, predictive and drugable biomarkers for pancreatic cancer have often failed due to inconsistencies in patient cohorts, study design and experimental protocols during early clinical phases (Kruger et al., 2014). As a result, DNA repair pathways, c-Myc or Kras have yet to join the list of targets in the spectrum of therapeutic approaches to pancreatic cancer (Hessmann, Schneider, Ellenrieder, & Siveke, 2016; Lin et al., 2013; Maginn, de Sousa, Wasan, & Stronach, 2014). Boeck et al. (2014) recommend prospective (instead of retrospective) biomarker evaluations, well defined patient populations and treatment regimes, and a higher standardization of methods for post-experimental analyses. Aside from the impact of study design, the special complexity and therapeutic challenge of pancreatic cancer complicate translational issues.

Just as important as the development of high standards for biomarkers and treatments are their implementation in preclinical research, too. In addition to standardizing protocols and methods, the choice of the right model is of essential importance.

3. Preclinical in vitro models of pancreatic cancer

3.1. Two-D (2D) cell cultures

Cell lines of various cancer entities have been widely used in basic research into cancer biology and in proof-of-concept studies for many years. Monolayer cultures are cost-effective and useful for highthroughput drug screening, but they do not reflect the holistic complexity of cancer. The in vitro propagation of pancreatic cancer cells was first described in the early sixties (Dobrynin, 1963). The intervening years have seen the establishment of more than 20 human PDAC cell lines for use in preclinical investigations in laboratories around the world. Deer et al. (2010) summarize the most prominent in vitro models and their differences in terms of origins, phenotypes, genotypes and tumorigenic characteristics. At the present time, Panc-1, MiaPaCa-2, AsPC-1, BxPC-3 and a few other lines have found prominent use in functional and pathway studies (Cao et al., 2015; Chen et al., 2016; Topalovski, Hagopian, Wang, & Brekken, 2016), biomarker validation (Yamaguchi et al., 2016; Yu, Ma, Shankar, & Srivastava, 2016), target identification (Borska et al., 2016; Pan et al., 2016; Wang et al., 2016) and drug screenings (Mura et al., 2016; Rochani et al., 2016; Spadavecchia et al., 2016). Panc-1 and MiaPaCa-2 cells are still being used, for example, as platforms to study gemcitabine resistance mechanisms and to improve the efficacy of gemcitabine in combinations with innovative drugs

(Bisht et al., 2016; Cao et al., 2015; Palam, Gore, Craven, Wilson, & Korc, 2015; Pan et al., 2016). The epithelial, mesenchymal, endocrine and stemness markers of both cell lines were recently characterized and found to have features of endocrine differentiation (Gradiz, Silva, Carvalho, Botelho, & Mota-Pinto, 2016).

New cell lines of both human and murine origin are still being established and characterized, and are being primarily recommended as tools for screening novel drug candidates. (Heller et al., 2016; Zechner et al., 2015). New cell lines often bear specific genotypic or phenotypic profiles. Murine PDAC cell lines are commonly established from genetically modified mice and are predominantly used in basic research to elucidate signaling pathways or (epi)genetic events involved in tumor development, progression and the outcomes of therapy (Deer et al., 2010).

Experimental data from in vitro studies are basic in nature and often lack reproducibility. Differences in growth environments and stress responses can alter signaling pathways and also the therapeutic sensitivity of cells. More standardized protocols as well as intensified access to published data are needed to strengthen the reliability of the data generated through their use. Domcke, Sinha, Levine, Sander, and Schultz (2013) exposed differences in genomic conservation between several ovarian cancer cell lines and patient ovarian tumors in a comparative data assessment of the Cancer Cell Line Encyclopedia and the Cancer Genome Atlas. Genomic variance is also true for other cancer types and emphasizes the importance of the means by which models for preclinical investigations are selected.

A number of valuable discoveries have been made from in vitro experiments, particularly in areas such as chromatin remodeling, microRNA activity and EMT signaling. But ultimately, in vitro cultures of PDAC cells cannot reflect either the complex genetic and epigenetic abnormalities found in this type of cancer type or the influence of the microenvironment, which means that they should not be the first option when carrying out experiments aimed at a direct translation of findings into the clinic.

In vitro models of pancreatic stellate cells (PSC) should be mentioned briefly; these cells are often used for co-culture experiments with PDAC cells to help elucidate microenvironmental issues (Haqq et al., 2014). They have been used in a number of ex vitro approaches to expose various mechanisms that have an impact on tumor metabolism, growth, invasion and blood vessel density (A.n.n., 2016; Di Maggio et al., 2016; Karnevi, Rosendahl, Hilmersson, Saleem, & Andersson, 2016; Sousa et al., 2016). Three-dimensional matrices of PSC cells have been created to simulate life-like settings. Such studies have shown that additional tumor stroma components (fibroblasts, macrophages, immune cells, endothelial cells) play an important role in the disease. While a focus on the interaction of PDAC and PSC cells touches only one piece of the puzzle, it has been valuable for basic research in functionality and mechanistic studies. Karnevi et al. (2016) investigated in co-cultures of Panc-1, MiaPaCa-2 and BxPC-3 with immortalized human stellate cells derived from patient PDAC tissue. These co-cultures down-regulated E-cadherin levels and an increased expression of vimentin in tumor cells, revealing that pancreatic stellate cells modulate EMT.

3.2. Three-D (3D) cell culture (organoids)

For several years, the generation and maintenance of spheres of normal and malignant cells in a semi-solid environment have offered several advantages over classical monolayers (Edmondson, Broglie, Adcock, & Yang, 2014). In current practice, scientists frequently create three-dimensional organoids by maintaining stem or progenitor cells in an advanced extra-cellular matrix that provides carefully defined additives. 3D organoids have been created using human and murine cells as models for normal (healthy) organs (Broutier et al., 2016; Hindley, Cordero-Espinoza, & Huch, 2016; Nguyen-Ngoc et al., 2015) and cancers of the colon, liver, prostate and pancreas (Bartucci et al., 2016; Boj, Hwang, Baker, Chio, et al., 2015; Skardal, Devarasetty, Rodman, Atala, & Soker, 2015; Weeber et al., 2015). All of these studies have produced organoids which preserve genetic and phenotypic features and attain a structural und functional resemblance to their in vivo counterparts, which suggests that organoids offer great potential for multifaceted preclinical investigations. 3D organoids are gaining increasing attention as a "playground" for simulations of malignant diseases in plastic dishes. The potential of 3D organoids in PDAC has been convincingly summarized by Coleman et al. (2014). The authors offer comprehensive descriptions of experiments in which stages of pancreatic organogenesis, carcinogenesis and - using multi-cellular approaches - interaction with the desmoplastic surroundings have been replicated. Further advantages include the high rate of success for generating organoids, even when low cell numbers from biopsies are used, and the relatively low cost in terms of time and effort (Boj, Hwang, Baker, Chio, et al., 2015). Boj, Hwang, Baker, Engle, et al. (2015) showed that xenograft tumors established from 3D PanIN organoids preserve neoplastic cell organization better than cells in a monolayer. Xenografts derived from 3D organoids likely bear a closer resemblance to the morphology and cellular architecture of clinical phenotypes than monolayer-derived counterparts.

Despite their capacity to mimic some malignant processes in organs, 3D organoids remain artificial models. The transfer of cells from the inside of the body onto plastic surfaces represents an act of selection that surely results in geno- and phenotypic modifications to an extent that has not yet been determined. Even if culture conditions are optimal, they fail to simulate the lifestyle and environmental factors of an individual patient. Ongoing trials are being conducted to determine the extent to which 3D organoids reflect the heterogeneity, genetic instability and strong barriers to therapy exhibited by pancreatic cancer. One deficiency is that current 3D organoids cannot be used to study metastatic and immunooncological aspects of PDAC.

From the translational point of view, 3D organoids are tools that potentially reflect the spatial architecture of tumors as adequately as patient-derived xenografts (PDX), and their applicability in personalized medicine approaches has recently been discussed (Bartucci et al., 2016; Francies & Garnett, 2015). Particularly in prostate cancer, organoids may compensate for the limited number of preclinical models: patient-derived xenografts of this type of tumor are difficult to maintain. In general it will be necessary to compare 3D organoids from patient tissues, their corresponding xenografts and PDX models from the same donor in terms of morphological, genetic and more general types of alterations. In addition to compare the three types of models to the original material, it will be necessary to observe how they respond to treatments.

4. In vivo preclinical models of pancreatic cancer

4.1. Syngeneic tumor grafts

Syngeneic mouse models, also known as allograft mouse tumor systems, were developed about 50 years ago but were relegated to the backseat of preclinical oncology as the concept of directly targeting human cancer genes or proteins gained prominence. Syngeneic tumor models have been developed and are commonly used in the study of malignancies such as colon cancer (CT-26), breast cancer (4T1), lung cancer (LewisLung), melanoma (B16F10) and leukemia (P388). The number of such robust models continues to grow; so far, their main application has been drug screening studies (Abolhassani et al., 2012; Vallespí et al., 2014).

The number of syngeneic mouse models for PDAC has been limited by both the availability of murine PDAC cell lines and the use of mouse models that have been genetically engineered to serve as models pancreatic diseases. Most of the cell lines have been derived from tumors of KC or KPC mice. The murine cell line 6606PDA, for example, was established from spontaneous tumors of C57BL/6J mice with a Kras^{G12D} transgene, whereas the common cell line Panc02 was developed by inducing PDAC based on 3-methyl-cholanthrene in C57BL/6J mice (Zechner et al., 2015). Both cell lines were transplanted ectopically and orthotopically into equivalent hosts (Jiang et al., 2014; Nikfarjam et al., 2013; Partecke et al., 2011) to generate models for use in sensitivity testing.

Liu, Li, et al. (2016) implanted Panc02 cells into C57BL/6J mice and demonstrated that Aspirin acts synergistically on gemcitabine efficacy, replicating results obtained in vitro. Gemcitabine induced the infiltration of B cells, dendritic cells and M2-polarized macrophages in the tumor tissue but reduced the influx of cytotoxic and helper T cells as well as myeloid-derived suppressor cells. The author emphasized the preventive effects of Aspirin and concluded that M2 macrophages might be involved in secondary treatment resistance. The same tumor model was used in an antitumoral vaccination study to investigate the glycoepitope C-ter-J28 +, which is known to be involved in pancreatic oncogenesis (Collignon et al., 2015). Ex vitro C-ter-J28 +-loaded mature dendritic cells from C57BL/6 hosts prevented subcutaneous Panc02 growth in 6 out of 12 mice and inhibited tumor progression in all other mice, which suggested that C-ter-28J might have potential for PDAC patients.

The Panc02 in vivo model was also used to show that a "comfortable" environment affects the outcome of in vivo studies (Wu et al., 2016). Both gemcitabine and 5-FU were more effective in mice housed in cages with enrichment. In another study, 6606PDA tumors revealed a differential intratumoral response to gemcitabine driven by locally confined equilibrative nucleoside transporter 1 (ENT1) expression (Zechner et al., 2016). Additionally, the authors detected ENT1 close to desmoplastic reaction, which they attributed to local differences in gemcitabine efficacy. These are just a few examples for the use of syngeneic PDAC models. Ongoing efforts for the generation of additional PDAC cell lines from suitable hosts will probably result in more new, reliable mouse tumor models.

The transplantation of murine tumor cells or tissues into genetically identical (syngeneic) hosts has the advantage that it does not require the immune depletion of the animals. This has promoted the revival of syngeneic models in cancer research over the last few years, particularly because of successful, promising results from immunotherapeutic approaches. So these "old", long-established animal models with a functional immune system are seen as a promising route in efforts to elucidate immunooncological interactions between hosts and tumors and to screen for novel relevant drugs. This trend could also serve as a new stimulus for the generation of novel, enhanced PDAC mouse models.

