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Swine Models for Translational Oncological Research: An Evolving Landscape and Regulatory Considerations

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Abstract

Swine biomedical models have been gaining in popularity over the last decade, particularly for applications in oncology research. Swine models for cancer research include pigs that have severe combined immunodeficiency for xenotransplantation studies, genetically modified swine models which are capable of developing tumors *in vivo*, as well as normal immunocompetent pigs. In recent years, there has been a low success rate for the approval of new oncological therapeutics in clinical trials. The two leading reasons for these failures are either due to toxicity and safety issues or lack of efficacy. As all therapeutics must be tested within animal models prior to clinical testing, there are opportunities to expand the ability to assess efficacy and toxicity profiles within the preclinical testing phases of new therapeutics. Most preclinical *in vivo* testing is performed in mice, canines, and non-human primates. However, swine models are an alternative large animal model for cancer research with similarity to human size, genetics, and physiology. Additionally, tumorigenesis pathways are similar between human and pigs in that similar driver mutations are required for transformation. Due to their larger size, the development of orthotopic tumors is easier than in smaller rodent models; additionally, porcine models can be harnessed for testing of new interventional devices and radiological/surgical approaches as well. Taken together, swine are a feasible option for preclinical therapeutic and device testing. The goals of this resource are to provide a broad overview on regulatory processes required for new therapeutics and devices for use in the clinic, cross-species differences in oncological therapeutic responses, as well as to provide an overview of swine oncology models that have been developed that could be used for preclinical testing to fulfill regulatory requirements.

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Keywords

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Introduction

In the last 10 years, a wide range of swine models have been in development as alternative large animal models for biomedical research, specifically for applications in cancer research. Such models include pigs with severe combined immunodeficiency (SCID) (Suzuki et al. 2012; Waide et al. 2015; Lei et al. 2016), pigs with inducible expression of $TP53^{R167}$ and/or $KRAS^{G12D}$ (Schook et al. 2015b; Li et al. 2015), and other genetically modified tumor-developing pigs (Donninger et al. 2015; Flisikowska et al. 2017). A variety of applications have been developed and tested in swine models. For example, SCID pigs can accept human xenografts, including human tumors (Basel et al. 2012; Ren et al. 2020; Hendricks-Wenger et al. 2021) and stem cells (Choi et al. 2017; Boettcher et al. 2020b). Additionally, pigs with inducible expression of oncogenes are capable of developing tumors in a wide range of tissues including pancreatic (Boas et al. 2020), hepatocellular carcinoma (Schachtschneider et al. 2017b), and others. Together, there are a variety of swine models available that could be developed for therapeutic testing (whether for small-molecule drug, biologic, or cellular-based therapies), device testing, or for assessing interventional procedures in oncological research prior to clinical trials.

Currently, rodent models are the animal of choice for many oncology researchers. However, there are many translational barriers from mice to humans including size, metabolism (Musther et al. 2014), and genetics (Groenen et al. 2012; Schook et al. 2015a). As such, many drugs that show efficacy and low toxicity in mice do not necessarily translate well to human responses (Van Norman 2019). Mice are valuable for first line screening and biological characterization of new therapeutics, as they are cheaper and smaller to handle. However, secondary follow-up studies in large animal models allow for better characterization of how new therapeutics and devices may function in a human. As such, the FDA requires that therapeutics be tested in a non-rodent species before consideration for clinical trial testing. Other large animal models in oncology typically include canines (Nguyen et al. 2018; Prouteau and André 2019) and non-human primates (League-Pascual et al. 2017; Velikyan and Lindhe 2018), both of which have societal ethical concerns. Together, swine models may overcome the translational barriers in mice and the ethical concerns in using other large animal models.

