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## Animal Models: Challenges and Opportunities to Determine Optimal Experimental Models of Pancreatitis and Pancreatic Cancer

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### Abstract

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#### Disclosure

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At the 2018 PancreasFest meeting, experts participating in basic research met to discuss the plethora of available animal models for studying exocrine pancreatic disease. In particular, the discussion focused on the challenges currently facing the field and potential solutions. That meeting culminated in this review that describes the advantages and limitations of both common and infrequently utilized models of exocrine pancreatic disease, namely pancreatitis and exocrine pancreatic cancer. The objective is to provide a comprehensive description of the available models, but also to provide investigators with guidance in the application of these models to investigate both environmental and genetic contributions to exocrine pancreatic disease. The content covers both nongenetic and genetically engineered models across multiple species (large and small). Recommendations for choosing the appropriate model as well as how to conduct and present results are presented.

## Keywords

pancreatitis; pancreatic cancer; animal models

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## 1. Introduction

Pancreatitis and pancreatic cancer are highly variable diseases with a range of etiologies and disease courses. The use of animal models in the scientific exploration of these diseases is critical. Animal models are vital tools for understanding pathophysiology and they are a key step in the drug development pipeline as well as disease biomarker discovery. In general, it has been difficult to translate findings from animal studies into meaningful changes in clinical care. During the 2018 PancreasFest conference, a working group met to discuss the challenges with animal models. The goal of this report is to emphasize issues that should be considered when performing animal studies of exocrine pancreatic disease.

Choosing the most appropriate model for a study is contingent upon the specific question being asked, what aspects of the disease are relevant as well as the time and resources one has available (**Fig. 1**). This report introduces nongenetic and genetic engineered models of pancreatitis and exocrine pancreatic cancer and identifies the key features of each model. In particular, it emphasizes the multiple factors contributing to inter-model and intra-model variability and their direct effects on the manifestation of disease.

## 2. Nongenetic models

### 2.1 Pancreatitis

Pancreatitis is an umbrella diagnosis that includes sub-classifications such as acute, recurrent and chronic. It can be further characterized by the presence or absence of necrosis. Multiple non-genetic factors have been identified that increase the risk of developing pancreatitis or of more severe disease; some can be recapitulated in animals. For example, exposure to a specialized (high fat) diet, alcohol feeding, endoscopic retrograde cholangio-pancreatography (ERCP), or duct obstruction can induce pancreatitis in animals in a manner similar to clinical pancreatitis in humans (Table 1). Models have been developed based on the known biological processes contributing to pancreatitis. For instance, acute pancreatitis

(AP) involves premature activation of trypsinogen within the pancreas. Administration of supraphysiologic concentrations of the cholecystokinin analog cerulein is a popular method of inducing experimental pancreatitis. Such a “hyperstimulation” model in rodents is generally used to induce mild to moderate injury and reflects the hyperstimulation pancreatitis observed in humans following exposure to certain scorpion bites and insecticides with cholinesterase activities. There are also several chemicals that, when infused into the pancreatic duct, may mimic the effects of bile reflux and its possible role in inducing biliary pancreatitis (Table 1). The technical challenges associated with bile acid and post-ERCP induced AP have likely prevented their wide spread adoption. Another group of animal models that are less commonly used are those that mimic uncommon forms of pancreatitis such as autoimmune pancreatitis. These models use foreign serum, adoptive cell transfer, or infectious pathogens to induce disease. There are potential advantages to using nongenetic models of pancreatitis, including their lower expense and, in many cases, the same method of induction can be achieved through multiple routes of administration of an agent and across both small and large animal species. Finally, the initiation of disease can often be highly synchronized across a cohort of animals.

Similar induction methods can sometimes be modified to phenocopy both acute and chronic types of pancreatitis. For example, the dosage, frequency, and route of administration of cerulein are regularly manipulated to experimentally induce both acute and chronic pancreatitis. Cerulein was first used to induce AP in rats using intravenous infusion, but it was later discovered that 6–8 hourly intraperitoneal injections also provoke mild AP whereas 12 hourly injections provoke more severe AP.<sup>1–4</sup> By repeating daily dosing with cerulein, one can replicate recurring episodes to develop a model of recurrent acute pancreatitis. However, many investigators use this paradigm, repeated dosing of cerulein, to generate chronic pancreatitis (CP) models. The dosing regimen is not standardized and the variability of the repeated cerulein paradigms was recently outlined by Klauss et al.<sup>5</sup> For example, bi-weekly sessions of 6–8 hourly injections for 6–10 weeks results in histological changes consistent with CP, but dramatic differences in fibrosis are seen in different mouse strains. Unlike clinical CP, when cerulein dosing is terminated, pancreata recover within 3–6 weeks.<sup>6</sup> Other groups have accelerated the repeated cerulein model and show that administering 6 hourly injections on 3 days per week for 3–4 weeks also recapitulates CP.<sup>7</sup> It is unclear at this time whether there are meaningful differences between the variable dosing paradigms for CP. It is also unknown whether a longer dosing schedule would generate irreversible pancreatic changes. Many laboratories currently choose the dosing paradigm based on what is most convenient for laboratory personnel; however, they have shown their chosen regimen works for their individual studies.

One step that must be taken to improve the comparability of results across studies and models is to standardize endpoints. The diagnosis and severity of pancreatitis relies heavily on pathologist-specific interpretations of histology: interstitial edema, acinar cell death (necrosis or vacuolization), parenchymal loss, hemorrhage, fat necrosis, inflammatory cell infiltrate, fat and fibrotic tissue replacement.<sup>8</sup> However, there are quantitative measures available for exocrine function, edema, immune responses, and endocrine function that should be employed alongside histological measures.<sup>9–14</sup> For example, serum enzyme levels (e.g. amylase or lipase) or intrapancreatic trypsinogen activation are standard exocrine-

related endpoints that should be included in all studies, particularly those focused on AP or early phases of CP. Furthermore, edema can be evaluated through objective comparisons of organ weight. Inflammation can be measured using a standard myeloperoxidase assay as well as pancreas-specific or systemic measures of cytokine expression. For severe or advanced disease models, measures of endocrine function such as glucose levels or glucose tolerance tests could also be informative. Though preliminary studies of stains for fibrosis are important, quantitative measures of collagen expression (collagen subtypes, hydroxyproline levels) should be performed when fibrosis is an endpoint. With all AP and CP models, the time course of pancreatitis can differ among species and within strains of the same species, as well as by age or by sex in some strains of rodents. Equally important is the time point at which a particular endpoint is measured. The peak and resolution of an endpoint is likely parameter specific and more thorough investigations are ongoing. For example, in the acute cerulein model initial zymogen activation within the acinar cell begins between 15 min and 1–2 h after the first cerulein injection, but inflammatory cells mediate this at later time points.<sup>15–18</sup> Thus, it is particularly important to consider previous literature and conduct preliminary studies to decide on the most appropriate time points to assess your chosen parameters (e.g. 1 h, 6 h, or 24 h after the first or last cerulein injection).<sup>19,20</sup>

## 2.2 Pancreatic Cancer

Multiple chemicals have been used to model tumorigenesis (Table 2). If these carcinogens are introduced systemically tumors develop across multiple organ systems. For instance, administration through oral gavage will incite lesions throughout the gastrointestinal tract whereas intraductal instillation will restrict tumor development to the pancreas.<sup>21–24</sup> More recently, investigators have largely moved away from chemically induced tumor models; instead, a variety of transplantation models have gained significant favor. There are several sources for the transplants that will be discussed below. The site of the transplant in the host animal can also vary, but the most popular locations are subcutaneous or directly into the pancreas. Investigators opt for subcutaneous placement because it is easy to observe tumor growth in the absence of advanced imaging technologies as well as to perform behavioral assays. For investigators interested in metastases, tumor cells can also be implanted into the target of interest such as liver or lung. Orthotopic transplantation of pancreatic tumor cells can be used to model primary tumor growth in a synchronized manner such that an entire cohort will have similar progression of disease.