4.2. Cell line-derived xenograft models

Immunodeficient mice bearing human tumor cells have a long history; their development was a milestone in preclinical cancer research. Innumerable studies involving the screening, pharmacodynamic and -kinetic, toxicity and functionality of drugs have been performed in tumor xenografts and produced significant insights to issues related to cancer. The impact of angiogenesis and inflammatory responses as well as metastasis and tumor relapse can be elucidated using appropriate in vivo models. The procedure has the advantages of reproducibility and statistical robustness; both cells and hosts are readily available and cells can be transplanted ectopically and orthotopically depending on the origin of the tumor and the scope of the intended study. Various stages of metastasis can be investigated by inoculating mice with monolayer-derived cells using different transplantation routes (subcutaneous, intravenous, intraperitoneal, intracranial, intratibial and in organs such as the spleen, pancreas, liver or lung). In some cases, such xenograft studies have failed to validate in vitro data for corresponding cell lines. There are several reasons, predominantly due to environmental factors and selection events that arise because long-term cultivation modifies the characteristics and pathways of tumor cells (Biau et al., 2016; Johnson et al., 2001; Peterson & Houghton, 2004; Voskoglou-Nomikos, Pater, & Seymour, 2003). This suggests that xenografts derived from cell lines have a limited predictive use. The results may also be impaired by the differences in the tumor stroma in mice, and the animals used for xenotransplantations are typically immunodeficient, which means they lack immunological components normally present in the natural setting. Nevertheless, cell line-derived xenografts undoubtedly have relevance for translational research, if these factors are carefully considered, but it is still necessary to validate and optimize them.

These models are still a popular tool for drug screening and proofof-concept studies in PDAC, as well as for studies of the tumors' chemoresistance. Our own facility has tested the efficacy of novel tumor growth-inhibiting compounds, applying derivatives of doxorubicin and gemcitabine in Panc-1 and MiaPaCa-2 bearing mice (Bergman et al., 2011; Graeser et al., 2010; Kratz, Azab, Zeisig, Fichtner, & Warnecke, 2013). D'Aronzo et al. (2015) showed that BxPC-3, MiaPaCa-2 and Panc-1 xenografts are more sensitive to gemcitabine when hENT1 levels were increased by nutritive starvation. The transporter hENT1 has been discussed as a predictive marker for gemcitabine sensitivity for guite some time (Jordheim & Dumontet, 2013). Liu et al. (2014) used Panc-1 and MiaPaCa-2 tumor bearing mice to demonstrate that the chromatin modulator BRG1 is involved in gemcitabine resistance. Another enzyme, SIRT-1, could be linked to gemcitabine responses in vitro, but its validation in corresponding xenografts failed (Oon, Strell, Yeong, Östman, & Prakash, 2015). Further novel therapeutic combinations have been tested in Mia-PaCa-2 (Dey et al., 2016) and Panc-1 (Suenaga et al., 2016) xenograft models to improve the efficacy of nab-Paclitaxel. Dey et al. (2016) showed enhanced tumor growth inhibition and synergistic interactions between the BCL2 and BCL-xl inhibitor ATB-263, and nab-paclitaxel and Panc-1 xenografts were more sensitive to nab-paclitaxel when combined with S-1, an orally applied prodrug of 5-FU (Suenaga et al., 2016). This exemplary set of xenograft studies documents the fact that acute clinical problems can still be successfully handled in these "old-fashioned" and often criticized platforms.

Nevertheless, predictions of therapeutic sensitivity using cell linederived xenografts have often lacked clinical relevance (Mak, Evaniew, & Ghert, 2014). This is mainly due to miscellaneous limitations that are inherent in using experimental animals to mimic a human disease, and also due to a lack of heterogeneity in xenografts originating from monolayer cell cultures. Achieving a partial remission of tumors in mice does not necessarily imply that a similar remission will occur in humans; a tumor's adaptive survival strategies and resistance mechanisms are likely impaired in most xenograft models. And many in vivo experiments are probably terminated too early for ethical reasons, which impedes an evaluation of either drug resistance that might arise during therapy or the recurrence of a tumor after a treatment has been halted.

4.3. Genetically engineered mouse models (GEMM)

A comprehensive review of the use of GEMMs in PDAC was recently written by Mohammed, Janakiram, Pant, and Rao (2015) and Gopinathan, Morton, Jodrell, and Sansom (2015). Preclinical models with high pathological relevance have been produced by specifically modifying genes that are associated with pancreatic cancer in the mouse genome. This creates tumors which are aggressive, heterogeneous and stromal (desmoplatic) in nature, making GEMMs an authentic, bridging model to patients. Beside their similarities to humans in terms of their genetic, phenotypic and physiological characteristics, recently developed GEMMs also suffer from typical PDAC symptoms (including body weight loss and cachexia) and the spontaneous formation of distant metastases in lung and liver. This has made it technically feasible to simulate different stages of PDAC tumorigenesis. The expression of oncogenic Kras^{G12D} can be specifically activated in the pancreas using the LSL (floxed STOP transcriptional cassette) targeting

construct under Cre recombinase control and the transcription factors Pdx-1 and p48 (Hingorani et al., 2003). Named KC mice (Kras^{LSLG12D/+} and PdxCre), these animals have normal pancreatic organogenesis and develop intraepithelial neoplasia (PanIN) at several weeks of age. The conditional expression of the R172H mutation in the p53 gene in the Kras^{G12D} context (=KPC mouse; Kras^{LSLG12D/+}, p53^{R172H/+} and PdxCre) removes some of the drawbacks of KC mice (long latency and infrequent tumor development). The latency time before developing PDAC is low in KPC mice, and all mice have invasive, metastatic tumors with a lethality of about 6 months (Westphalen & Olive, 2012). In fact, most of what we know about the influence of Kras comes from studies of GEMMS.

Several other genes known to be associated with pancreatic cancer have manipulated in these mice to study their impact in tumorigenesis and progression. Aguirre et al. (2003) demonstrated that the loss of functional CDKN2A has a stimulating effect on PDAC development in KC mice; Ijichi et al. (2006) found similar results for transforming growth factor receptor β 2 (TGF β 2). Skoulidis et al. (2010) investigated the impact of BRCA2 in KC and KPC mice. The inactivation of p16 (Ink4A) and SMAD4 plays a role in pancreatic malignancy (Bardeesy et al., 2006; Kojima et al., 2007). Overall, about 40 genetically modified mouse models have been generated for the analysis of gene functions in the biology of PDAC (Westphalen & Olive, 2012); while their significance varies, the KPC mouse model currently is most pertinent for preclinical investigations. Just as in clinical studies, the enrollment of genetically modified mice into preclinical trials should be subject to precise, standard protocols and distinct inclusion criteria. The outcome of a study can be influenced by animal age, tumor stage and treatment schedules, as has been found for gemcitabine efficacy in KPC mice (Frese et al., 2012; Yip-Schneider et al., 2013). Unsurprisingly, the same situation is found in other experimental models and can be overcome by standardized experimental conditions. Rhim et al. (2014) have also shown that the sophistication of study designs using GEMM have an important impact: whether the stroma barrier of PDAC in KPC mice can be effectively targeted using gemcitabine depends on the tumor burden and mode of treatment.

An interesting novel approach to using GEMM for drug screening has been the generation of a reporter mouse model in which tumoral Kras-G12D activation can be directly observed through GFP expression (Ocal et al., 2015). An Rsg16:GFP transgene was inserted into KC mice with a CDKN2A mutation (termed KIC mice). These mice were treated for 2 weeks at different ages, which permitted researchers to precisely quantify the anti-tumoral effects of compounds under investigation by means of levels of GFP expression (Ocal et al., 2015). Another innovative concept in validating the importance of genes is the multiplexed transient CRISPR/Cas9 targeting of the pancreas in adult mice (Maresch et al., 2016). Still at an early stage of development, this technology can be used to carry out high-throughput analyses of drivers of pancreatic cancer and to identify mechanisms which lead to PDAC in humans. More "smart tools" for preclinical oncology are novel KC and KPC mice that express easily detectable luciferase in proliferating neoplastic cells of the pancreas, also useful in monitoring carcinogenesis in living animals (De Latouliere et al., 2016; Majumder et al., 2016). In contrast to xenografts, GEMMs can also be employed in pursuing questions related to immunooncology, tumorigenesis and aspects of tumor stroma (D'Alincourt Salazar et al., 2016). Majumder et al. (2016) transplanted tumor fragments of KPC into the pancreas of wild type mice as a fast and economical alternative to genuine GEMM that preserves tumor heterogeneity and desmoplastic stroma.

Yet GEMMs have drawbacks as well as advantages in terms of clinical relevance. Generating genetically modified mice is time- and laborintensive. It is expensive to monitor abdominal tumor growth, requiring highly sophisticated devices specifically created for animal imaging. Inducible and reversible conditional Kras^{G12D} constructs were developed (Collins et al., 2012), but in current practice they are still encoded by a transgene (resulting in an extra copy of Kras) and not driven from an endogenous promoter (Gopinathan et al., 2015). Tissue-specific promoters, such as Pdx1-Cre in KPC mice, are sometimes causing undesirable off-target disorders like papillomas or lymphomas (Gades et al., 2008; Gopinathan et al., 2015). Additionally, the artificial genetic incidence of pancreatic cancer development and progression in GEMM is not directly comparable to the true situation in patients. In contrast to human tumors, the activation of Kras^{G12D} and impairment of tumor suppressor p53 take place at the same time in KPC mice. Another difference is the degree of an uploidy in human tumors, which results in a great variety of gene modifications from one cell to the other within the same tumor, but does not occur in the same way in the mouse. Furthermore, the 5% of human pancreatic cancers without Kras mutations cannot be mimicked by GEMM. Alongside these technical and systematic issues, cancer in mice is not completely identical with cancer in humans. Even with a general genetic homology between the species of about 80%, gene structures and functions can vary. Finally, the metabolism of humans and mice differs. Many of these species-specific discrepancies in both the normal and the malignant pancreas have been previously described (Logsdon, Arumugam, & Ramachandran, 2015).

In summary, GEMMs are highly valuable preclinical models for the elucidation of pancreatic tumorigenesis, associated genetic alterations and for the evaluation of targeted therapies. However, species-related issues restrict their capacity to predict therapeutic responses for patients with pancreatic cancer. Ongoing experimental advances which more closely consider the genetic complexity of PDAC should provide scientific platforms of increased translational relevance.

4.4. Patient-derived xenografts (PDX)

Cell-line derived xenografts lack the cellular interactions and structural properties of their donor tissues, resulting in differences in spatial organization and intra-tumor heterogeneity as well as in discrepancies in gene expression profiles and drug response read-outs (Cree, Glaysher, & Harvey, 2010).