Swine are gaining popularity for use in oncology research due to their similarities in size, genome sequence similarity of immune system genes and responses (Kapetanovic et al. 2012; Dawson et al. 2013), and physiology (Meurens et al. 2012), as well as gene sequence, expression, and activity of relevant CYP gene families to humans (Helke and Swindle 2013; Burkina et al. 2017; Millecam et al. 2018). Many porcine models are now transitioning into a space where they can be effectively used in the preclinical assessment of the safety and efficacy of new drugs, cellular therapies, and well as oncological imaging and interventional

devices. Their larger size allows for the development of surgical and ablation models as well. In addition, the driver mutations required for tumorigenesis in swine and human result in similar cancer pathway perturbations. In earlier tumor studies in swine, multiple mutagenic "hits" (hTERT p53, cyclin D1, CDK4, c-Myc, and H-Ras were assessed in this particular study) were required for transformation, similar to human cells (Adam et al. 2007). Recent examples of swine models of cancer for testing of cancer interventions include testing pressurized intra-peritoneal aerosolized chemotherapy (Tan et al. 2020), high-frequency ultrasound ablation of the pancreas (Huang et al. 2019), as well as microwave ablation testing for breast tumors (Ortega-Palacios et al. 2018).

With this context, this perspective and resource will focus on three main topics: *(a)* descriptions of the regulatory processes for new drugs, devices, and procedures; *(b)* a high-level overview of cross-species differences in preclinical animal models in the context of therapeutic testing; and *(c)* a review on porcine models that have been developed thus far for oncological research. In all, our goal with this short perspective is to provide a resource on the current state of swine biomedical models in oncological research within the context of translational applications.

Regulatory Processes for Clinical Testing of New Drugs and Devices

New drug and devices for clinical testing must first be assessed by federal agencies (FDA in the US, European Medicines Agency in Europe, etc.) prior to clinical testing and/or marketing. In this discussion, we will focus on information provided in FDA guidance documents, however we expect that many of these concepts are translatable to other governmental agencies in other countries. There are many guides that are available from the FDA that are useful for planning and executing preclinical studies. These references can be used for organizational planning prior to experimentation to ensure all standards are met to help facilitate regulatory processes at a later date. We will focus on three main aspects of the regulatory process and associated documentation: Good Laboratory Practices (GLP), Investigational New Drugs (INDs), as well as processes for new devices (Table 1).

GLP Guidelines

The FDA guidelines provide an overview on laboratory standards that should be met during the execution of nonclinical experimental processes. Testing should be performed under GLP conditions, which describe the conditions of the experimental processes ranging from personnel who performed the experiment, equipment used, protocols that were executed, and reports that were derived from the studies. These GLP standards are also critical for consistent and safe manufacturing of new therapeutics as well. GLP guidelines fall under Title 21 Part 58 of the Code of Federal Regulations.

IND Guidelines and Considerations

IND clearance is required to assess new therapeutics in human trials. The IND takes into consideration a variety of toxicity and efficacy tests from prior preclinical, or smaller clinical studies. Pharmacological studies are required in animal models to assess the effects and potential toxicities of new drugs and therapeutics. Pharmacological studies involve both

pharmacokinetic and pharmacodynamic assessments. Pharmacokinetic studies evaluate how body functions affect the drug, while pharmacodynamic studies assess how the drug affects the body. Both study types can help clinicians determine dosing schedules and regimens.

Pharmacodynamic studies assess how a drug or therapeutic affects the organism (i.e. organ systems). Collected data from these studies may include how long it takes for the body to respond to the drug, documenting specific ways the body responds (i.e. increased heart rate), or monitoring blood markers in response to the drug. In collected data for these studies, it is important to note if there are any acute, subacute, or chronic toxicities as well.

Pharmacokinetic studies assess how the body metabolizes, cycles, and excretes a given drug. The IND has specific sections outlined for details related to the absorption, distribution, metabolism, and excretion profiles of the therapeutic of interest. These studies require monitoring of the concentration(s) of the drug, metabolites, or therapeutic (cellular and biologics) in the body after administration. Depending on the specific therapeutic being tested, in preclinical assessment of new therapeutics, these studies may require repeat blood tests for determining absorption and metabolism, organ assessment for determining distribution and metabolism, and urine and/or feces collection to assess metabolism and excretion.