The source of pancreatic tumor cells that are transplanted is critical. There are a multitude of human pancreatic tumor cell lines available.<sup>25–27</sup> To perform xenografts (interspecies transplantation), nude or SCID mice must be used so the foreign cells will not be rejected. More recently, the development of the patient-derived xenograft (PDX) provided a great hope toward the development of personalized therapies since drugs could be directly tested on each individual patient's tumor. The PDX model of pancreatic ductal adenocarcinoma (PDAC) has facilitated translational (from mouse to man) studies on pancreatic cancer. Manegold et al recently reported on “serial” subcutaneous implantations of PDX PDAC tissue into immunocompromised NOD/SCID/IL2r $\gamma$ <sup>-/-</sup> (NSG) mice to study the role of CBP/ $\beta$ -catenin-induced pancreatic cancer cell stemness in human PDAC carcinogenesis.<sup>28</sup> The investigators found that the first subcutaneous implantation of human PDAC into mice

that were treated with a specific small molecule CBP/ $\beta$ -catenin antagonist, ICG-001, did not inhibit the growth of the xenograft. However, the ICG-001-treated tumor (which grew during the first implantation) failed to grow during the second implantation. The investigators concluded that CBP/ $\beta$ -catenin antagonism of pancreatic cancer cell stemness was able to prevent propagation of human PDAC. Indeed and as previously reviewed,<sup>29</sup> such PDX models provide a couple of key advantages because the PDAC xenografts are comprised of cells which: 1) largely retain the appropriate genetic profile even after initial selection in the mouse; 2) display a high degree of heterogeneity characteristic of PDAC at least initially; and 3) are derived from humans.

Xenograft models do have several limitations; the key one being that transplant recipients must have a compromised immune system.<sup>30</sup> A relatively more physiological approach is the syngeneic or allograft model in which the donor cells and recipient are of the same species. Like xenografts, either primary tumor cells or cell lines can be used for allografts. Utilizing a syngeneic approach allows a better assessment of how the intact immune system contributes to tumor growth. Previously, the Panc02 cell line was the primary cell line readily available for such syngeneic mouse models. However, the major limitation of the Panc02 cell line is the finding by Logsdon and colleagues that they do not harbor mutations in Kras or p53 which represent the most common, classical mutations in PDAC,<sup>29</sup> hence severely diminishing the translational relevance of such models. More recently, Kras mutant cell lines, 6606PDA and 6606L, isolated from PDAC and liver metastasis, respectively, from KrasG12D (KC) mouse<sup>31</sup>, as well as Kras mutant and p53 mutant cell lines UN-KPC-960 and UN-KPC-961 derived from KrasG12D;Trp53R172H;Pdx1-Cre (KPC) mice<sup>32</sup>, have been developed and have been used in syngeneic models. Given these recently developed mouse cell lines which harbor relevant PDAC mutations as well as methods for harvesting primary tumor cells<sup>33</sup>, the syngeneic mouse model should facilitate more in-depth assessment of how the immune system contributes to the genesis of PDAC. Overall, transplantation models are useful because they are relatively easy and quick to set up. However, most transplant models are based on the implantation of differentiated tumor cells, which could be viewed as a drawback of this approach. In patients, there is a natural progression from normal to neoplastic cells. Transplantation models lack the opportunity to study how bodily systems function together to drive the transformation of 'normal' cells.

### 2.3 Environmental Modulators

Although nongenic models have weaknesses, there are advantages to using them for the study of exocrine pancreatic diseases. Nongenic models provide an opportunity to study the impact of environmental modulators including alcohol and tobacco, major risk factors for both pancreatitis and pancreatic cancer.<sup>34</sup> Combining exposure to these factor(s) with the nongenic models is a useful way to examine the role of the environment in the development and progression of pancreatic disease. The most common method to introduce alcohol exposure is through the Lieber-Decarli liquid diet that allows for easy modifications of the amount of ethanol.<sup>35</sup> Ethanol (EtOH) administration alone does not cause a pancreatitis phenotype; however, combined with chemical (e.g. dibutyltin dichloride (DBTC), oleic acid) or diet models, it can accelerate disease and increase severity.<sup>36-41</sup> Furthermore, EtOH in combination with its non-oxidative metabolites, fatty acid ethyl esters or a subclinical

stimulus dose of cerulein or lipopolysaccharide is also sufficient to produce pancreatitis.<sup>42–45</sup> EtOH use has also been associated with development of adenocarcinoma in the dimethylbenzanthracene (DMBA) model.<sup>46</sup> EtOH increases multiplicity, but not incidence in the rat azaserine model, but not the hamster N-Nitrosobis(2-oxopropyl)amine (BOP) model.<sup>47</sup> Interestingly, dietary fat enhances carcinogenesis in both the azaserine and BOP models. Cigarette smoke has also been shown to exacerbate nongenetic models of pancreatitis and pancreatic cancer.<sup>48–50</sup> By combining EtOH, smoke, and diet with nongenetic models of exocrine pancreatic disease, investigators have been able to confirm the degree that these environmental exposures affect the risk for development or increased severity of disease.

### 3. Genetic Models

To study pancreatitis and pancreatic cancer, investigators have developed a plethora of genetically engineered animal models. Three key aspects for developing a genetically engineered model include choosing a method to produce the animal, choosing the genetic alteration, and choosing the gene promoter that will drive expression of that genetic alteration. In addition to the CRISPR-Cas9 technology, several classical methodologies to generate genetically engineered models are well-described.<sup>51</sup> There are several gene promoters that are popular for use in genetically engineered models of exocrine pancreatic disease because they are enriched in the pancreas and, when combined with a conditional technology (e.g. Cre-Loxp), are thought to restrict recombination specifically to the pancreas (Table 3). However, it is important to realize that the method and the gene targeted have implications for differing interpretations of results. Specifically, using technologies that are not inducible means that the animal will express the genetic change in all cell types that express the gene at any time throughout development, including extra pancreatic tissues. In instances where the mutation of interest is a known germ line mutation, global recombination approaches may be optimal because they mimic hereditary disease. However, this could be considered a negative when the goal is to achieve pancreas or even cell-type specific mutations. PDX1 and p48/pft1a, the two most widely used gene promoters in exocrine pancreatic disease, are expressed in multiple cell types within the pancreas and can result in multiple foci of disease.<sup>52,53</sup> PDX1 is also expressed in duodenum and antrum of the stomach.<sup>54–56</sup> Moreover, both PDX1 and p48 are expressed in various divisions of the nervous systems. Pft1a/p48 is a key regulator in the differentiation of spinal cord dorsal horn neurons, determining GABAergic versus glutamatergic cell fate.<sup>57</sup> PDX1 is expressed in a subset of sensory and proprioceptive neurons and regulates neuronal calcium homeostasis.<sup>58</sup> Neuronal PDX1 is also involved in hypothalamic control of glucose metabolism.<sup>59,60</sup> Thus, extra pancreatic expression and/or function of ‘pancreas specific’ genes could contribute to disease phenotype and should be considered when interpreting data.