To better mimic attributes of human malignancies, patient-derived xenografts (PDX) of various solid tumor entities have been established and studied with increasing interest. In most cases chemotherapynaïve tumor tissue, obtained during surgery or from biopsies, is transplanted directly into a immuno-deficient mice without any in vitro propagation. The preferential transplantation route is subcutaneous, but some groups also implant the tumor orthotopically or in other locations to improve the engraftment (Cho et al., 2016). In vivo growth in the mouse usually takes one to four months with take rates ranging from 20 to 80%. The successful generation of a PDX is influenced by the nature of the tumor entity, the quality of the transplanted material (tumor cell/stroma ratio, degree of necrosis), the transplantation site and aspects of the recipient mouse strain. Often a failure of engraftment is correlated with a good prognosis for the patient; aggressive tumors grow faster in mice (Cho et al., 2016; Klinghammer et al., 2015). Established PDX can be transplanted serially into subsequent mouse cohorts, generating a renewable resource for biomarker screening and drug efficacy studies. Ovarian, lung, head-and-neck and colorectal cancer samples have demonstrated that PDX established through orthotopic or subcutaneous/ectopic transplantation resemble the genome of the donor tumor (Fichtner et al., 2008; Garralda et al., 2014; Klinghammer et al., 2015; Monsma et al., 2012). Such tumors conserve genetic and morphological homologies in vivo for several generations (Fig. 1). They overcome growth delays and metastasize (Bankert et al., 2011; DeRose et al., 2011; Justilien & Fields, 2013; Liu et al., 2010; Rozenberg, Monahan, Torrice, Bear, & Sharpless, 2010; Sicklick et al., 2014), leading to metastases in lymph nodes, lungs, liver or peritoneum (Hoffmann, Orthmann, Hoffmann, Reiner, & Fichtner, 2014; Park, Kim, McCauley, & Gallick, 2010, chap. 14).

Correlations between the sensitivity of the response of PDX models to therapies and the individual outcomes for corresponding patients have been demonstrated for ovarian, breast and colon cancers



151



Fig. 1. Conserved morphology between patient tumor and corresponding PDX (3rd generation) tissues of PDAC. Tissue sections were histologically analyzed by H/E staining.

(Fichtner et al., 2004; Weroha et al., 2014; Zhang et al., 2013). Garralda et al. (2014) used exome sequencing in a study of various types of incurable, advanced cancers to demonstrate that PDX tumors which responded to therapeutic interventions maintained genetic and phenotypic characteristics of the tumors of patients in the clinic. Unfortunately, the small number of patients and PDX models in such preclinical studies have been too small to have statistical power. To truly estimate the capacity of PDX models in predicting drug responses – and as tools for personalized medicine and translational research – such co-clinical trials will have to be considerably broadened. But there is a basic conflict between the long times required for successful engraftments and the restricted life expectancy of patients. And the generation, maintenance and application of patient-derived xenografts require funding and appropriate facilities.

A publication in Nature Medicine in 2015 demonstrated the powerful reproducibility and clinical relevance of PDX models in a large-scale setting, Gao et al. (2015) included 277 PDX models - of breast, colon, pancreatic, lung and gastric cancer as well as melanoma tumor entities - in a global drug screening study involving 62 treatment groups that comprised one mouse with one patient tumor for one drug. They identified significant correlations between specific genetic characteristics and sensitivity to therapies in patient populations rather than in individuals. This approach is indeed very close to the clinical situation, but presumably considers failures as the growth and take rate of even established PDX is heterogeneous. On the other hand, large-scale work significantly can reduce a skewing of results due to variation in tumor growth. The Biomedical Research PDX Enclyclopedia (PDXE) established by the Novartis Institute comprises 1,057 PDX models of more than 16 tumor entities with comprehensive genomic and response data (Gao et al., 2015) and constitutes an excellent resource for translational research. Within the framework of the Pediatric Preclinical Testing Program (PPTP), Murphy et al. (2016) provided evidence that single mouse approaches more precisely predict therapeutic outcomes than the median response of treatment groups with n = 8 to 10 mice. Through the use of fewer animals and a greater number of models, this study captured a heterogeneous view of childhood cancer and identified responsive tumor types. A similar approach has recently been launched in Europe as an initiative of 16 cancer centers in 10 European countries. The consortium has virtually assembled about 1500 characterized PDX models of 30 different malignancies (www.europdx.eu). Within a collaborative framework, these models are available for academic research and pharmaceutical companies with the aim of standardizing preclinical oncology and drawing better conclusions.

Patient-derived xenografts have also been developed to investigate the complexity of pancreatic cancer and the effectiveness of novel treatment strategies. Walters et al. (2013) established 15 orthotopic PDX from the tissues of pancreatic cancer patients, demonstrating their genetic and phenotypic homology as well as correlating engraftment rates and patient survival. Another cohort of 11 patient-derived orthotopic PDX models was examined histologically using a tissue microarray technique (Pérez-Torras et al., 2011). These models showed that tumor growth kinetics and protein expression patterns are preserved over serial in vivo passages. A panel of 20 PDX models with a take rate of 72% was generated by subcutaneous transplantations (Jung et al., 2016), and the tumors recapitulated the histological and molecular characteristics of those of the patients. A comprehensive analysis of the PDX tissues revealed that the content of murine cells lay between 1–12%, confirming that the human cell content was high in the xenografts. In a larger-scale study, 102 tumor tissues from patients with PDAC were transplanted subcutaneously into NOD/SCID mice, resulting in 57 propagable PDX models. This led to the conclusion that patient tumor size is the only significant predictor for PDX engraftment (Jun et al., 2016). In contrast, neither the conditions of surgery conditions, the degree of tumor differentiation nor the metastatic status correlated with successful in vivo growth.

In another study by Damhofer et al. (2015), 47 PDAC patient tissues were transplanted subcutaneously into highly immune-deficient NSG mice (NOD/SCID with knock-down in IL2R γ), yielding twelve successfully engrafted tumors whose phenotypic attributes closely and repeatedly reflected those of the donor tumor. The human microenvironment of PDX tumors is replaced by murine stroma early in the engraftment process. Another approach by Allaway et al. (2016) generated subcutaneous PDX models using PDAC material from fine needle aspiration biopsies. Surprisingly, in light of the low number of tumor cells that this method yields, 9 out of 24 samples successfully engrafted in NSG

mice. For some patients, biopsies from metastases were even transplanted in parallel. A characterization of the genomic and histological properties of these metastasized tumors revealed a high degree of homology to both the primary PDX and the original donor.

Biopsy-derived xenografts likely lack the full clonal diversity found in heterogeneous tumors, which means that their genomic features should be carefully analyzed in order to determine how findings relate to specific oncological questions. Thomas et al. (2015) generated 56 PDX models through the subcutaneous implantation of 70 PDAC patient tumors into NOD/SCID mice. Tissue microarray analyses revealed a high correlation of IHC markers (β -catenin, caspase-3, E-cadherin, p16 and SMAD4) between xenografts and patient samples. This PDX panel revealed that successful engraftment in mice is a predictive factor for tumor recurrence in patients, but no correlation was found between demographic or clinicopathological parameters and effective in vivo growth. Finally, in a study conducted on a cohort of thirty PDAC subcutaneous PDX models, Kras and PIK3CA genes were found to undergo a steady rate of mutation over 10 in vivo passages (Tignanelli, Herrera Loeza, & Yeh, 2014). Even though this work focused solely on these two mutations, the authors proposed that PDX models preserve crucial features of patient tumor progression. All of these cases reveal genetic similarities and morphological as well as biological conservation of patient tumors through the process of engraftment, leading to the conclusion that PDAC PDX models are recommended for translational auestions.

In our facility a well characterized panel of 18 PDAC PDX models has been established over the last few years (Behrens et al., 2015). Our data indicate that the take rate is strongly dependent on the viability of the patient sample, the tumor cell content and the mouse strain chosen for xenotransplantation. Further conclusions drawn from this PDX panel include the observation of a distinct inherent resistance to gemcitabine as shown by the fact that 50% continued to progress, 39% were arrested and remission was only noticed in 11% of the cases. But, combining gemcitabine with Abraxane resulted in 56% complete or partial remission in this PDX cohort.

In general, PDX models are the favored method used to identify drugs that significantly inhibit tumor growth - in hopes that they can be used to cure patients - or to validate eligible prognostic biomarkers. Preclinical efforts to date have predominantly been small-scale, with a limited clinical impact. Recent examples have been published for melanoma (Yamamoto et al., 2016) and cancer of the ovaries (Liu, Palakurthi, et al., 2016), pancreas (MacLaughlin et al., 2016), bladder (Chang et al., 2016), brain (Crommentuijn et al., 2016), breast (Ter Brugge et al., 2016), head-and-neck (Klinghammer et al., 2015), lung (Rolff, Becker, Merk, Hoffmann, & Fichtner, 2016) and kidney (Diaz-Montero et al., 2016). Most publications regarding pancreatic cancer PDX models have described the establishment, characterization and preclinical application of these xenografts, but these models have yet to be comprehensively applied for companion clinical studies. Two publications from the Hidalgo group describe a parallel implementation of preclinical and clinical trials in patients with advanced PDAC (Laheru et al., 2012; Von Hoff et al., 2011). In each study, a small cohort of PDX-bearing mice was treated in methods paralleling those applied to patients enrolled into clinical phase I/II trials. Even though the PDX models were not generated from corresponding patients, the outcomes of both platforms yielded a trend toward correlation and generated valuable information toward the validation of biomarkers.

Eng et al. (2016) identified a cancer stem cell (CSC) population in three subcutaneous PDAC PDX models which expressed high levels of death receptors 4 and 5. Targeting these CS cells with a specific anti-DR5 monoclonal antibody (drozitumab) resulted in apoptosis and the regression of PDX tumors, which offers a promising perspective for patients, due to the role that CS cells are known to play in metastasis. Although there have been repeated reports that Metformin has protective and cancerostatic effects (Bhaw-Luximon & Jhurry, 2016), tumor growth inhibition could not be detected in a set of four subcutaneous PDX models of pancreatic cancer treated with different doses of Metformin (Lipner et al., 2016). However, because Metformin constrained the proliferation of four pancreatic cancer cell lines in vitro (Capan-2, CFPAC-1, HPAF-II, SW1990), the authors concluded that its efficacy could be influenced by factors such as tumor heterogeneity, microenvironment, size and growth rate – questions to be taken up in further studies.

In summary, human PDAC malignancies acquire broad heterogeneity due to the constant accumulation of genetic and molecular abnormalities. The development of large panels of well characterized, molecularly defined PDX models reflecting this tumor heterogeneity have increased their impact in terms of predicting patients' responses to new therapeutic agents in the clinic and defining powerful biomarkers. This method has undisputed advantages over others in terms of reproducibility and the renewability and availability of tumor material. On the other hand, the development of PDX models takes time, and not all patient tumors engraft, which restricts their current applicability for personalized medicine. The most likely reasons for the low number of co-clinical studies that have been carried out to date are the intensive time and cost of the procedures required to generate PDAC PDX cohorts. The failure of PDX models to predict outcomes can likely be attributed in part to the fact that only a fraction of the whole patient tumor is propagated in mice, and only 20% of tumors that have been diagnosed are resectable. Xenotransplantation represents to some degree an event of selection that reduces intratumoral clonal diversity and creates a lack of homology between PDX and patient tissue. The degree of divergence is influenced by both the surgical procedure that is used and the properties of a specific tumor. Further obstacles to success in setting up a preclinical study with the best conditions for predicting outcomes are the partial loss of the native tumor stroma that occurs through xenotransplantation (especially in subcutaneous PDX) and the fact that the tumors are propagated in immune-deficient hosts, which leads to basic differences in the immunological context of the tumors (Lodhia et al., 2015).

5. Future perspectives for PDX models

Patient-derived xenografts currently come closest to addressing the urgent needs that arise in late preclinical research: due to their use of the right species, renewable resources and their preservation of the initial clonal heterogeneity of pancreatic tumors. There is abundant evidence that they recapitulate neoplastic cell architecture and conserve genetic and phenotypical biology at the histological and molecular levels. This makes PDX models pivotal platforms for therapeutic screening, the validation of tumor biomarkers and predictions for treatment outcomes. These models have the further potential to address issues related to metastasis and tumor relapse, if experiments are carefully designed.