Other considerations in the IND application include genetic toxicology and carcinogenicity (which typically play a larger role in non-oncological therapeutic development), as well as reproductive and developmental toxicology. Taken together, all these different studies combined can be used to determine an overall toxicity and efficacy profiles for new drugs, which are associated with specific doses and dosage timings. Table 2 outlines sections of the IND application which relate to preclinical evaluation of new therapeutics. Readers are referred to Choiden et al. for a more in-depth guide on IND and clinical trial applications (Chiodin et al. 2019).

Devices and Procedures

Medical devices fall under three different regulatory categories: Class I, II, and III. In general, Class I devices are at a low risk of causing patient injury and these devices are not intended to save lives. Class II devices carry a higher risk to patients as they may come in contact with internal bodily tissues and blood. Class III devices pose the highest risk to patients as they are designed to sustain life or are implanted in patients longer term (Jin 2014). For new devices to gain clearance for marketing, they either need to have 510(k) clearance (most Class I and II devices) or go through the premarket approval process (Class III devices). An Investigational Device Exemption (IDE) allows a device to be assessed for safety and efficacy in a clinical study. Most oncological devices in development fall under Category II; a few examples are circulating tumor cell enumeration systems (FDA 2018), high intensity ultrasound system for prostate tissue ablation (FDA 2017), and other imaging devices (Jin 2014). Class II and III devices may be assessed in animal models prior to FDA clearance.

Cross-species Therapeutics Responses

Clinical trial success rates in oncology are low compared to other clinical fields (Wong et al. 2019). In a report that assessed clinical trials and Food and Drug Administration (FDA) approval for therapeutic agents from 2009 to 2018 (for oncological therapeutics), only 3.4% of Phase 1 therapeutics made it to approval; this proportion was higher for therapeutics in Phase 2 (6.7% made it to approval) and Phase 3 (35.5% made it to approval) (Wong et al. 2019). There are a variety of reasons for clinical trial failure including the lack of funds and poor participant recruitment and compliance. However, the leading reasons are due to safety issues and failure to show efficacy in later phases (Fogel 2018). All therapies being considered by the FDA must have been assessed in animal models prior to entering a clinical trial. Taken together, there are opportunities to optimize preclinical assessment of new cancer therapeutics; particularly using animals that demonstrate similar drug metabolism as humans, and organs with approximate size and anatomical structures to those found in humans. Understanding the specific shortcomings of animal models during the preclinical testing phases is not trivial, as many years may elapse between the initial preclinical testing phases and unsuccessful clinical trials. A few examples of species-specific responses and animal model shortcomings are presented within this section.

In any type of animal model, there will be differences in the tumor microenvironment and associated vasculature compared to human tumors, which contribute to the species-specific differences in drug responses. For example, DMXAA, a STING agonist, was assessed in a combination with carboplatin and paclitaxel in patients with advanced NSCLC in a Phase III trial (Lara et al. 2011). The addition of DMXAA did not improve outcomes over carboplatin and paclitaxel alone. Further re-evaluation found that DMXAA disrupted murine vessels efficiently, but did not have a strong effect in human cells (Conlon et al. 2013). As preliminary studies had originally been performed in mice (Roberts et al. 2008), these results exemplify the importance of testing therapeutics in more than one animal model.