#### 3.1 Pancreatitis

There are several genetically engineered mouse models (GEMMs) that spontaneously exhibit pancreatitis-related phenotypes (Table 4). These models can be useful for questions regarding the course of idiopathic pancreatitis as well as testing pharmacological interventions. Although the majority of genetically engineered pancreatitis models are in the mouse, non-murine models are also available. The Wistar Bonn/Kobori (WBN/KOB) rat

exhibits degeneration of pancreatic parenchyma, widely distributed fibrosis, and infiltration of lymphocytes.<sup>61</sup> Overexpressing Sonic hedgehog or Indian hedgehog at the Ptf1a domain in zebrafish results in morphological changes in developing pancreas.<sup>62</sup> With age, these zebrafish show progressive pancreatic fibrosis intermingled with proliferating ductular structures and destruction of acinar structures. Our current understanding of hereditary pancreatitis has been significantly improved through the development of GEMMs. Animal models expressing mutations in trypsinogen or SPINK genes exhibit spontaneous pancreatitis or a predisposition to develop more severe disease following experimentally induced pancreatitis.<sup>63–66</sup> Like the combinatorial approach introduced earlier (nongenic method of induction plus exposure to environmental factors), a similar strategy is often utilized to study the role of a specific gene or signaling pathway in the development or severity of pancreatitis. In this scenario, a nongenic method of inducing pancreatitis is applied to GEMMs that do not exhibit a spontaneous disease phenotype. Investigators have successfully used this approach to implicate numerous signaling pathways in the development and severity of pancreatitis, including the complement system,<sup>67–71</sup> cytokine signaling,<sup>72–77</sup> immunoglobulins,<sup>78,79</sup> and, of course, protease (e.g. trypsin) pathways.<sup>15,16,80,81</sup> Based on the prior evidence implicating trypsin pathways, Sahin-Toth and colleagues recently created a gain of function trypsinogen mutant that effectively mimics hereditary pancreatitis.<sup>66</sup>

### 3.2 Pancreatic Cancer

The field of basic pancreatic cancer research has exploded with the development of GEMMs for pancreatic cancer. Mutations inducing a gain of function in the GTPase *Kras* occur in nearly all human pancreatic intraepithelial neoplasia (PanIN) and pancreatic ductal adenocarcinoma (PDAC).<sup>82,83</sup> Several basic GEMMs, described in Table 5, take advantage of oncogenic *Kras* expression combined with ‘pancreas specific’ promoters.<sup>82–89</sup> Although multiple mutations in *Kras* have been detected in PDAC patients, the most prevalent is G12D.<sup>90–92</sup> Thus, *Kras*<sup>G12D</sup> has become the backbone of PDAC GEMMs. The pathophysiology, histology, molecular, and clinical aspects of GEMMs that parallel the disease course of human pancreatic cancers are detailed elsewhere.<sup>83,93–96</sup> Briefly, GEMMs exhibit a slower disease course compared to transplant models. Lesions (e.g. PanIN or mucinous) develop followed by tumor formation, and in some cases, metastases.

Mutations in additional oncogenes such as TP53, CDKN2A, and SMAD4, occur in more than 50% of human PDAC cases.<sup>52,83,86–89,93</sup> The contribution of these genes to the development and progression of disease can be assessed with the complex *Kras*-based GEMMs described in Table 6.<sup>97–101</sup> Often one of the most obvious results of introducing additional pathological gene mutations is an acceleration of disease progression. The most popular complex *Kras*-based GEMM is the KPC model (*Kras*<sup>P53Cre</sup>). KPC was originally coined to describe a GEMM in which PDX1-cre drives *Kras*<sup>G12D</sup> and p53<sup>R172H</sup> mutations.<sup>102,103</sup> Over time, however, it has been more loosely applied to GEMMs that use either PDX1-cre or p48/Ptf1a-cre as well as those deleting a p53 allele (p53<sup>fl/+</sup>).<sup>104–107</sup> For mice to be viable, the *Kras* mutation must be heterozygous in these models. However, it is possible to make viable mice that express different zygosity for other gene alterations. The zygosity of any additional genetic alterations incorporated into a model can directly affect the time



course of disease progression. For instance, mice expressing a single mutant p53 allele ( $Kras^{G12D};p53^{fl/+}$ ) develop tumors around 16 weeks whereas dual mutant p53 alleles ( $Kras^{G12D};p53^{fl/fl}$ ) develop lethal tumors by 8 weeks.<sup>98</sup> We encourage authors to follow this example and provide explicit descriptions of their models in every publication to avoid confusion in the field.

A key challenge with making GEMMs is the complexity of the genetics. As one moves from the basic to more complex models, there is a reduction in both cost-effectiveness and efficiency in breeding and utilizing these models (e.g. only 1 in 8 or 1 in 16 mice could have the genotype of interest). Many investigators have also opted to incorporate reporters into their models to allow for tracking of pancreatic-lineage cells as they transform, disseminate, and metastasize.<sup>104–106,108,109</sup> Though a creative improvement on this already valuable technology, the resulting genetic complexity presents an increased risk for unknown or unexpected side effects. Bearing in mind the caveats of complex genetic models, GEMMs are a powerful tool to aid in our investigation of PDAC.

Although the majority of current pancreatic cancer models are Kras based, there are non-Kras based models. The first transgenic mouse models of pancreatic neoplasia were generated by expressing the full-length or truncated oncogenic simian virus 40 T antigen (SV40) under the rat elastase-1 promoter.<sup>110,111</sup> These mice exhibit acinar dysplasia that progresses to neoplasia in adulthood. Survival is approximately 4–6 months. More recently, non-Kras based models have been developed to study the contribution of specific tumor suppressor genes to the development of PDAC. Mutations in *pten* are commonly found in several tumor types. Stanger et al developed  $Pdx1-CreER^{TM};Pten^{lox/lox}$  in which *pten* is deleted from the pancreas and cre activity is confirmed through the Z/AP reporter.<sup>112</sup> Beginning at 3 weeks of age, mice exhibit an age-dependent phenotype with multifocal architectural changes. Acini are progressively replaced by proliferative mucin-expressing ductal structures, centroacinar cells proliferate, and ductal adenocarcinoma develops at 11 weeks. In order to parse out the contribution of the tumor suppressor genes Ink4a/Arf (p16<sup>Ink4a</sup>/p19<sup>Arf</sup>) and TP53, Bardeesy et al, examined the incidence, latency, and histological phenotype of PDAC following either hemi- or homozygous loss of p16 and p19 or p16 and p53.<sup>113</sup> These authors demonstrated that PDAC can develop in a non-Kras based GEMM, but also that specific tumor suppressor genotypes directly influence the phenotypes of resulting tumors.

### 3.3 Environmental Modulators

To explore the interactions between genetics and the environment, factors such as alcohol, diet, and tobacco can be paired with genetic models of disease. For instance, long-term ethanol feeding in the  $Mist1-creERT2;XBP1^{+/-}$  model of spontaneous pancreatitis causes increased endoplasmic reticulum stress, thereby enhancing acinar cell pathology.<sup>114</sup> Similarly, exposure to tobacco induces flattening of ductal epithelial cells and significantly increases atrophy in the  $rEla1;sshIL-1\beta$  model of spontaneous pancreatitis.<sup>76</sup> Ethanol and smoking have also been introduced into genetic models of pancreatic cancer. Exposing KC ( $Pdx1-Cre; K-Ras^{+}/LSLG12D$ ) mice to the Lieber-DeCarli alcohol diet in combination with cerulein results in synergistic and additive effects on PanIN formation.<sup>104,115</sup> Exposing KC

mice to tobacco smoke also stimulates PanIN development, fibrosis, activation of stellate cells and M2 macrophages, as well as increased expression of both stem cell and epithelial-mesenchymal transition markers.<sup>116,117</sup> Smoking was found to activate histone deacetylases and regulate cytokines such as interleukin-6 and interleukin-4, which promote cancer development at the early stage.<sup>116</sup> Smoking also activates stem cell features of pancreatic cells, through activation of PAF1 expression in KC mice.<sup>117</sup> The same pro-cancer effects were observed in *Kras*<sup>+/LSLG12V*geo*</sup>;Ela-tTA/tetO-Cre (Ela-KRAS) mice.<sup>118</sup> The effect of alcohol alone on promoting PDAC is less obvious than smoking in animal models. However, the combination of alcohol with pancreatitis further promotes the progression of the disease in KC mice.<sup>115</sup> Analogous to the association between pancreatitis and diet, a high fat diet and obesity are associated with increased risk for pancreatic cancer. Several groups have taken advantage of GEMMs in order to study the biological mechanisms linking obesity to pancreatic cancer. A high-fat lard-based diet in the Ela-CreERT; K-Ras<sup>+/LSLG12D</sup> GEMM promotes immune infiltration in addition to PanIN and PDAC development through regulating inflammation in a COX2 and lipocalin-2 dependent manner.<sup>119,120</sup> Similarly, a high-fat, high-calorie, corn oil-based diet given to EL-Kras and KC mice is associated with accrual of additional genetic mutations, as well as more extensive inflammation, fibrosis and neoplasia.<sup>121,122</sup> The incidence of cancer was particularly higher in males, which is thought to be associated with a sex-dependent localization of adipose (visceral vs. subcutaneous). Interestingly, a high calorie diet based on fish oil (menhaden) significantly delays PanIN progression.<sup>123</sup> Using these early stage models, including KC mice, is sometimes criticized because PDAC has not yet developed and never develops in most of these mice. However, understanding the changes in the cancer precursor cells is equally important to understanding changes in cancer cells as it will help develop a preventive strategy. Moreover, the data published in KC mice was confirmed in the *Pdx1-Cre;LSL-Kras*<sup>G12D</sup>; *Trp53*<sup>R172H/+</sup> (KPC) mice confirming that smoking and high fat diet up-regulate tumor growth and metastasis.<sup>118,124</sup> It is very important to use the best model to study the effect of environmental factors on PDAC promotion; it is equally important to choose the best time to start or stop treatments as the disease progresses with different kinetics in each model.