Two main weaknesses persist in patient-derived xenografts and the strategies by which they are handled: 1) inherent tumor stroma are lost during xenotransplantation and replaced by those of the new host; and 2) avoiding rejection of the xenograft requires that the tumor be placed into an animal that lacks a functional immune system. Each of these factors – and their combination – alters the environment in which the disease develops and is treated and can therefore interfere with the degree to which results can be translated from the model to the clinical situation.

Cellular interactions of tumor cells with components of the extra cellular matrix can regulate the gene expression programs, differentiation and general behavior of tumor cells. The partial loss of an intact human tumor microenvironment during engraftment of patient material may affect tumor progression and is considered a reason for the low rate at which breast and prostate xenografts take in the new host (Hidalgo et al., 2014). Several authors have discussed the impact of the microenvironment on tumor biology (Fang & DeClerk, 2013; Knudsen, Balaji, Freinkman, McCue, & Witkiewicz, 2016) and its therapeutic potential, for example in cancers of the pancreas (Mei, Du, & Ma, 2016; Rossi, Rehman, & Gondi, 2014), breast (Nwabo Kamdje et al., 2014) and prostate (Chiarugi, Paoli, & Cirri, 2014). After xenotransplantation, human stromal components are replaced by murine tissue within 3 to 9 weeks (Hylander et al., 2013). Investigations of murine stroma in pancreatic cancer PDX by our own group have revealed an environment expressing α -SMA, SPARC, collagen I and FAP in subcutaneous tumors (Behrens, Pfohl, Hallas, Buettner, & Hoffmann, 2016, Fig. 2). Recent findings show that murine cells reflect stromal architecture and functionality homologous to that of humans to a certain degree, but speciesrelated differences (ligand-receptor profile) can affect cellular crosstalk and therefore tumor biology. A recent innovative concept has been the idea of directly co-transplanting human mesenchymal stem cells (MSC) and cancer cells. MS cells can acquire environmental activities in co-culture studies in vitro and in vivo (Barcellos-de-Souza et al., 2016; Mandel et al., 2013; Melzer, Yang, & Hass, 2016). Since MSCs can be isolated from a range of tissues and organs, future experiments should permit a careful investigation of the influence of cell sources on the stromal character of tumor tissues. Additionally, PDX models are maintained as tumor fragments that may suffer from technical limitations during co-transplantation.

Another recent approach has been to co-implant stroma cells derived from patients (cancer-associated fibroblasts, CAF) that may prevent the invasion of murine environmental components and configure the xenograft in as human a manner as possible. Knudsen et al. (2016) successfully propagated CAFs from six different patients in monolayer cultures and studied metabolic events in these cells that promote tumor growth. Orthotopic co-transplantation with Capan-2 pancreatic cancer cells in NSG mice revealed that patient-derived CAF have tumor- and metastasis-promoting activities. The degree to which such human stroma cells become fully spatially and functionally integrated into the tumor topology or not remains to be determined, as well as the ways in which artificial stroma might alter the genetic and phenotypic attributes of the tumor mass.

Tumor stroma are directly linked to the larger immunooncological context of an animal because immunologically active players such lymphocytes, macrophages, dendritic cells and regulatory T-cells enter the microenvironment of the tumor, take up residence there, and interact with it. Because patient-derived xenografts are established in immunodeficient mice, these models lack the native immune response. To limit the effects of this disadvantage, scientists are working intensively to create a humanized mouse in which species-specific interactions between tumor and immune cells can be analyzed. One of the most promising strategies is the intrahepatic implantation of CD34 + cells from the human umbilical cord blood into the neonatal NSG (NOD/SCID-IL2rynull) mouse, which results in a multilineage engraftment of human CD4 + and CD8 + T cells and B cells (Wulf-Goldenberg, Stecklum, Fichtner, & Hoffmann, 2015). We have used these mice in xenotransplantations of PDAC PDX53 and observed that tumors exhibit a growth rate that is similar to that of non-humanized mice;



Fig. 2. Immunohistochemical staining of murine collagen I and α -SMA in PDX (3rd generation) tissue reveals activated stroma components in human xenograft tumors.

additionally, the tumors respond to Nivolumab and Ipilimumab in similar ways, exhibiting an inhibition of tumor growth of 32% and 62%, respectively (unpublished results).

As a means of improving the humanization of mice, several groups have tried and evaluated variations in CD34 + cell sources, the mouse strain, or mode of application (Holzapfel, Wagner, Thibaudeau, Levesque, & Hutmacher, 2015). Currently, these humanized mice produce a lower number of immune cells and different rations of B to T cells than humans. Since T cells aren't educated by the mouse thymus, the functions of both B and T cells are limited; the mice therefore lack adaptive responses and their B cells remain immature. Recent advances in mouse humanization include the production of human cytokines through genetic engineering (Billerbeck et al., 2011) and knockouts of MHC class I and II genes (Covassin et al., 2011) in mice to improve the function of human immune cells. Another method by which human immune cells can be established in a mouse is through the intravenous cotransplantation of human PBMCs (peripheral blood mononuclear cells) into immunodeficient mice. These cells are easy to obtain and comprise around 75% CD4/CD8 + T cells; the rest are primarily B and NK cells.

Several studies have proved their immunooncological capacity (Guichelaar et al., 2013). Our own group demonstrated this through the successful application of a bispecific EpCAM/CD3 antibody to SW480 colon cancer xenografts in PMBC-bearing mice (Wulf-Goldenberg, Eckert, & Fichtner, 2011). The major weakness of this approach is that it leads to the development of lethal GvHD (graft versus host disease) within a few weeks; processes involved in GvHD can influence the outcome of a study in various ways. Also crucial to experimental reliability is the HLA mismatch between human tumor cells and inoculated T cells (PBMC). It might be possible to avoid this problem by simultaneously preparing tumor tissue and blood from the same patient, which would enhance co-transplantation studies.

Further advances in humanizing mice may be crucial given the rising number of strategies aimed at targeting immunological interactions in cancer, and the successes that these approaches have had in prolonging survival. Since 2011, the CTLA-4 inhibitor Ipilimumab and the anti-PD1 antibodies Pembrolizumab and Nivolumab have been globally approved for the treatment of cancers, including melanoma as well as renal and lung cancer. Further candidates for immunotherapy are

Table 1

Comparison and application of different PDAC preclinical models

| Type of model | Application | Advantages | Limitations | References |
|--|---|--|---|--|
| <i>In vitro</i> 2D cell culture | Drug development and high-throughput screening Analysis of signaling pathways | Low costs Low time effort Easy handling and propagation Highly standardized | Lack of tumor heterogeneity Lack of structural organisation and functional differentiation Lack of tumor environment Genetic drift due to in vitro propagation High degree of dedifferentiation | Hwang, Boj, Clevers, and Tuveson (2016) Biau et al. (2016) Gillet, Varma, and Gottesman (2013) |
| 3D organoids | High-throughput drug screening | Moderate costs Moderate time effort Structural organisation and functional differentiation | Unclear relationship to tumor type Lack of tumor environment Technically sophisticated | Boj, Hwang, Baker, Chio, et al. (2015) Edmondson et al. (2014) |
| In vivo Cell line-derived xenografts | Drug screening Biomarker discovery | Moderate time effort | Neither intra- nor inter-tumoral heterogeneity Lack of stroma and immune components | Wilding and Bodmer (2014) Logsdon et al. (2015) |
| Syngeneic models | Drug screening | Competent immune system Low time effort Species analogy | Limited relation to tumor type Different biomarkers and drug targets Not suited for species (human)-specific approaches | Murphy (2015) Vallespí et al. (2014) |
| Genetically engineered mouse models | Genetic analyses of tumor development and progression | Fast growth Competent immune system | Expensive Time intensive Lack of genetic complexity | Westphalen and Olive (2012) McDonald et al. (2012) |
| Patient-derived xenografts | Drug screening Prediction of drug responses in preclinical "phase II" studies Biomarker identification and validation | Inter-tumoral heterogeneity Conserved geno- and phenotype Suitable for individualized therapy predictions | Dependent on pre-defined tumor-associated genes Time- and cost-intensive Lack of human tumor stroma Lack of immune components Engraftment rate | Logsdon et al. (2015) Hwang et al. (2016) Witkiewicz et al. (2010) Jung et al. (2016) |

Hodgkin lymphomas as well as head-and-neck tumors. With the development of suitable animal models and studies that lead to preclinical approval, patients with pancreatic cancer could benefit as well.

6. Conclusions

Pancreatic cancer is a malignancy with a rather low incidence, leading to the regrettable fact that early diagnostic screenings (as established for breast and colorectal cancer) have not yet become routine or feasible in normal public health care settings. Thus diagnosis is usually only made at a point when the disease has reached a high degree of malignancy that will likely be fatal. The consequences – which put a strain on both patients and clinicians – are treatment resistance, metastasis, recurrence and worst of all, a lack of therapeutic strategies that lead to cures. While preclinical oncology efforts have been mounted to identify and validate novel drugs that can control the disease, we can only expect to successfully translate laboratory findings into clinical practice through the development of experimental platforms that holistically mimic the complexity of this type of tumor.

6.1. Novel approaches for better models

The best model answers questions.

Basic research and applied science have different overall aims and thus if the aim is translation, it is crucial to choose experimental models that fit a particular type of study. Here we have provided an overview of current preclinical models and their particular advantages and drawbacks, as a means of identifying the uses to which they can best be applied (Table 1). All models have limitations in their ability to reflect the true course of a disease in human subjects, yet we have seen a continual development of novel approaches that aim to bridge experimental gaps. Roife et al. (2016) for example developed a live-tissue sensitivity assay (LTSA) in which slices from PDAC patient tumors were maintained in 96-well plates. The slices are about 200 µm thick and viable for up to 5 days in an agarose-containing environment. Authors used this platform for high throughput drug screening and reported a significant correlation of their results with patient outcomes. While this method has the advantages of low cost and effort, its results are restricted to initial tumor responses rather than those that would be expected later. Another experimental design is based on the cultivation of PDAC patient-derived, primary tumor cells on feeder monolayers that are treated with a specific Rho inhibitor to conditionally reprogram epithelial features (Beglyarova et al., 2016). These unselected cultures stayed vital for up to 60 days and were used for systematic analysis of drug sensitivity. Authors found correlating chemosensitivity between independent cultures from primary material and F1 generation xenografts and identified drugs that acted cytotoxically in vitro and in vivo. Furthermore, the specific regulatory cross-signaling between MYC and the transcription factor ERCC3 was evaluated and found to be involved in cell viability and resistance to triptolide of pancreatic cancer cells (Beglyarova et al., 2016).

For the moment, resolving these issues may well require breaking the complex and multifaceted disease of cancer into single questions; choosing the best preclinical platform and model to address each of them, and then integrating the results with the assistance of more global data assessment tools.

6.2. Closing remark

Data can only be translated from bench to bedside if they are highly reproducible and reliable. This makes it essential to develop models that fit these criteria in exploring any sort of innovative concept whose final aim is to produce clinical applications. Regardless of the status of progress in research into a process or disease, whether for PDAC or another health threat, the clinical impact and benefit to patients will ultimately depend on standardized protocols and statistical methods, intensive collaborations and the transparency of data. One crucial step toward these goals would be to establish a public database for preclinical studies, along the lines of those already available for clinical trials (such as https://clinicaltrails.gov/). This resource would be an immensely valuable tool for translational researchers everywhere.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgment

The authors would like to thank Russ Hodge for proofread and amendment of the manuscript.