Spontaneous tumors in companion dogs can act as a model for certain tumor types. Canine tumors are a valuable model for human tumors as they develop spontaneously, are larger in size, and are heterogenous in terms of the tumor microenvironment and therapeutic responses. However, most canine tumors develop over a course of 2 years, whereas human tumors can develop over 5 to 10 years (Gardner et al. 2016). Companion dogs are typically treated with drugs in a similar manner as humans, and the National Cancer Institute (NCI) manages the Comparative Oncology Trials Consortium (COTC), which enrolls dogs in clinical trials to assess new therapeutics. One example of a successful finding from a COTC trial was safety and efficacy of NHS-IL12 immunocytokine in dogs (Paoloni et al. 2015), which was later found to be well-tolerated in human patients (Strauss et al. 2019); NHS-IL12 is currently still in Phase 2 trials as of mid-2021. While these advances are important, there still may be variations in therapeutic response given the shorter lifespan of canines and size variations between different breeds (Lawrence et al. 2015).

Comparison of therapeutic responses between different species is an important aspect of assessing the most viable models for cancer models. For example, human, murine, and porcine HCC cell lines were subjected to doxorubicin, cisplatin, mitomycin C, and sorafenib

to assess therapeutic responses; 5-fluorocil was used as a negative control as it is ineffective for treating HCC (Gaba et al. 2020). Murine HCC had increased susceptibility to 5-fluorocil compared to both human and porcine HCC, providing an example of a murine response that did not align with human responses. Responses to all therapeutics between human and porcine were more similar than between human and murine responses (Gaba et al. 2020). Further studies are needed to examine different therapeutic responses between porcine and murine models.

Genetic variation of animal samples can also impact the observed therapeutic responses in preclinical studies. Mouse models are inbred and can contribute to a skewed interpretation of how broadly mice respond to cancer therapeutics; thus outbred models are preferable for answering certain oncological questions (Chatzistamou et al. 2018). Livestock species, like pigs, are outbred and can reflect more diverse biological responses. However, genetically modified swine and other swine biomedical models may be less genetically diverse than wildtype production swine depending on breeding practices. We recognize that swine have not historically been used in preclinical efficacy evaluation, and thus there are not many examples available of therapeutic translation from initial studies performed in swine. However, swine models are a new avenue to investigate for potentially improved clinical translation, both as a stand-alone model and as an additional model that can augment our translational understanding from rodent experimentation.

Porcine Models for Cancer Research and Future Perspectives

There are a variety of porcine models that are in development or have previously been used in oncological research. These models fall into three main categories: pigs with SCID, immunocompetent genetically modified pigs, and normal immunocompetent pigs. Each of these different models can be leveraged for different cancer applications during the preclinical assessment of new drugs, therapeutics, and devices. Table 3 provides an overview of different porcine cancer models and Table 4 shows studies in which swine were used in the preclinical assessment of an oncological intervention.

In general, most oncological studies in recent years have focused on surgical, ablation, imaging, and small-molecule testing applications in normal or genetically modified swine models. SCID pig models are still in the early stages of development. The next sections will provide more detail on each of these three different pig models.

SCID Models

SCID pig models have been naturally discovered (Waide et al. 2015) or genetically modified (for example, Ito et al. 2014; Suzuki et al. 2016). Humans and animals with SCID can have varying deficiencies in T cells, B cells, and/or natural killer (NK) cells depending on the mutation (Tasher and Dalal 2012). Existing SCID pig models include T− B− NK+ (mutations in *Artemis*, *RAG1,* or *RAG2*), T−B+NK− (mutations in *IL2RG*), or T−B−NK− phenotype (mutations in two genes; Table 3). Because these animals lack adaptive immune systems, they can accept human xenografts for study. SCID mice are the model of choice for studying human tumors *in vivo* as they are able to grow human tumors and support the development of human immune systems (Yin et al. 2020). Swine SCID models could also

be developed for these oncological applications (Boettcher et al. 2018). To note, non-human primates with SCID were developed for similar biomedical purposes as well (Sato et al. 2016).