#### 4. Inducible Genetic models

Despite the ‘identical’ genetic background in models that use inbred strains, disease development and progression is variable across animals. Some investigators minimize this issue by utilizing inducible technologies to improve synchronization of experiments and avoid complications of expressing mutations throughout development of the animal. Many of the models described in Tables 4–6 take advantage of these inducible technologies. The most popular choice is to employ tamoxifen-dependent cre recombinase systems (e.g. CreER, CreERT, CreERT2) in which transgene expression is not induced until tamoxifen treatment. There are also models that take advantage of the tetracycline transactivator (tTA) method in which expression is triggered by the removal of doxycycline from drinking water or food.<sup>118,125–127</sup>

Viruses provide an alternative inducible method that allows more control over the localization of recombinant gene expression. For instance, adeno-associated virus serotype 6 (AAV6) encoding Ela-iCre infused into the pancreatic duct of calcineurin B1 (CnB1)<sup>fl/fl</sup>

mice results in acinar cell specific loss of CnB1 gene expression.<sup>128</sup> In newborn pigs, ductal expression of genes can be achieved by injecting AAV9 vectors into the celiac artery, accessed via umbilical catheterization.<sup>129</sup> In Swiss Webster mice, intraductal infusion of lentivirus has been used to drive expression of shRNAp53, *Kras*<sup>G12D</sup> and luciferase.<sup>130</sup> These mice develop pathology similar to human PDAC without requiring alterations of embryonic development. They develop PanINs with increasing severity followed by tumor formation 28 weeks post-virus injection. Mice also have elevated levels of cancer markers and, in some cases, liver and lung metastases. Inducible methods have advanced the field of GEMMs by improving temporal and spatial control over these models.

Viruses also have the advantage of enabling the development of genetically engineered animal models in larger species. Viral models are becoming a popular route for developing large animal models because of their importance in drug development and improved resemblance to humans. Previously, large animal (e.g. dog, non-human primate) studies have been restricted to case reports of spontaneous disease. One type of pancreatic disease that has benefited from a viral approach is cystic fibrosis associated pancreatitis. Mutations in the *CFTR* gene lead to dysregulation of fluid transport in multiple organs, primarily lung and pancreas. Low flow of secretions leads to duct obstruction, acini destruction, severe inflammation, fibrosis, and fat replacement. With the advent of recent advances in genetics including viral-mediated gene targeting and somatic cell nuclear transfer, global manipulation of the *CFTR* gene in larger species has become possible. Two pig models, global *CFTR*-null and *CFTR*-508, were generated using homologous recombination and somatic cell nuclear transfer.<sup>131,132</sup> Fetal and neonatal CF pigs have pancreatic lesions seen typically in humans with CF including progressive acinar cells loss, ductal plugging and fibrosis.<sup>133,134</sup> Although islets are morphologically normal, CF pigs demonstrate abnormal glycemic responses and decreased insulin secretion at birth.<sup>135</sup> A similar approach has been executed to generate *CFTR*-null ferrets.<sup>136</sup> CF ferrets exhibit a milder exocrine pancreatic phenotype compared to CF pigs. Newborn CF ferrets have only minor histological changes in the pancreas including dilated acini and ductules with inspissated, eosinophilic zymogen secretions, but no acinar atrophy and otherwise preserved pancreatic architecture.<sup>137</sup> The pancreatic disease progresses rapidly in CF ferrets after birth (>1 month) with loss of acini, inflammation, fibrosis, islet destruction/remodeling and hyperglycemia.<sup>138,139</sup> Another model that is worthwhile to mention is the *CFTR*-null zebrafish model.<sup>140</sup> Loss of *CFTR* leads to exocrine pancreatic destruction in zebrafish larva. The *CFTR* models are based on expression of global alterations, but viral-mediated approaches can also be used to make tissue specific manipulations. The oncopig cancer model (OCM) is a novel model in which pigs have combined *Kras*<sup>G12D</sup> and *TP53*<sup>R167H</sup> mutations under control of a cre-inducible vector.<sup>141</sup> Introduction of virus containing Ad-Cre directly into the pancreatic duct drives locally invasive disease that has the hallmarks of human PDAC including a dense fibroblastic stroma and acinar-to-ductal metaplasia.<sup>142</sup> The development of large species models creates a unique opportunity in which not only pharmacological therapies, but also novel surgical approaches, can be tested.

## 5. Pain

Pain is a prominent feature associated with both pancreatitis and pancreatic cancer. The role of the nervous system, neuroplasticity and neurogenic inflammation in exocrine pancreatic disease has been reviewed before.<sup>143–146</sup> Chronic pain, such as that associated with pancreatic disease, is often a result of sensitization of peripheral neurons. Several studies have used molecular tools to examine the expression of molecules involved in neurogenic inflammation and peripheral sensitization including growth factors, neuropeptides, and TRP channels.<sup>147–154</sup> A few labs have also performed functional studies (e.g. calcium imaging, electrophysiology) to directly assess sensory neuron excitability and sensitization.<sup>148,150,155–161</sup> Most studies, however, have focused on pain-associated behaviors (Table 7). There are two types of pain that can be assessed, experimentally evoked or ongoing pain. Experimentally evoked mechanical pain involves applying Von Frey monofilaments of increasing force to determine withdrawal thresholds. Application of radiant heat or placing the animal on a hot plate can be used to assess latency to respond to a noxious temperature. Finally, direct electrical stimulation of the pancreas evokes an abdominal muscle contraction called the visceromotor reflex (VMR). This is thought to be a model of referred pain. This technique can be used to determine the threshold to evoke a VMR or changes in the size of the VMR in controls versus animals with pancreatic disease. If the thresholds or latencies to evoke a response using any of these tests are reduced, this is interpreted as the animal experiencing pain. There are no strong models for directly measuring ongoing pain. In humans, however, ongoing pancreatic pain leads to hunching posture as well as a reduction in quality of life and spontaneous activity. Several investigators have taken advantage of this and used a variety of measures thought to indicate ongoing pain: reduced rearing, grooming, wheel running and ambulation as well as increased hunching, vocalization and catalepsy. Unfortunately, pancreatic pain related studies have been limited to rats and mice whose neural innervation, ductal structure, location, and gross anatomy is quite different from humans. Future studies need to address pain in a wider variety of animal models, including larger species, in order to improve our understanding of pain within the context of exocrine pancreatic disease.