References

- A.n.n (2016). Autophagic pancreatic stellate cells promote pancreatic tumor growth. Cancer Discovery (2016 Aug 19, [Epub ahead of print] PubMed PMID: 27543586).
- Abolhassani, M., Guais, A., Sanders, E., Campion, F., Fichtner, I., Bonte, J., ... Schwartz, L. (2012). Screening of well-established drugs targeting cancer metabolism: Reproducibility of the efficacy of a highly effective drug combination in mice. *Investigational New Drugs* 30, 1331–1342.
- Aguirre, A. J., Bardeesy, N., Sinha, M., Lopez, L., Tuveson, D. A., Horner, J., ... DePinho, R. A. (2003). Activated Kras and Ink4a/Arf deficiency cooperate to produce metastatic pancreatic ductal adenocarcinoma. *Genes and Development* 17, 3112–3126.
- Allaway, R. J., Fischer, D. A., de Abreu, F. B., Gardner, T. B., Gordon, S. R., Barth, R. J., ... Smith, K. D. (2016). Genomic characterization of patient-derived xenograft models established from fine needle aspirate biopsies of a primary pancreatic ductal adenocarcinoma and from patient-matched metastatic sites. *Oncotarget* 7, 7087–7102.
- Bankert, R. B., Balu-Iyer, S. V., Odunsi, K., Shultz, L. D., Kelleher, R. J., Jr., Barnas, J. L., ... Yokota, S. J. (2011). Humanized mouse model of ovarian cancer recapitulates patient solid tumor progression, ascites formation, and metastasis. *Public Library of Science: One* 6, e24420.
- Barcellos-de-Souza, P., Comito, G., Pons-Segura, C., Taddei, M. L., Gori, V., Becherucci, V., ... Chiarugi, P. (2016). Mesenchymal stem cells are recruited and activated into carcinoma-associated fibroblasts by prostate cancer microenvironment-derived TGF-β1. *Stem Cells*. http://dx.doi.org/10.1002/stem.2412 (Epub ahead of print).
- Bardeesy, N., Aguirre, A. J., Chu, G. C., Cheng, K. H., Lopez, L. V., Hezel, A. F., ... Depinho, R. A. (2006). Both p16(Ink4a) and the p19(Arf)-p53 pathway constrain progression of pancreatic adenocarcinoma in the mouse. Proceedings of the National Academy of Sciences of the United States of America 103, 5947–5952.
- Barone, E., Corrado, A., Gemignani, F., & Landi, S. (2016). Environmental risk factors for pancreatic cancer: An update. Archives of Toxicology (PubMed PMID: 27538405 [Epub ahead of print]).
- Bartucci, M., Ferrari, A. C., Kim, I. Y., Ploss, A., Yarmush, M., & Sabaawy, H. E. (2016). Personalized medicine approaches in prostate cancer employing patient derived 3D organoids and humanized mice. Frontiers in Cell and Development Biology 4, 64.
- Beglyarova, N., Banina, E., Zhou, Y., Mukhamadeeva, R., Andrianov, G., Bobrov, E., ... Astsaturov, I. (2016). Screening of conditionally reprogrammed patient-derived carcinoma cells identifies ERCC3-MYC interactions as a target in pancreatic cancer. *Clinical Cancer Research* (pii: clincanres.0149.2016. [Epub ahead of print]).
- Behrens, D., Hallas, C., Anders, D., Hoffmann, J., Lawlor, R., Scapa, A., & Fichtner, I. (2015). In vivo models of pancreatic cancer for translational medicine. Proceedings of the AACR-NCI-EORTC international conference: Molecular targets and cancer therapeutics; 2015 Nov 5–9; Boston, MA. Philadelphia (PA): AACR. Molecular Cancer Therapeutics 14, 12 Suppl 2. (Abstract nr A100).
- Behrens, D., Pfohl, U., Hallas, C., Buettner, B., & Hoffmann, J. (2016). Analysis of murine stromal components in patient derived xenograft (PDX) models of pancreatic cancer. *European Journal of Cancer* 61(Suppl. 1), S9–S218 (EACR24 Poster Sessions, Abstract no. 309).
- Bergman, A. M., Adema, A. D., Balzarini, J., Bruheim, S., Fichtner, I., Noordhuis, P., ... Peters, G. J. (2011). Antiproliferative activity, mechanism of action and oral antitumor activity of CP-4126, a fatty acid derivative of gemcitabine, in in vitro and in vivo tumor models. *Investigational New Drugs 29*, 456–466.
- Bhaw-Luximon, A., & Jhurry, D. (2016). Metformin in pancreatic cancer treatment: From clinical trials through basic research to biomarker quantification. *Journal of Cancer Research and Clinical Oncology* 142, 2159–2171.
- Biau, J., Chautard, E., Court, F., Pereira, B., Verrelle, P., Devun, F., ... Dutreix, M. (2016). Global conservation of protein status between cell lines and xenografts. *Translational Oncology* 9, 313–321.
- Billerbeck, E., Barry, W. T., Mu, K., Dorner, M., Rice, C. M., & Ploss, A. (2011). Development of human CD4 + FoxP3 + regulatory T cells in human stem cell factor-, granulocytemacrophage colony-stimulating factor-, and interleukin-3-expressing NOD-SCID IL2Rgamma(null) humanized mice. *Blood* 117, 3076–3086.
- Bisht, S., Schlesinger, M., Rupp, A., Schubert, R., Nolting, J., Wenzel, J., ... Feldmann, G. (2016). A liposomal formulation of the synthetic curcumin analog EF24 (Lipo-EF24) inhibits pancreatic cancer progression: Towards future combination therapies. *Journal of Nanobiotechnology* 14, 57.

- Boeck, S., Ormanns, S., Haas, M., Bächmann, S., Laubender, R. P., Siveke, J. T., ... Heinemann, V. (2014). Translational research in pancreatic cancer: KRAS and beyond. *Pancreas* 43, 150–152.
- Boj, S. F., Hwang, C. I., Baker, L. A., Chio, I. I., Engle, D. D., Corbo, V., ... Tuveson, D. A. (2015a). Organoid models of human and mouse ductal pancreatic cancer. *Cell 160*, 324–338.
- Boj, S. F., Hwang, C. I., Baker, L. A., Engle, D. D., Tuveson, D. A., & Clevers, H. (2015b). Model organoids provide new research opportunities for ductal pancreatic cancer. *Molecular* and Cellular Oncology 3, e1014757.
- Borska, S., Pedziwiatr, M., Danielewicz, M., Nowinska, K., Pula, B., Drag-Zalesinska, M., ... Dziegiel, P. (2016). Classical and atypical resistance of cancer cells as a target for resveratrol. Oncology Reports 36, 1562–1568.
- Broutier, L., Andersson-Rolf, A., Hindley, C. J., Boj, S. F., Clevers, H., Koo, B. K., & Huch, M. (2016). Culture and establishment of self-renewing human and mouse adult liver and pancreas 3D organoids and their genetic manipulation. *Nature Protocols* 11, 1724–1743.
- Cao, J., Yang, J., Ramachandran, V., Arumugam, T., Deng, D., Li, Z., ... Logsdon, C. D. (2015). TM4SF1 promotes gemcitabine resistance of pancreatic cancer in vitro and in vivo. *PloS One* 10, e0144969.
- Chang, N., Lee, H. W., Lim, J. E., Jeong, D. E., Song, H. J., Kim, S., ... Jeon, H. G. (2016). Establishment and antitumor effects of dasatinib and PKI-587 in BD-138T, a patient-derived muscle invasive bladder cancer preclinical platform with concomitant EGFR amplification and PTEN deletion. *Oncotarget*. http://dx.doi.org/10.18632/oncotarget. 10539 (Epub ahead of print).
- Chen, J., Wang, S., Su, J., Chu, G., You, H., Chen, Z., ... Zhou, M. (2016). Interleukin-32α inactivates JAK2/STAT3 signaling and reverses interleukin-6-induced epithelial-mesenchymal transition, invasion, and metastasis in pancreatic cancer cells. OncoTargets and Therapy 9, 4225–4237.
- Chiarugi, P., Paoli, P., & Cirri, P. (2014). Tumor microenvironment and metabolism in prostate cancer. Seminars in Oncology 41, 267–280.
- Cho, S. Y., Kang, W., Han, J. Y., Min, S., Kang, J., Lee, A., ... Park, H. (2016). An integrative approach to precision cancer medicine using patient-derived xenografts. *Molecules and Cells* 39, 77–86.
- Coleman, S. J., Watt, J., Arumugam, P., Solaini, L., Carapuca, E., Ghallab, M., ... Kocher, H. M. (2014). Pancreatic cancer organotypics: High throughput, preclinical models for pharmacological agent evaluation. World Journal of Gastroenterology 20, 8471–8481.
- Collignon, A., Perles-Barbacaru, A. T., Robert, S., Silvy, F., Martinez, E., Crenon, I., ... Béraud, E. (2015). A pancreatic tumor-specific biomarker characterized in humans and mice as an immunogenic onco-glycoprotein is efficient in dendritic cell vaccination. *Oncotarget* 6, 23462–23479.
- Collins, M. A., Bednar, F., Zhang, Y., Brisset, J. -C., Galban, S., Galban, C. J., ... Pasca di Magliano, M. (2012). Oncogenic Kras is required for both the initiation and maintenance of pancreatic cancer in mice. *Journal of Clinical Investigation* 122, 639–653.
- Covassin, L., Laning, J., Abdi, R., Langevin, D. L., Phillips, N. E., Shultz, L. D., & Brehm, M. A. (2011). Human peripheral blood CD4 T cell-engrafted non-obese diabetic-scid IL2rgamma(null) H2-Ab1 (tm1Gru) Tg (human leucocyte antigen D-related 4) mice: A mouse model of human allogeneic graft-versus-host disease. *Clinical and Experimental Immunology* 166, 269–280.
- Cree, I. A., Glaysher, S., & Harvey, A. L. (2010). Efficacy of anti-cancer agents in cell lines versus human primary tumour tissue. *Current Opinion in Pharmacology* 10, 375–379.
- Crommentuijn, M. H., Kantar, R., Noske, D. P., Vandertop, W. P., Badr, C. E., Würdinger, T., ... Tannous, B. A. (2016). Systemically administered AAV9-sTRAIL combats invasive glioblastoma in a patient-derived orthotopic xenograft model. *Molecular Therapy Oncolytics* 3, 16017.
- D'Alincourt Salazar, M., Manuel, E. R., Tsai, W., D'Apuzzo, M., Goldstein, L., Blazar, B. R., & Diamond, D. J. (2016). Evaluation of innate and adaptive immunity contributing to the antitumor effects of PD1 blockade in an orthotopic murine model of pancreatic cancer. Oncoimmunology 5, e1160184.
- Damhofer, H., Ebbing, E. A., Steins, A., Welling, L., Tol, J. A., Krishnadath, K. K., ... Bijlsma, M. F. (2015). Establishment of patient-derived xenograft models and cell lines for malignancies of the upper gastrointestinal tract. *Journal of Translational Medicine* 13, 115.
- D'Aronzo, M., Vinciguerra, M., Mazza, T., Panebianco, C., Saracino, C., Pereira, S. P., ... Pazienza, V. (2015). Fasting cycles potentiate the efficacy of gemcitabine treatment in in vitro and in vivo pancreatic cancer models. *Oncotarget* 6, 18545–18557.
- De Latouliere, L., Manni, I., Iacobini, C., Pugliese, G., Grazi, G. L., Perri, P., ... Piaggio, G. (2016). A bioluminescent mouse model of proliferation to highlight early stages of pancreatic cancer: A suitable tool for preclinicalstudies. *Annals of Anatomy 207*, 2–8.
- Deer, E. L., González-Hernández, J., Coursen, J. D., Shea, J. E., Ngatia, J., Scaife, C. L., ... Mulvihill, S. J. (2010). Phenotype and genotype of pancreatic cancer cell lines. *Pancreas* 39, 425–435.
- DeRose, Y. S., Wang, G., Lin, Y. C., Bernard, P. S., Buys, S. S., Ebbert, M. T., ... Welm, A. L. (2011). Tumor grafts derived from women with breast cancer authentically reflect tumor pathology, growth, metastasis and disease outcomes. *Nature Medicine* 17, 1514–1520.
- Dey, J., Kerwin, W. S., Grenley, M. O., Casalini, J. R., Tretyak, I., Ditzler, S. H., ... Klinghoffer, R. A. (2016). A Platform for Rapid, Quantitative Assessment of Multiple Drug Combinations Simultaneously in Solid Tumors In Vivo. *PloS One* 11, e0158617.
- Di Maggio, F., Arumugam, P., Delvecchio, F. R., Batista, S., Lechertier, T., Hodivala-Dilke, K., & Kocher, H. M. (2016). Pancreatic stellate cells regulate blood vessel density in the stroma of pancreatic ductal adenocarcinoma. *Pancreatology S1424-3903*(16), 30461–30466. Dira Montene, C. M. K., B. (2016). Conteness of the strong statement of the strong strong
- Diaz-Montero, C. M., Mao, F. J., Barnard, J., Parker, Y., Zamanian-Daryoush, M., Pink, J. J., ... Lindner, D. J. (2016). MEK inhibition abrogates sunitinib resistance in a renal cell carcinoma patient-derived xenograft model. *British Journal of Cancer*. http://dx.doi.org/ 10.1038/bjc.2016.263 (Epub ahead of print).
- Dobrynin, Y. V. (1963). Establishment and characteristics of cell strains from some epithelial tumors of human origin. *Journal of the National Cancer Institute* 31, 1173–1195.