## 6. Choosing a Model

One must consider the strengths and weaknesses implicit to each model as well as available resources and expectations regarding experiments and findings. Just as important, however, is which animal system to choose. Nongenetic models can be applied to a greater variety of species, but there are a variety of potential responses. Indeed, it is important to choose the most appropriate method (+/- additional environmental factors) to induce pancreatitis and/or pancreatic cancer. For instance, the nitrosamine BOP preferentially drives pancreatic tumorigenesis in rodents, but not dogs (Table 2). There are also species differences associated with the nongenetic models of pancreatitis. For example, cerulein administration to rats results in more interstitial edema and intracellular vacuolization while mice develop more acinar cell necrosis: a difference that may be explained by species-dependent roles of apoptosis and autophagy.<sup>162,163</sup> Mechanical models of pancreatitis have been applied across the widest range of species, from mice to non-human primates, and a diversity of responses have been reported.<sup>164</sup> In most species, ductal ligation and bile acid infusion evokes mild

disease; however, pigs and opossums exhibit severe disease. The variability in mechanical models is likely a consequence of the anatomical and functional differences across species.<sup>165–167</sup> Grossly, mouse and rabbit, for example, have diffuse pancreata scattered within the mesentery whereas dog, hamster, and pig have compact pancreata more closely resembling the retroperitoneal solid pancreas found in humans. Additionally, the ductal structure varies widely across species. Rats have a single outflow channel that may explain why rats develop fibrosis more rapidly than dogs following ductal ligation. Mice, on the other hand, have multiple ducts. This can provide an internal control within each animal if desired, but it also makes it technically challenging to execute a complete obstruction. When discussing data, it is important to consider how the anatomy of the animal may contribute to observed phenotypes.

Within a species, there are also important considerations when choosing a model (e.g. age, sex, strain, body fat). For instance, mouse strain directly impacts the severity of pancreatitis and systemic inflammation following intraductal infusion of taurocholate.<sup>168</sup> Serum enzymes and morphological damage are increased compared to controls in nine different strains; however, NOD/SHILT and AKR/J mice had enzyme activity significantly higher than the other strains. Further, only half of the strains exhibited elevated IL-6, a marker of inflammation. Even within substrains, differences in the severity of disease can be observed. C57BL/6J mice, for example, are more susceptible to cerulein-induced pancreatitis than their C57BL/6NHsd counterparts with regard to atrophy, morphological changes, and fibrosis.<sup>169</sup> Strain differences have also been reported in GEMMs. A study examining the effects of high fat diets on PDAC in the *Ela-Kras<sup>G12D</sup>* GEMM, reported that the incidence, frequency, and size of pancreatic neoplasia were significantly increased in mice on a F1 background as compared to those on the FVB background.<sup>170</sup> Differences are not restricted to strain, but also appear with respect to sex. Females, for instance, exhibit a much greater sensitivity to the choline-deficient ethionine-supplemented (CDE) diet because CDE induces hemorrhagic necrosis in an estrogen-dependent manner.<sup>171,172</sup> Another consideration is body fat status. The co-administration of interleukin-12 and interleukin-18, cytokines elevated in AP patients, drives edematous AP in wild type or lean mice.<sup>173</sup> However, the same doses induce necrotizing pancreatitis in both diet-induced obese mice as well as a GEMM of obesity called *ob/ob* mice.<sup>173,174</sup> Finally, age understandably plays a role in the development of disease in non-inducible genetically engineered models. However, age has also been implicated in non-genetic models. For example, in the cerulein paradigm for AP, a loss of uncoupling protein 2 aggravated the severity of disease in older but not young mice.<sup>175</sup>

## 7. Conclusion

The goal of animal models is to reproduce human disease including etiology, histopathology, pathophysiology, and therapeutic responsiveness. All of the animal models presented have both strengths and weaknesses with regard to disease phenotype. There are also considerations with respect to time and cost. The rationale for which model is chosen for a study should be clearly explained in publications. With the complex nature and variability of these models, it is essential to be overly transparent and provide explicit details regarding the design of the model used in a particular study, including which features of disease it

successfully recapitulates. Several of the models available provide an opportunity to examine the synergy between environmental and genetic contributors to disease, which would expand our understanding of the pathogenesis and progression of exocrine pancreatic diseases. However, neuroplasticity and pain are key features of both pancreatitis and pancreatic cancer. If models are going to be truly translational moving forward, they should also recapitulate alterations in the nervous system. At this time, how the nervous system is affected is unknown for most of the available models. To improve translation of basic pancreas research to clinically relevant therapies, there must be methods in place to ensure thorough interpretation of data, comparison across studies, and validation of findings. Towards that end, all future studies should incorporate objective quantitative measures to allow for these direct comparisons. Furthermore, in the event that potential therapeutics is identified, we strongly recommend simultaneous testing and/or validation by an independent laboratory at another institution. Indeed, such cross-validation should be incorporated into studies during the design phase, as potential collaborators are easily identified through the numerous pancreas associations and consortiums. Although we have made much progress, continued refinement of currently available models along with development of newer models will be important for bridging the gap between basic science and the clinic.

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1. What is your hypothesis? Specific Aims?
2. Within that scope, what questions are you asking?
3. Is there a particular gene or protein of interest?
4. Which cell compartment are you interested in?
5. Does this entail cell-cell or protein-protein interactions?
6. Do you have in vivo/in vitro preliminary data as a guide?
7. Are you focused on signal transductions, pathology, therapy, or combo?
8. What is your time frame? Budget?

**FIGURE 1.**

Questions that should be considered during study design when choosing an animal model.

TABLE 1.

## Nongenetic Models of Pancreatitis

Model	Examples	Type	Species	Features	References
Secretagogue	cerulein	AP RAP/CP	rat mouse hamster dog	<p>Acute:</p> <ul style="list-style-type: none"> <li>5–12 hrly injections</li> <li>Atrophy, fibrosis, hyperamylasemia, edema, acinar vacuolization, leukocyte infiltrate, duct distortion, pain</li> <li>Simulates AP in humans that is induced by Trinidadian scorpion toxin and anti-cholinesterase insecticides</li> </ul> <p>RAP/Chronic:</p> <ul style="list-style-type: none"> <li>5–8 hrly injections, 1–3 times/wk, 3–10 wks.</li> <li>fibrosis, atrophy, decreased lipase, adipose replacement, necrosis, elevated glucose, leukocyte infiltrate</li> </ul> <p>Routes of administration: i.v., s.c., i.p.</p>	AP <sup>1–4,8–11,13,14,150,176–178</sup> RAP/CP <sup>5–7,12,179–181</sup>
Amino Acid	L-arginine L-lysine L-ornithine	AP CP	rat mouse hamster	<p>Acute:</p> <ul style="list-style-type: none"> <li>dose-dependent acinar necrosis</li> <li>edema, hyperamylasemia, leukocyte infiltrate, high morbidity/mortality, including organ failure.</li> </ul> <p>Chronic:</p> <ul style="list-style-type: none"> <li>long-term administration</li> <li>pancreatic atrophy, fibrosis</li> </ul> <p>Routes of administration: i.p.</p>	L-arginine <sup>8,14,153,182–186</sup> L-lysine <sup>187</sup> L-ornithine <sup>188</sup>
Cytokine	Interleukin-12 + Interleukin-18	AP	mouse	<p>Acute:</p> <ul style="list-style-type: none"> <li>2 injections, 24 hr apart of interleukin-12 (150 ng) and interleukin-18 (750 ng)</li> <li>100% lethal in ob/ob (B6.V-<i>Lep<sup>ob</sup></i>) mice within 48 hrs after 2<sup>nd</sup> injection</li> <li>Lean mice develop edematous AP whereas ob/ob mice or diet-induced obese mice develop necrotizing AP.</li> <li>Obese mice show greater increase in serum amylase, lipase, and calcium, heightened acute-phase responses, and do not engage repair pathways (e.g. Reg I and PAP).</li> </ul> <p>Routes of administration: i.p.</p>	173,174
Diet	Lieber-DeCarli High Fat CDE	AP CP	rat mouse hamster	<p>Lieber-DeCarli:</p> <ul style="list-style-type: none"> <li>EtOH containing liquid diet</li> </ul>	Lieber-DeCarli <sup>14,35,189,190</sup> CDE <sup>4,171,191,192</sup> High Fat <sup>36,177,193</sup>

Model	Examples	Type	Species	Features	References
Chemical (bile)	TNBS DBTC LPS NaTC Oleic Acids	AP CP	rat mouse hamster rabbit dog cat pig	<ul style="list-style-type: none"> <li>• EtOH must be paired with an additional stimulus</li> </ul> <p>High Fat:</p> <ul style="list-style-type: none"> <li>• high fat diet combined with other stimuli (e.g. EtOH)</li> <li>• high fat alone induces slight increases in acinar hypertrophy, autophagy, apoptosis, necrosis, edema/inflammation, vascular injury, and duct changes</li> </ul> <p>CDE:</p> <ul style="list-style-type: none"> <li>• can be adjusted to achieved desired mortality rate</li> <li>• Constant CDE results in AP featuring severe hemorrhagic necrosis, local acinar/fat necrosis, transient atrophy, mild fibrosis; female-specific</li> <li>• Intermittent CDE results in CP phenotype.</li> <li>• Requires young female mice</li> </ul> <p>Routes of administration: oral</p>	TNBS <sup>161,194,195</sup> DBTC <sup>196-198</sup> LPS <sup>199,200</sup> NaTC <sup>14,168,201-204</sup> Oleic acids <sup>205-211</sup>
Mechanical	Ligation/Obstruction Vascular/Ischemia	AP RAP/CP	rat mouse hamster rabbit dog opossum pig cat	<p>Early phase: leukocyte infiltrate, edema, hyperamylasemia, apoptosis</p> <p>Late phase: atrophy, fibrosis, necrosis</p> <p>Invasive procedure</p>	ligation/obstruction <sup>4,8,21,2-224</sup> vascular/ischemia <sup>25-230</sup>
Post-ERCP	radiocontrast hydrostatic pressure acidic saline	Post-ERCP	rat mouse	<p>edema, increased serum amylase and markers of inflammation, neutrophil infiltration</p> <p>Routes of administration: intraductal infusion of radiocontrast or acidic saline; 100 mm Hg for 10 min</p>	128,231-234
Infectious	E. coli Meningococci Coxsackie B	AIP	mouse rabbit goat	<p>fatty tissue replaces acini; leukocyte infiltrate</p> <p>Routes of administration: i.p. or intraductal</p>	190,235-237
Immune	Adoptive transfer Foreign serum	AIP	rabbit rat	<p>necrosis, inflammation, hyperamylasemia</p> <p>Routes of administration: i.p. or intraductal</p>	238-241

AP indicates acute pancreatitis; RAP, recurrent acute pancreatitis; CP, chronic pancreatitis; i.v., intravenous; s.c., subcutaneous; i.p., intraperitoneal; ob/ob, leptin-deficient obese mouse; CDE, choline-deficient ethionine-supplemented; TNBS, 2,4,6-trinitrobenzenesulfonic acid; DBTC, dibutyltin dichloride; LPS, lipopolysaccharide; NaTC, sodium taurocholate; mm HG, millimeters mercury; Post-ERCP, post endoscopic retrograde cholangio-pancreatography; AIP, autoimmune pancreatitis



TABLE 2.

## Chemical Models of Pancreatic Cancer

Chemical	Key Features	References
Nitrosamines	<ul style="list-style-type: none"> <li>• N-Nitrosobis(2-oxopropyl)amine (BOP)/N-nitroso(2-hydroxypropyl)(2-oxopropyl)amine (HPOP)               <ul style="list-style-type: none"> <li>– Neoplasms around 14–17 wks.</li> <li>– lesions develop in ducts</li> <li>– 50% acquire genetic mutations associated with PDAC</li> <li>– dietary fat enhances carcinogenesis</li> </ul> </li> <li>• methyl-2-oxopropyl nitrosamine (MOP)               <ul style="list-style-type: none"> <li>– more potent, shorter latency than BOP</li> <li>– 80% penetrance</li> </ul> </li> <li>• N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG)               <ul style="list-style-type: none"> <li>– Administered to canines</li> <li>– Results in duct dilation, hyperplasia, ductal adenocarcinoma</li> </ul> </li> <li>• Species Differences               <ul style="list-style-type: none"> <li>– Most effective in Syrian golden hamsters</li> <li>– Sprague-Dawley rats more sensitive in respiratory/upper digestive tract</li> <li>– Wistar-MRC rats commonly develop thyroid tumors</li> <li>– Mice commonly have liver and lung involvement</li> <li>– i.p. BOP does not cause pancreatic tumors in dogs</li> </ul> </li> </ul>	21–23,47,242–247
DMBA	<ul style="list-style-type: none"> <li>• 7,12-Dimethylbenzanthracene (DMBA)               <ul style="list-style-type: none"> <li>– Found in cigarette smoke, charbroiled foods, exhaust</li> </ul> </li> <li>• Phenotypic PDAC               <ul style="list-style-type: none"> <li>– Local administration leads to PanINs and PDAC at 30–60 days</li> </ul> </li> <li>• Alcohol enhances adenocarcinoma development</li> <li>• Nicotine promotes DMBA carcinogenesis</li> <li>• High fat/high protein diet promotes DMBA carcinogenesis</li> </ul>	46,50,248,249
Azaserine	<ul style="list-style-type: none"> <li>• Wistar/Lewis Rats               <ul style="list-style-type: none"> <li>– Atypical acini/nodules form at 4mo</li> <li>– Acinar cell carcinomas and adenomas at 12–18 mo.</li> </ul> </li> </ul>	47,250–253

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Chemical	Key Features	References
	<ul style="list-style-type: none"><li>- Dietary fat enhances carcinogenesis</li><li>• Outbred CD-1 mouse strain does not develop advanced lesions</li><li>• Routes of administration: i.p.; s.c.</li></ul>	

**TABLE 3.**  
Common Genes Used to Drive Expression in Genetic Models of Pancreatic Disease

Gene	Conditional/Inducible Expression Systems Available	Target Cell Population
<i>PDX1</i>	Cre CreER	Acinar Ductal Endocrine Centroacinar
<i>Ptf1a/p48</i>	Cre CreERT	Acinar Ductal Endocrine Centroacinar
<i>Nestin</i>	Cre	Acinar Acinar progenitor
Elastase-1 (Ela)	Kras <sup>G12D</sup> Cre CreERT2 tTA	Acinar
<i>proCPA1</i>	CreERT2	Acinar
<i>Mist1</i>	Cre CreERT2	Acinar
<i>Rip</i>	CreERT	Insulin-expressing cells
<i>Insulin</i>		Islet
<i>Neuregulin 3 (Ngn3)</i>	Cre	Islet

TABLE 4.

## GEMMs With a Spontaneous Pancreatitis Phenotype

GEMM	Phenotype	References
rEla1:sshL-1 $\beta$	<ul style="list-style-type: none"> <li>Pancreatic atrophy, dilation of ducts occurred secondary to proximal fibrotic stenosis, increase acinar proliferation and apoptosis, increased expression of chemokines and cytokines</li> <li>Crossing in a p53 mutation to obtain Elastase sshL-1<math>\beta</math>*p53R172H<sup>+/+</sup> results in the development of PanINs and lesions indicative of acinar to ductal metaplasia</li> <li>Exposure to aqueous extract of cigarette smoke or Snus containing diet results in ductal epithelia flattening and proliferation, and glandular atrophy</li> <li>High penetrance</li> </ul>	76,254
rEla1;mPRSS1 <sup>R122H</sup> (hereditary pancreatitis)	<ul style="list-style-type: none"> <li>7 wks-acinar damage</li> <li>12 wks-inflammatory infiltrate</li> <li>24 wks fibrosis</li> <li>40% penetrance by 1yr</li> </ul>	63
rEla1;hPRSS1 <sup>R122H/N29I</sup> (hereditary pancreatitis)	<ul style="list-style-type: none"> <li>Fibrosis</li> <li>Pathology consistent with chronic pancreatitis (&gt;9month)</li> <li>10% penetrance</li> </ul>	64
PACE-try(on) Ela1-creERT;ratPRSSII (hereditary pancreatitis)	<ul style="list-style-type: none"> <li>AP only, rapid caspase-3 activation, apoptosis with delayed necrosis, loss of acinar cells leads to replacement with fatty tissue.</li> <li>Chronic inflammation or fibrosis does not develop.</li> </ul>	65
Ela1-cre;PERK <sup>-/-</sup>	<ul style="list-style-type: none"> <li>Hyperglycemic by 4 wks</li> <li>Induction of ER stress response, nonapoptotic acinar cells death pronounced inflammation</li> <li>Exocrine and endocrine insufficiency</li> </ul>	255
Ela1-LT $\alpha$ b (Ela1-LT $\beta$ )	<ul style="list-style-type: none"> <li>Model of AIP, overexpression of lymphotoxin beta</li> <li>Formation of B and T cell zones (tertiary lymphoid tissues)</li> <li>Systemic autoimmune response</li> <li>Chronic inflammation (in pancreas)</li> </ul>	256
PDX1-cre;IKK $\alpha$ <sup>-/-</sup>	<ul style="list-style-type: none"> <li>Elevated serum amylase and lipase</li> <li>progressive acinar cell vacuolization and death, interstitial fibrosis, inflammation, and compensatory proliferation</li> </ul>	257