Domcke, S., Sinha, R., Levine, D. A., Sander, C., & Schultz, N. (2013). Evaluating cell lines as tumour models by comparison of genomic profiles. *Nature Communications* 4, 2126.

- Edmondson, R., Broglie, J. J., Adcock, A. F., & Yang, L. (2014). Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. Assay and Drug Development Technologies 12, 207–218.
- Eng, J. W., Mace, T. A., Sharma, R., Twum, D. Y., Peng, P., Gibbs, J. F., ... Hylander, B. L. (2016). Pancreatic cancer stem cells in patient pancreatic xenografts are sensitive to drozitumab, an agonistic antibody against DR5. *Journal of Immunotherapy of Cancer* 4, 33.
- Falasca, M., Kim, M., & Casari, I. (2016). Pancreatic cancer: Current research and future directions. *Biochimica et Biophysica Acta 1865*, 123–132.
 Fang, H. & DeClerk, Y. A. (2012). The second second
- Fang, H., & DeClerk, Y. A. (2013). Targeting the tumor microenvironment: From understanding pathways to effective clinical trails. *Cancer Research* 73, 4965–4977. Feday I. Stollarous Provement Content on Content of Content on Content on
- Ferlay, J., Steliarova-Foucher, E., Lortet-Tieulent, J., Rosso, S., Coebergh, J. W., Comber, H., ... Bray, F. (2013). Cancer incidence and mortality patterns in Europe: Estimates for 40 countries in 2012. European Journal of Cancer 49, 1374–1403.
- Fichtner, I., Rolff, J., Soong, R., Hoffmann, J., Hammer, S., Sommer, A., ... Merk, J. (2008). Establishment of patient-derived non-small cell lung cancer xenografts as models for the identification of predictive biomarkers. *Clinical Cancer Research* 14, 6456–6468.
- Fichtner, I., Slisow, W., Gill, J., Becker, M., Elbe, B., Hillebrand, T., & Bibby, M. (2004). Anticancer drug response and expression of molecular markers in early-passage xenotransplanted colon carcinomas. *European Journal of Cancer* 40, 298–307.
- Francies, H. E., & Garnett, M. J. (2015). What role could organoids play in the personalization of cancer treatment? *Pharmacogenomics* 16, 1523–1526.
- Frese, K. K., Neesse, A., Cook, N., Bapiro, T. E., Lolkema, M. P., Jodrell, D. I., & Tuveson, D. A. (2012). nab-Paclitaxel potentiates gemcitabine activity by reducing cytidine deaminase levels in a mouse model of pancreatic cancer. *Cancer Discovery* 2, 260–269.
- Gades, N. M., Ohash, A., Mills, L. D., Rowley, M. A., Predmore, K. S., Marler, R. J., & Couch, F. J. (2008). Spontaneous vulvar papillomas in a colony of mice used for pancreatic cancer research. *Comparative Medicine* 58, 271–275.
- Gao, H., Korn, J. M., Ferretti, S., Monahan, J. E., Wang, Y., Singh, M., ... Sellers, W. R. (2015). High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. *Nature Medicine* 21, 1318–1325.
- Garralda, E., Paz, K., López-Casas, P. P., Jones, S., Katz, A., Kann, L. M., ... Hidalgo, M. (2014). Integrated next generation sequencing and avatar mouse models for personalized cancer treatment. *Clinical Cancer Research 20*, 2476–2484.
- Gillet, J. P., Varma, S., & Gottesman, M. M. (2013). The clinical relevance of cancer cell lines. *Journal of the National Cancer Institute* 105, 452–458.
- Gopinathan, A., Morton, J. P., Jodrell, D. I., & Sansom, O. J. (2015). GEMMs as preclinical models for testing pancreatic cancer therapies. *Disease Models & Mechanisms* 8, 1185–1200.
- Gradiz, R., Silva, H. C., Carvalho, L., Botelho, M. F., & Mota-Pinto, A. (2016). MIA PaCa-2 and PANC-1 - pancreas ductal adenocarcinoma cell lines with neuroendocrine differentiation and somatostatin receptors. *Scientific Reports* 6, 21648.
- Graeser, R., Esser, N., Unger, H., Fichtner, I., Zhu, A., Unger, C., & Kratz, F. (2010). INNO-206, the (6-maleimidocaproyl hydrazone derivative of doxorubicin), shows superior antitumor efficacy compared to doxorubicin in different tumor xenograft models and in an orthotopic pancreas carcinoma model. *Investigational New Drugs* 28, 14–19.
- Guichelaar, T., Emmelot, M. E., Rozemuller, H., Martini, B., Groen, R. W., Storm, G., ... Mutis, T. (2013). Human regulatory T cells do not suppress the antitumor immunity in the bone marrow: A role for bone marrow stromal cells in neutralizing regulatory T cells. *Clinical Cancer Research* 19, 1467–1475.
- Haqq, J., Howells, L. M., Garcea, G., Metcalfe, M. S., Steward, W. P., & Dennison, A. R. (2014). Pancreatic stellate cells and pancreas cancer: Current perspectives and future strategies. *European Journal of Cancer* 50, 2570–2582.
- Heller, A., Angelova, A. L., Bauer, S., Grekova, S. P., Aprahamian, M., Rommelaere, J., ... Giese, N. A. (2016). Establishment and characterization of a novel cell line, ASAN-PaCa, derived from human adenocarcinoma arising in intraductal papillary mucinous neoplasm of the pancreas. *Pancreas* (2016 Aug 11. [Epub ahead of print]).
- Hessmann, E., Schneider, G., Ellenrieder, V., & Siveke, J. T. (2016). MYC in parcreatic cancer: Novel mechanistic insights and their translation into therapeutic strategies. *Oncogene* 35, 1609–1618.
- Hidalgo, M., Amant, F., Biankin, A. V., Budinská, E., Byrne, A. T., Caldas, C., ... Villanueva, A. (2014). Patient-derived xenograft models: An emerging platform for translational cancer research. *Cancer Discovery* 4, 998–1013.
- Hidalgo, M., Cascinu, S., Kleeff, J., Labianca, R., Löhr, J. M., Neoptolemos, J., ... Heinemann, V. (2015). Addressing the challenges of pancreatic cancer: Future directions for improving outcomes. *Pancreatology* 15, 8–18.
- Hindley, C. J., Cordero-Espinoza, L., & Huch, M. (2016). Organoids from adult liver and pancreas: Stem cell biology and biomedical utility. *Developmental Biology S0012-*1606, 30277–30279.
- Hingorani, S. R., Petricoin, E. F., Maitra, A., Rajapakse, V., King, C., Jacobetz, M. A., ... Tuveson, D. A. (2003). Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. *Cancer Cell* 4, 437–450.
- Hoffmann, J., Orthmann, A., Hoffmann, A., Reiner, R., & Fichtner, I. (2014). Establishment and validation of models for metastasis developed from patient xenogragrafts (PDX). Proceedings of the 105th annual meeting of the American Association for Cancer Research; 2014 Apr 5–9; San Diego, CA. Philadelphia (PA): AACR; 2014. Abstract 4953.
- Holzapfel, B. M., Wagner, F., Thibaudeau, L., Levesque, J. P., & Hutmacher, D. W. (2015). Concise review: humanized models of tumor immunology in the 21st century: Convergence of cancer research and tissue engineering. *Stem Cells* 33, 1696–1704.
- Hwang, C. I., Boj, S. F., Clevers, H., & Tuveson, D. A. (2016). Preclinical models of pancreatic ductal adenocarcinoma. *Journal of Pathology* 238, 197–204.
- Hylander, B. L., Punt, N., Tang, H., Hillman, J., Vaughan, M., Bshara, W., ... Repasky, E. A. (2013). Origin of the vasculature supporting growth of primary patient tumor xenografts. *Journal of Translational Medicine* 11, 110.
- Ijichi, H., Chytil, A., Gorska, A. E., Aakre, M. E., Fujitani, Y., Fujitani, S., ... Moses, H. L. (2006). Aggressive pancreatic ductal adenocarcinoma in mice caused by pancreas-specific

blockade of transforming growth factor-beta signaling in cooperation with active Kras expression. *Genes and Development 20*, 3147–3160.

- Jiang, Y. J., Lee, C. L., Wang, Q., Zhou, Z. W., Yang, F., Jin, C., & Fu, D. L. (2014). Establishment of an orthotopic pancreatic cancer mouse model: Cells suspended and injected in Matrigel. World Journal of Gastroenterology 20, 9476–9485.
- Johnson, J. I., Decker, S., Zaharevitz, D., Rubinstein, L. V., Venditti, J. M., Schepartz, S., ... Sausville, E. A. (2001). Relationships between drug activity in NCI preclinical in vitro and in vivo models and early clinical trials. *British Journal of Cancer* 84, 1424–1431.
- Jordheim, L. P., & Dumontet, C. (2013). Do hENT1 and RRM1 predict the clinical benefit of gemcitabine in pancreatic cancer? *Biomarkers in Medicine* 7, 663–671.
- Jun, E., Jung, J., Jeong, S. Y., Choi, E. K., Kim, M. B., Lee, J. S., ... Kim, S. C. (2016). Surgical and oncological factors affecting the successful engraftment of patient-derived xenografts in pancreatic ductal adenocarcinoma. *Anticancer Research* 36, 517–521.
- Jung, J., Lee, H. C., Seol, H. S., Choi, Y. S., Kim, E., Lee, E. J., ... Chang, S. (2016). Generation and molecular characterization of pancreatic cancer patient-derived xenografts reveals their heterologous nature. *Oncotarget*. http://dx.doi.org/10.18632/oncotarget. 11530 (Epub ahead of print).
- Justilien, V., & Fields, A. P. (2013). Utility and applications of orthotopic models of human non-small cell lung cancer (NSCLC) for the evaluation of novel and emerging cancer therapeutics. *Current Protocols in Pharmacology* 62, 27.
- Karandish, F., & Mallik, S. (2016). Biomarkers and targeted therapy in pancreatic cancer. Biomarkers in Cancer 8(Suppl. 1), 27–35.
- Karnevi, E., Rosendahl, A. H., Hilmersson, K. S., Saleem, M. A., & Andersson, R. (2016). Impact by pancreatic stellate cells on epithelial-mesenchymal transition and pancreatic cancer cell invasion: Adding a third dimension in vitro. *Experimental Cell Research* 346, 206–215.
- Kelloff, G. J., & Sigman, C. C. (2012). Cancer biomarkers: Selecting the right drug for the right patient. *Nature Reviews Drug Discovery* 11, 201–214.
- Klinghammer, K., Raguse, J. D., Plath, T., Albers, A. E., Joehrens, K., Zakarneh, A., ... Fichtner, I. (2015). A comprehensively characterized large panel of head and neck cancer patient-derived xenografts identifies the mTOR inhibitor everolimus as potential new treatment option. *International Journal of Cancer* 136, 2940–2948.
- Knudsen, E. S., Balaji, U., Freinkman, E., McCue, P., & Witkiewicz, A. K. (2016). Unique metabolic features of pancreatic cancer stroma: Relevance to the tumor compartment, prognosis, and invasive potential. *Oncotarget*. http://dx.doi.org/10.18632/oncotarget. 11893 (Epub ahead of print).
- Kojima, K., Vickers, S. M., Adsay, N. V., Jhala, N. C., Kim, H. G., Schoeb, T. R., ... Klug, C. A. (2007). Inactivation of Smad4 accelerates Kras(G12D)-mediated pancreatic neoplasia. *Cancer Research* 67, 8121–8130.
- Kratz, F., Azab, S., Zeisig, R., Fichtner, I., & Warnecke, A. (2013). Evaluation of combination therapy schedules of doxorubicin and an acid-sensitive albumin-binding prodrug of doxorubicin in the MIA PaCa-2 pancreatic xenograft model. *International Journal of Pharmacology* 441, 499–506.
- Kroetz, D. L. (2016). Best practices for clinical and translational research and implementation. *Clinical and Translational Science*. http://dx.doi.org/10.1111/cts.12411 (Epub ahead of print).
- Kruger, S., Haas, M., Ormanns, S., Bächmann, S., Siveke, J. T., Kirchner, T., ... Boeck, S. (2014). Translational research in pancreatic ductal adenocarcinoma: Current evidence and future concepts. World Journal of Gastroenterology 20, 10769–10777.
- Laheru, D., Shah, P., Rajeshkumar, N. V., McAllister, F., Taylor, G., Goldsweig, H., ... Hidalgo, M. (2012). Integrated preclinical and clinical development of S-trans, trans farnesylthiosalicylic acid (FTS, Salirasib) in pancreatic cancer. *Investigational New Drugs* 30, 2391–2399.
- Le, N., Sund, M., & Vinci, A.GEMS collaborating group of Pancreas 2000. (2016). Prognostic and predictive markers in pancreatic adenocarcinoma. *Digestive and Liver Disease* 48, 223–230.
- Lin, W. C., Rajbhandari, N., Liu, C., Sakamoto, K., Zhang, Q., Triplett, A. A., ... Wagner, K. U. (2013). Dormant cancer cells contribute to residual disease in a model of reversible pancreatic cancer. *Cancer Research* 73, 1821–1830.
- Lipner, M. B., Marayati, R., Deng, Y., Wang, X., Raftery, L., O'Neil, B. H., & Yeh, J. J. (2016). Metformin treatment does not inhibit growth of pancreatic cancer patient-derived xenografts. *PloS One 11*, e0147113.
- Liu, Q., Li, Y., Niu, Z., Zong, Y., Wang, M., Yao, L., ... Zhao, Y. (2016b). Atorvastatin (Lipitor) attenuates the effects of aspirin on pancreatic cancerogenesis and the chemotherapeutic efficacy of gemcitabine on pancreatic cancer by promoting M2 polarized tumor associated macrophages. *Journal of Experimental & Clinical Cancer Research* 35, 33.
- Liu, J. F., Palakurthi, S., Zeng, Q., Zhou, S., Ivanova, E., Huang, W., ... Drapkin, R. (2016a). Establishment of patient-derived tumor xenograft models of epithelial ovarian cancer for preclinical evaluation of novel therapeutics. *Clinical Cancer Research* (pii:clincanres.1237. 2016).
- Liu, H., Patel, M. R., Prescher, J. A., Patsialou, A., Qian, D., Lin, J., ... Clarke, M. F. (2010). Cancer stem cells fromhuman breast tumors are involved in spontaneous metastases in orthotopic mouse models. *Proceedings of the National Academy of Sciences of the United States of America* 107, 18115–18120.
- Liu, X., Tian, X., Wang, F., Ma, Y., Kornmann, M., & Yang, Y. (2014). BRG1 promotes chemoresistance of pancreatic cancer cells through crosstalking with Akt signalling. *European Journal of Cancer* 50, 2251–2262.
- Lodhia, K. A., Hadley, A. M., Haluska, P., & Scott, C. L. (2015). Prioritizing therapeutic targets using patient-derived xenograft models. *Biochimica et Biophysica Acta* 1855, 223–234.
- Logsdon, C. D., Arumugam, T., & Ramachandran, V. (2015). Animal models of gastrointestinal and liver diseases. The difficulty of animal modeling of pancreatic cancer for preclinical evaluation of therapeutics. *American Journal of Physiology – Gastrointestinal* and Liver Physiology 309, G283–G291.
- MacLaughlin, C. M., Ding, L., Jin, C., Cao, P., Siddiqui, I., Hwang, D. M., ... Hedley, D. W. (2016). Porphysome nanoparticles for enhanced photothermal therapy in a patient-derived orthotopic pancreas xenograft cancer model: A pilot study. *Journal of Biomedical Optics* 21, 84002.

- Maginn, E. N., de Sousa, C. H., Wasan, H. S., & Stronach, E. A. (2014). Opportunities for translation: Targeting DNA repair pathways in pancreatic cancer. *Biochimica et Biophysica Acta* 1846, 45–54.
- Majumder, K., Arora, N., Modi, S., Chugh, R., Nomura, A., Giri, B., ... Dudeja, V. (2016). A novel immunocompetent mouse model of pancreatic cancer with robust stroma: A valuable tool for preclinical evaluation of new therapies. *Journal of Gastrointestinal Surgery* 20, 53–65.
- Mak, I. W., Evaniew, N., & Ghert, M. (2014). Lost in translation: animal models and clinical trials in cancer treatment. American Journal of Translational Research 6, 114–118.
- Mandel, K., Yang, Y., Schambach, A., Glage, S., Otte, A., & Hass, R. (2013). Mesenchymal stem cells directly interact with breast cancer cells and promote tumor cell growth in vitro and in vivo. *Stem Cells and Development 22*, 3114–3127.
- Maresch, R., Mueller, S., Veltkamp, C., Öllinger, R., Friedrich, M., Heid, I., ... Rad, R. (2016). Multiplexed pancreatic genome engineering and cancer induction by transfectionbased CRISPR/Cas9 delivery in mice. *Nature Communications* 7, 10770.
- McDonald, O. G., Maitra, A., & Hruban, R. H. (2012). Human correlates of provocative questions in pancreatic pathology. Advances in Anatomic Pathology 19(6), 351–362.
- Mei, L, Du, W., & Ma, W. W. (2016). Targeting stromal microenvironment in pancreatic ductal adenocarcinoma: controversies and promises. *Journal of Gastrointestinal Oncology* 7, 487–494.
- Melzer, C., Yang, Y., & Hass, R. (2016). Interaction of MSC with tumor cells. Cell Communication and Signaling: CCS 14(1), 20.
- Mohammed, A., Janakiram, N. B., Pant, S., & Rao, C. V. (2015). Molecular targeted intervention for pancreatic cancers. *Cancers (Basel)* 7, 1499–1542.
- Monsma, D. J., Monks, N. R., Cherba, D. M., Dylewski, D., Eugster, E., Jahn, H., ... Webb, C. P. (2012). Genomic characterization of explant tumorgraft models derived from fresh patient tumor tissue. *Journal of Translational Medicine* 10, 125.
- Mura, S., Buchy, E., Askin, G., Cayre, F., Mougin, J., Gouazou, S., ... Couvreur, P. (2016). In vitro investigation of multidrug nanoparticles for combined therapy with gemcitabine and a tyrosine kinase inhibitor: Together is not better. *Biochimie S0300-9084*(16), 30151.
- Murphy, J. F. (2015). Pre-clinical murine models: Syngeneic models for immuno-oncology. Medcrave Online Journal of Immunology 2, 52.
- Murphy, B., Yin, H., Maris, J. M., Kolb, E. A., Gorlick, R., Reynolds, C. P., ... Houghton, P. J. (2016). Evaluation of alternative in vivo drug screening methodology: A single mouse analysis. *Cancer Research* 76, 5798–5809.
- Nguyen-Ngoc, K. V., Shamir, E. R., Huebner, R. J., Beck, J. N., Cheung, K. J., & Ewald, A. J. (2015). 3D culture assays of murine mammary branching morphogenesis and epithelial invasion. *Methods in Molecular Biology* 1189, 135–162.
- Nikfarjam, M., Yeo, D., He, H., Baldwin, G., Fifis, T., Costa, P., ... Christophi, C. (2013). Comparison of two syngeneic orthotopic murine models of pancreatic adenocarcinoma. *Journal of Investigative Surgery 26*, 352–359.
- Nwabo Kamdje, A. H., Seke Etet, P. F., Vecchio, L., Tagne, R. S., Amvene, J. M., Muller, J. M., ... Lukong, K. E. (2014). New targeted therapies for breast cancer: A focus on tumor microenvironmental signals and chemoresistant breast cancers. World Journal of Clinical Cases 2, 769–786.
- Ocal, O., Pashkov, V., Kollipara, R. K., Zolghadri, Y., Cruz, V. H., Hale, M. A., ... Wilkie, T. M. (2015). A rapid in vivo screen for pancreatic ductal adenocarcinoma therapeutics. *Disease Models & Mechanisms 8*, 1201–1211.
- Oon, C. E., Strell, C., Yeong, K. Y., Östman, A., & Prakash, J. (2015). SIRT1 inhibition in pancreatic cancer models: Contrasting effects in vitro and in vivo. *European Journal of Pharmacology* 757, 59–67.
- Palam, L. R., Gore, J., Craven, K. E., Wilson, J. L., & Korc, M. (2015). Integrated stress response is critical for gemcitabine resistance in pancreatic ductal adenocarcinoma. *Cell Death & Disease* 6, e1913.
- Pan, M. R., Hsu, M. C., Luo, C. W., Chen, L. T., Shan, Y. S., & Hung, W. C. (2016). The histone methyltransferase G9a as a therapeutic target to override gemcitabine resistance in pancreatic cancer. *Oncotarget*. http://dx.doi.org/10.18632/oncotarget.11256 (Epub ahead of print).
- Park, S. I., Kim, S. J., McCauley, L. K., & Gallick, G. E. (2010). Pre-clinical mouse models of human prostate cancer and their utility in drug discovery. *Current Protocols in Pharmacology* (Unit 14.15).
- Partecke, L. I., Sendler, M., Kaeding, A., Weiss, F. U., Mayerle, J., Dummer, A., ... Stier, A. (2011). A syngeneic orthotopic murine model of pancreatic adenocarcinoma in the C57/BL6 mouse using the Panc02 and 6606PDA cell lines. *European Surgical Research* 47, 98–107.
- Pérez-Torras, S., Vidal-Pla, A., Miquel, R., Almendro, V., Fernández-Cruz, L., Navarro, S., ... Mazo, A. (2011). Characterization of human pancreatic orthotopic tumor xenografts suitable for drug screening. *Cellular Oncology (Dordrecht)* 34, 511–521.
- Peterson, J. K., & Houghton, P. J. (2004). Integrating pharmacology and in vivo cancer models in preclinical and clinical drug development. *European Journal of Cancer* 40, 837–844.
- Rhim, A. D., Oberstein, P. E., Thomas, D. H., Mirek, E. T., Palermo, C. F., Sastra, S. A., ... Stanger, B. Z. (2014). Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. *Cancer Cell* 25, 735–747.
- Rochani, A. K., Balasubramanian, S., Ravindran Girija, A., Raveendran, S., Borah, A., Nagaoka, Y., ... Kumar, D. S. (2016). Dual mode of cancer cell destruction for pancreatic cancer therapy using Hsp90 inhibitor loaded polymeric nano magnetic formulation. *International Journal of Pharmaceutics* 511, 648–658.
- Roife, D., Dai, B., Kang, Y., Rios Perez, M. V., Pratt, M., Li, X., & Fleming, J. B. (2016). Ex vivo testing of patient-derived xenografts mirrors the clinical outcome of patients with pancreatic ductal adenocarcinoma. *Clinical Cancer Research* (pii: clincanres.2936. 2015. [Epub ahead of print]).
- Rolff, J., Becker, M., Merk, J., Hoffmann, J., & Fichtner, I. (2016). Preclinical study of a combination of erlotinib and bevacizumab in early stages of unselected non-small cell lung cancer patient-derived xenografts. *Targeted Oncology* 11, 507–514.
- Rossi, M. L., Rehman, A. A., & Gondi, C. S. (2014). Therapeutic options for the management of pancreatic cancer. World Journal of Gastroenterology 20, 11142–11159.