GEMM	Phenotype	References
PDX1-cre;Kif3a <sup>-/-</sup>	<ul style="list-style-type: none"> <li>By 2 wks, acinar to ductal metaplasia and periductal fibrosis is evident</li> <li>By 12wks, acinar cells are replaced by adipose</li> <li>By 6mo, pancreata composed of enlarging cysts</li> </ul>	258
PDX1-cre;SRF <sup>-/-</sup>	<ul style="list-style-type: none"> <li>After weaning, mice develop profound morphological alterations of the exocrine pancreas, which were reminiscent of severe pancreatitis.</li> <li>massive acinar injury, Nuclear Factor Kappa B activation and proinflammatory cytokine release leads to complete destruction of the exocrine pancreas and its replacement by adipose tissue</li> </ul>	259
Ptf1a-cre <sup>ex1</sup> ; Atg5 <sup>-/-</sup>	<ul style="list-style-type: none"> <li>Greater penetrance in male</li> <li>Inflammation, necrosis, acinar-to-ductal metaplasia, acinar hypertrophy and degeneration, increased ROS, decreased glutamate metabolism, reduced autophagy, increased p62, ER and mitochondrial stress</li> </ul>	260
Mist1-creERT2;XBP1 <sup>+/-</sup>	<ul style="list-style-type: none"> <li>Activation of the unfolded protein response</li> <li>Extensive apoptosis followed by recovery and regeneration of acini</li> </ul>	114,261
hInsulin;TGFB	<ul style="list-style-type: none"> <li>Fibrosis and inflammation</li> </ul>	262
hK5; COX-2	<ul style="list-style-type: none"> <li>Overexpression of COX2 under the bovine K5 promoter induces CP (fibrosis and chronic inflammation) by 3 mo.</li> <li>At 5–6 mo pancreatic hypertrophy</li> <li>At 6–8 mo, dysplasia</li> </ul>	263
hK8	<ul style="list-style-type: none"> <li>Overexpression of human keratin 8</li> <li>Exocrine pancreas alterations, includes dysplasia, loss of acinar architecture, redifferentiation of acinar to ductal cells, inflammation, fibrosis, and substitution of exocrine by adipose tissue, as well as increased cell proliferation and apoptosis.</li> <li>Extra pancreatic epithelial changes also observed</li> </ul>	264,265
CPA1 <sup>N356K</sup>	<ul style="list-style-type: none"> <li>C57Bl/6 mice harbor human p.N256K mutation in exon 7 of mouse <i>Cpa1</i> locus</li> <li>At 3 mo., pancreata begin to atrophy (decrease weight) with 35–40% decrease @ 6 mo</li> <li>Loss of acini (30% by 12 mo), inflammatory infiltrate, presence of pseudotubular complexes (acinar-to ductal metaplasia), widespread fibrotic changes</li> <li>At 1 mo., elevated circulating amylase that is lost by 3 mo</li> <li>Elevated intrapancreatic trypsin and markers of endoplasmic reticulum stress</li> </ul>	266
SPINK3 <sup>-/-</sup> (hereditary pancreatitis)	<ul style="list-style-type: none"> <li>Pancreata initially develop normally</li> <li>acinar degeneration starts around E16.5</li> </ul>	267–269

GEMM	Phenotype	References
	<ul style="list-style-type: none"> <li>• Rapid onset cell death begins within days of birth</li> <li>• Mice die by P14.5</li> </ul>	
D23A (hereditary pancreatitis)	<ul style="list-style-type: none"> <li>• T7D23A knock-in mouse carries a heterozygous p.D23A mutation in mouse cationic trypsinogen (isoform T7) on a C57BL/6N background</li> <li>• Pancreata are normal for first 2–3 weeks of life, with diminution in size visible by 2 months</li> <li>• 4–5 weeks of age               <ul style="list-style-type: none"> <li>– 40% exhibit typical AP at 4–5 weeks, characterized by edema, inflammatory infiltrate (dominated by neutrophils and macrophages), and localized necrosis</li> <li>– &gt;50% exhibit minimal parenchyma and widespread regeneration including fibrosis and stellate cell activation</li> <li>– Elevated amylase in 40% of mice</li> </ul> </li> <li>• 2–12 months of age               <ul style="list-style-type: none"> <li>– Elevated intrapancreatic trypsin activity</li> <li>– Adipose replacement dominated, while inflammatory infiltrate decreased</li> <li>– Distorted ducts surrounded by prominent fibrosis</li> <li>– No calcifications and no loss of islets observed</li> </ul> </li> </ul>	66
LXR $\beta$ <sup>-/-</sup>	<ul style="list-style-type: none"> <li>• Liver X receptor <math>\beta</math> was removed using cre-transgenic <i>deleter</i> mouse strain</li> <li>• Inflammation, duct dilation, fibrosis</li> <li>• Pancreatic exocrine insufficiency</li> </ul>	270
LAMP2 <sup>-/-</sup>	<ul style="list-style-type: none"> <li>• Begins with acinar cell vacuolization and progresses to severe damage, macrophage infiltration, and acinar cell death</li> <li>• Decreased amylase, increased MPO, Caspase-3</li> </ul>	271
MHCII <sup>-/-</sup> (aged)	<ul style="list-style-type: none"> <li>• At 6 mo, periductal lymphocytic infiltrate observed that progresses to selective acinar cell destruction</li> <li>• Significant decrease in amylase and lipase</li> <li>• Adoptive transfer of splenic mononuclear cells confers disease upon recipient</li> </ul>	272
E2F1 <sup>-/-</sup> /E2F2 <sup>-/-</sup>	<ul style="list-style-type: none"> <li>• endocrine and exocrine cell dysplasia</li> <li>• a reduction in the number and size of acini and islets, and their replacement by ductal structures and adipose tissue.</li> <li>• Mutant pancreatic cells exhibit increased rates of DNA replication but also of apoptosis, resulting in severe pancreatic atrophy</li> </ul>	273
IKK2 <sup>CA</sup> constitutively active, inducible	<ul style="list-style-type: none"> <li>• CMVrtTA X tetO-IKK2-EE(IKK2)<sup>CA</sup> <ul style="list-style-type: none"> <li>– Doxycycline induces constitutive IKK2 activity via S177E and S181E mutations</li> <li>– only minor damage, leukocyte infiltration, elevated RANTES and TNF<math>\alpha</math></li> </ul> </li> </ul>	126,127,274

GEMM	Phenotype	References
	<ul style="list-style-type: none"> <li>• Ela1-rTA X Luciferase-(tetO)<sup>+</sup>-IKK2<sup>CA</sup>, Ela-creERT X LSL-IKK2</li> <li>– Tamoxifen induces constitutive IKK2 activity in acinar cells</li> <li>– Results in edema, immune infiltration, necrosis, elevated lipase, and fibrosis</li> <li>– Severity increased by co-expressing p65</li> </ul>	
MRL-Mp <sup>lpr/lpr</sup> (autoimmune pancreatitis, AIP)	<ul style="list-style-type: none"> <li>• Express lymphoproliferative gene (lpr)</li> <li>• Incidence of pancreatitis is significantly greater in females, with the highest incidence (74%) in mice 34–38 weeks</li> <li>• spontaneous inflammatory lesions characterized by mononuclear cell infiltration, acini destruction, and replacement of parenchyma by adipose</li> <li>• Treating MRL-Mp mice with repeated injections of polyinosinic-polycytidylic acid drives a Type I AIP, characterized by enhanced levels of IgG4 antibodies which is associated with systemic IgG4-related disease.</li> </ul>	275–277

TABLE 5.