Rozenberg, G. I., Monahan, K. B., Torrice, C., Bear, J. E., & Sharpless, N. E. (2010). Metastasis in an orthotopic murine model of melanoma is independent of RAS/RAF mutation. *Melanoma Research 20*, 361–371.

Rubio, D. M., Schoenbaum, E. E., Lee, L. S., Schteingart, D. E., Marantz, P. R., Anderson, K. E., ... Esposito, K. (2010). Defining translational research: implications for training. *Academic Medicine* 85, 470–475.

Sicklick, J. K., Leonard, S. Y., Babicky, M. L., Tang, C. M., Mose, E. S., French, R. P., ... Lowy, A. M. (2014). Generation of orthotopic patient-derived xenografts from gastrointestinal stromal tumor. *Journal of Translational Medicine* 10(12), 41.

Siegel, R. L., Miller, K. D., & Jemal, A. (2016). Cancer statistics, 2016. CA: A Cancer Journal for Clinicians 66, 7–30.

Skardal, A., Devarasetty, M., Rodman, C., Atala, A., & Soker, S. (2015). Liver-tumor hybrid organoids for modeling tumor growth and drug response in vitro. *Annals of Biomedical Engineering* 43, 2361–2373.

- Skoulidis, F., Cassidy, L. D., Pisupati, V., Jonasson, J. G., Bjarnason, H., Eyfjord, J. E., ... Venkitaraman, A. R. (2010). Germline Brca2 heterozygosity promotes Kras(G12D) – driven carcinogenesis in a murine model of familial pancreatic cancer. Cancer Cell 18, 499–509.
- Sousa, C. M., Biancur, D. E., Wang, X., Halbrook, C. J., Sherman, M. H., Zhang, L., ... Kimmelman, A. C. (2016). Pancreatic stellate cells support tumour metabolism through autophagic alanine secretion. *Nature* 536, 479–483.
- Spadavecchia, J., Movia, D., Moore, C., Maguire, C. M., Moustaoui, H., Casale, S., ... Prina-Mello, A. (2016). Targeted polyethylene glycol gold nanoparticles for the treatment of pancreatic cancer: From synthesis to proof-of-concept in vitro studies. *International Journal of Nanomedicine* 11, 791–822.
- Strobel, O., & Büchler, M. W. (2016). Pancreatic cancer: Clinical practice guidelines What is the evidence? Nature Reviews. Clinical Oncology 13, 593–594.
- Suenaga, M., Yamada, S., Fujii, T., Tanaka, C., Kanda, M., Nakayama, G., ... Kodera, Y. (2016). S-1 plus nab-paclitaxel is a promising regimen for pancreatic cancer in a preclinical model. *Journal of Surgical Oncology* 113, 413–419.
- Ter Brugge, P., Kristel, P., van der Burg, E., Boon, U., de Maaker, M., Lips, E., ... Jonkers, J. (2016). Mechanisms of therapy resistance in patient-derived xenograft models of BRCA1-deficient breast cancer. *Journal of the National Cancer Institute 108* (pii: djw148).
- Thomas, R. M., Truty, M. J., Kim, M., Kang, Y., Zhang, R., Chatterjee, D., ... Fleming, J. B. (2015). The canary in the coal mine: The growth of patient-derived tumorgrafts in mice predicts clinical recurrence after surgical resection of pancreatic ductal adenocarcinoma. *Annals of Surgical Oncology 22*, 1884–1892.
- Tignanelli, C. J., Herrera Loeza, S. G., & Yeh, J. J. (2014). KRAS and PIK3CA mutation frequencies in patient-derived xenograft models of pancreatic and colorectal cancer are reflective of patient tumors and stable across passages. *The American Journal of Surgery* 80, 873–877.
- Topalovski, M., Hagopian, M., Wang, M., & Brekken, R. A. (2016). Hypoxia and transforming growth factor beta cooperate to induce fibulin-5 expression in pancreatic cancer. *Journal of Biological Chemistry* (pii: jbc.M116.730945. [Epub ahead of print]).
- Vallespí, M. G., Pimentel, G., Cabrales-Rico, A., Garza, J., Oliva, B., Mendoza, O., ... Reyes, O. (2014). Antitumor efficacy, pharmacokinetic and biodistribution studies of the anticancer peptide CIGB-552 in mouse models. *Journal of Peptide Science* 20, 850–859.
- Von Hoff, D. D., Ramanathan, R. K., Borad, M. J., Laheru, D. A., Smith, L. S., Wood, T. E., ... Hidalgo, M. (2011). Gemcitabine plus nab-paclitaxel is an active regimen in patients with advanced pancreatic cancer: A phase I/II trial. *Journal of Clinical Oncology* 29, 4548–4554.
- Voskoglou-Nomikos, T., Pater, J. L., & Seymour, L. (2003). Clinical predictive value of the in vitro cell line, human xenograft, and mouse allograft preclinical cancer models. *Clinical Cancer Research* 9, 4227–4239.
- Walters, D. M., Stokes, J. B., Adair, S. J., Stelow, E. B., Borgman, C. A., Lowrey, B. T., ... Bauer, T. W. (2013). Clinical, molecular and genetic validation of a murine orthotopic

xenograft model of pancreatic adenocarcinoma using fresh human specimens. *PloS One 8*, e77065.

- Wang, H., Hang, C., Ou, X. L., Nie, J. S., Ding, Y. T., Xue, S. G., ... Zhu, J. X. (2016). MiR-145 functions as a tumor suppressor via regulating angiopoietin-2 in pancreatic cancer cells. *Cancer Cell International* 16, 65.
- Weeber, F., van de Wetering, M., Hoogstraat, M., Dijkstra, K. K., Krijgsman, O., Kuilman, T., ... Voest, E. E. (2015). Preserved genetic diversity in organoids cultured from biopsies of human colorectal cancer metastases. *Proceedings of the National Academy of Sciences of the United States of America* 112, 13308–13311.
- Weroha, S. J., Becker, M. A., Enderica-Gonzalez, S., Harrington, S. C., Oberg, A. L., Maurer, M. J., ... Haluska, P. (2014). Tumorgrafts as in vivo surrogates for women with ovarian cancer. *Clinical Cancer Research 20*, 1288–1297.
- Westphalen, C. B., & Olive, K. P. (2012). Genetically engineered mouse models of pancreatic cancer. Cancer Journal 18, 502–510.
- Wilding, J. L., & Bodmer, W. F. (2014). Cancer cell lines for drug discovery and development. Cancer Research 74, 2377–2384.
- Witkiewicz, A. K., Balaji, U., Eslinger, C., McMillan, E., Conway, W., Posner, B., ... Knudsen, E. S. (2016). Integrated patient-derived models delineate individualized therapeutic vulnerabilities of pancreatic cancer. *Cell Reports* 16, 2017–2031.
- Wu, Y., Gan, Y., Yuan, H., Wang, Q., Fan, Y., Li, G., ... Tu, H. (2016). Enriched environment housing enhances the sensitivity of mouse pancreatic cancer to chemotherapeutic agents. *Biochemical and Biophysical Research Communications* 473, 593–599.
- Wulf-Goldenberg, A., Eckert, K., & Fichtner, I. (2011). Intrahepatically transplanted human cord blood cells reduce SW480 tumor growth in the presence of bispecific EpCAM/ CD3 antibody. *Cytotherapy* 13, 108–113.
- Wulf-Goldenberg, A., Stecklum, M., Fichtner, I., & Hoffmann, J. (2015). Preclinical model of patient-derived tumor xenograft in humanized mice. [Abstract]. Proceedings of the 107th annual meeting of the American Association for Cancer Research. Cancer Research 76(14 Suppl). (Abstract nr 5200).
- Yamaguchi, M., Osuka, S., Weitzmann, M. N., El-Rayes, B. F., Shoji, M., & Murata, T. (2016). Prolonged survival in pancreatic cancer patients with increased regucalcin gene expression: Overexpression of regucalcin suppresses the proliferation in human pancreatic cancer MIA PaCa-2 cells in vitro. International Journal of Oncology 48, 1955–1964.
- Yamamoto, M., Zhao, M., Hiroshima, Y., Zhang, Y., Shurell, E., Eilber, F. C., ... Hoffman, R. M. (2016). Efficacy of tumor-targeting Salmonella A1-R on a melanoma patient-derived orthotopic xenograft (PDOX) nude-mouse model. *PloS One* 11(8), e0160882.
- Yip-Schneider, M. T., Wu, H., Stantz, K., Agaram, N., Crooks, P. A., & Schmidt, C. M. (2013). Dimethylaminoparthenolide and gemcitabine: A survival study using a genetically engineered mouse model of pancreatic cancer. *BMC Cancer* 13, 194.
- Yu, W., Ma, Y., Shankar, S., & Srivastava, R. K. (2016). Role of SATB2 in human pancreatic cancer: Implications in transformation and a promising biomarker. *Oncotarget*. http:// dx.doi.org/10.18632/oncotarget.10860 (Epub ahead of print).
- Zechner, D., Bürtin, F., Albert, A. C., Zhang, X., Kumstel, S., Schönrogge, M., ... Vollmar, B. (2016). Intratumoral heterogeneity of the therapeutical response to gemcitabine and metformin. *Oncotarget*. http://dx.doi.org/10.18632/oncotarget.10892 (Epub ahead of print).
- Zechner, D., Bürtin, F., Amme, J., Lindner, T., Radecke, T., Hadlich, S., ... Vollmar, B. (2015). Characterization of novel carcinoma cell lines for the analysis of therapeutical strategies fighting pancreatic cancer. *Cell & Bioscience* 5, 51.
- Zhang, X., Claerhout, S., Prat, A., Dobrolecki, L. E., Petrovic, I., Lai, Q., ... Lewis, M. T. (2013). A renewable tissue resource of phenotypically stable, biologically and ethnically diverse, patient-derived human breast cancer xenograft models. *Cancer Research* 73, 4885–4897.