## Basic Kras-based GEMMs

GEMM	Lesions (Incidence %)			Observations	References
	PanIN	PDAC	MET		
Elastase-TGF- $\alpha$	100	10	No	KrasG12D accelerates progression of PanIN	278
Elastase-KRAS <sup>G12D</sup>	100 (CPN)	No	No	Acinar expression of human KRASG12D drives CPN with ductal histotype	279
Pdx1-Cre; K-Ras <sup>+/LSL</sup> G12D (KC model)	100	<20	Yes	Impossibility to assess cell of origin Inflammation accelerates EMT and PanIN	102,104,280
Ptf1a-Cre; K-Ras <sup>+/LSL</sup> G12D	100	<20	Yes	Impossibility to assess cell of origin	102
Elastase-tTA; Tet-O-Cre; K-Ras <sup>+/LSL</sup> G12D <sup>geo</sup>	90	10	No	Pancreatitis accelerates PanIN development	125
Nestin-Cre; K-Ras <sup>+/LSL</sup> G12D	100	No	No	Short survival due to CNS complications. PDAC initiation & progression accelerated by AP	281,282
Elastase-tTA; Tet-O-Cre; K-Ras <sup>+/LSL</sup> G12D <sup>geo</sup> plus cerulein	100	20	No	PanIN development requires pancreatitis	125
Elastase-CreER; K-Ras <sup>+/LSL</sup> G12D	36	NA	No	Leaky sperm. Short-term study.	283
Elastase-CreERT2; K-Ras <sup>+/LSL</sup> G12D	63	No	No	PanIN development does not require pancreatitis	284
Mist1-CreERT2; K-Ras <sup>+/LSL</sup> G12D	30	No	No	PanIN development does not require pancreatitis	284
Pdx1-CreER; K-Ras <sup>+/LSL</sup> G12D	55	Rare	No	The system may target acinar and precursor cells	285
proCPA1CreERT2; K-Ras <sup>LSL</sup> G12D	10	No	No	PanIN development requires pancreatitis in older mice (5–8 weeks; PanIN in 33% mice)	285
RipCreER; K-Ras <sup>+/LSL</sup> G12D	0	No	No	Cooperation with pancreatitis	285

NA indicates not analyzed; CPN, cystic papillary neoplasms.



TABLE 6.

Complex Kras-based GEMMs

GEMM	Lesions	Metastasis	Survival, mo	References
Pdx1-Cre; K-Ras+/LSLGG12D; Ink4a/Arflox/lox	PanIN and PDAC	Yes	2	286
Pdx1-Cre; K-Ras +/LSLGG12D; p53R172H/+ (KPC model)	PanIN and PDAC	Yes	5	102
Pdx1-Cre; K-Ras +/LSLGG12D; Ink4a/Arf-/-	PanIN and PDAC	Yes	5	287
Pdx1-Cre; K-Ras +/LSLGG12D; p53lox/lox	PanIN and PDAC	No	3	287
Pdx1-Cre; K-Ras +/LSLGG12D; p53lox/+; YFP	PanIN and PDAC	Yes	5	104
Pdx1-Cre; K-Ras +/LSLGG12D; p53lox/lox; Ink4a/Arf-/-	PanIN and PDAC	Yes	2	287
Pdx1-Cre; K-Ras +/LSLGG12D; Smad4lox/lox	IPMN and PDAC	Yes	9	287
Pdx1-Cre; K-Ras +/LSLGG12D; Bcr2Tr/ 11	PanIN and PDAC	NA	NA	288
Pdx1-Cre; K-Ras +/LSLGG12D; p53R270H/+; Bcr2Tr/+	PanIN and PDAC	NA	<5	288
Pdx1-Cre; K-Ras +/LSLGG12D; p53R270H/+; Bcr2Tr/ 11	PanIN and PDAC	Yes	2.5	288
Pdx1-Cre; K-Ras +/LSLGG12D; Lkb1lox/lox	PanIN and PDAC	NA	4.5	289
Pdx1-Cre; K-Ras +/LSLGG12D; Notch1lox/lox	PanIN (high grade)	No	3	290
Pdx1-Cre; K-Ras +/LSLGG12D; Usp9x+/lox	PanIN and PDAC	NA	NA	291
Pfl1a(p48)-Cre; K-Ras+/LSLGG12D; p53lox/+	PanIN and PDAC	Yes	5	107
Pfl1a(p48)-Cre; K-Ras+/LSLGG12D; p53lox/+; tdTomato	PanIN and PDAC	Yes	5	53
Pfl1a-Cre; K-Ras+/LSLGG12D; TGFβ1Rlox/lox	PanIN and PDAC	Yes	2	292
Pfl1a-Cre; K-Ras+/LSLGG12D; Elastase-TGF-α	PanIN, IPMN, PDAC	Yes	7	293
Pfl1a-Cre; K-Ras+/LSLGG12D; Smad4lox/lox	MCN and PDAC	Yes	8	294
Pfl1a-Cre; K-Ras+/LSLGG12D; Notch2lox/lox	MCN; anaplastic, PDAC	No	>12	295
Elastase-tTA; Tet-O-Cre; K-Ras+/LSLGG12Vgeo; p53-/-	PanIN and PDAC	Yes	3-4	125
Elastase-tTA; Tet-O-Cre; K-Ras+/LSLGG12Vgeo; p53lox/lox	PanIN and PDAC	Yes	3-4	296
Elastase-tTA; Tet-O-Cre; K-Ras+/LSLGG12Vgeo; Ink4a/Arflox/lox	PanIN and PDAC	Yes	3-4	296

NA indicates not analyzed; IPMN, intraductal papillary mucinous neoplasm; MCN, mucinous cystic neoplasm

**TABLE 7.**

**Pain Associated Behavior Analysis in Animal Models of Pancreatic Disease**

<b>Model</b>	<b>Species</b>	<b>Measure</b>	<b>References</b>
Pancreatitis			
Cerulein: mild acute (6–8 injections)	Mouse Rat	Abdominal Von Frey Open Field Activity	150,151,297,298
Cerulein: severe acute (12 injections)	Mouse	Abdominal Von Frey	148
Cerulein: chronic (6 inj./2days/6wks)	Mouse	Abdominal Von Frey Open field activity	156,179
L-arginine	Rat	Abdominal Von Frey	299
DBTC (i.v.)	Rat	Abdominal Von Frey Abdominal Hargreaves Hot plate	300–303
TNBS (intraductal)	Mouse Rat	Abdominal and hind paw Von Frey Electrically-evoked visceromotor reflex Open field activity Voluntary wheel running Vocalizations Catalepsy Elevated plus maze	147,149,152,156,158,159,195,304
EtOH + HFD chronic (10 wks.) [Lieber DeCarli + high fat diet]	Rat	Abdominal Von Frey Hot plate	36
Wistar/Bonn Kobori (WBN/Kob)	Rat	Home cage activity	305
Pancreatic cancer			
Orthotopic xenograft	Mouse	Hunching Abdominal Von Frey Electrically-evoked visceromotor reflex	155,306
Subcutaneous or intraneural xenograft	Mouse Rat	Hind paw Von Frey	307,308
Orthotopic allograft	Mouse	Abdominal Von Frey Open field activity Home cage activity Voluntary wheel running	309
rEla1:SV40	Mouse	Hunching Vocalization	310,311
KC mice (p48/pf1a-cre)	Mouse	Abdominal Von Frey	312,313
KPC mice (p48/pf1a-cre)	Mouse	Hunching Open field activity	53