Xenograft studies in pigs could be particularly useful for clinical researchers who are interested in studying large human tumors *in vivo*, targeted radioligand therapies, immunotherapeutics, and others. In an example use case, SCID mouse models with patient derived xenograft tumors and human immune cells were used to study the efficacy of drugs and therapeutics in human tumors (Okada et al. 2019). A main limitation in these models is that the small tumor sizes may limit translatability for modeling how a larger human tumor may respond. A potential application of SCID pigs would be transplantation of human tumors, which could be grown to larger sizes. In this scenario, the SCID pig model could provide information on both the safety and efficacy of a new therapeutic. SCID pigs with human immune systems would help to expand the versatility of this model as well (Boettcher et al. 2018, 2020b). Humanization of these animals will be necessary to critically evaluate therapeutics, as immune cell stimulation plays a substantial role in therapeutic responses. Table 5 outlines cancer models that have already been characterized in SCID pigs.

Another important consideration of SCID pig models is the requirement of using clean biocontainment facilities (Powell et al. 2018) or isolators (Hara et al. 2018) for rearing. SCID pigs that are housed in conventional settings succumb to disease at approximately 6–8 weeks of age (Powell et al. 2018); however they can be raised for up to 6 months in biocontainment facilities (Boettcher et al. 2020a). The lack of widespread SCID pig facilities is currently a limitation in broader scale use of this model. In addition, requirement of longer-term studies (> 1 year) is not yet feasible in SCID pig models due to the increased likelihood of disease susceptibility over time.

Genetic Porcine Cancer Models

A variety of genetic pig models have been developed with different mutagenesis approaches for targeting oncogenes within the pigs. One of these models, the Oncopig model, contains Cre inducible transgenes which encode $KRAS^{G12D}$ and $TP53^{R167H}$, which are both commonly mutated genes in human cancers. As all cells in this pig model contain this transgene construct, administration of Cre (in the form of an adenoviral vector encoding Cre) to tissues results in tumor induction. Thus far, soft tissue sarcomas (Schachtschneider et al. 2017a), pancreatic ductal adenocarcinoma (Principe et al. 2018), and hepatocellular carcinoma (Schachtschneider et al. 2017b; Gaba et al. 2020) have been validated in vivo in the Oncopig model.

Other genetic models have been developed for specific types of cancer as well. For example, a breast cancer cell line was created by transfecting primary porcine breast epithelial cells with SV40LT and subsequent transfection with a vector containing miRNA for targeted *BRCA1* knockdown. These transformed cells had an increased growth rate and were capable of being grown in suspension (Donninger et al. 2015). Additionally, porcine cells with *BRCA1* knocked down had enhanced growth rate, developed acini in culture (similar to immortalized human breast cells), and had a cancer stem cell phenotype with an

overexpression of EPCAM and ALDH1. This study was a proof-of-concept study to assess if the porcine model would be of value for studying human breast cancer. These findings warrant further investigation into the development of a breast cancer model in swine. In addition, the development of swine cell-based models could be used for syngeneic transplant models in swine with the same genetic background as the original cell line, as is commonly performed in mice models (Park et al. 2018).

In addition to breast cancer, many other porcine cancer models have been developed. For example, a colorectal cancer model has been developed by inducing a stop codon at codon 1311 in the porcine adenomatous polyposis coli (*APC*) gene ($APC^{1311/+}$). One-year old $APC^{1311/+}$ pigs had visible lesions within the colon and rectum ranging in size from 1 mm to 1 cm (Flisikowska et al. 2012). Other developed models include B cell lymphomas (Andrews et al. 2019; Schenk et al. 2019), gliomas (Tora et al. 2020), as well as melanomas (Oxenhandler et al. 1979; Horák et al. 1999). Researchers have also been able to use porcine melanoma models for studying melanoma-associated T cell populations to understand the mechanisms of spontaneous regression, which could be used for defining new therapeutic targets for melanoma (Cizkova et al. 2019). Readers are referred to a recent review for an update on swine biomedical T cell research (Käser 2021).

These genetically modified porcine models are all immunocompetent, which allows them to be reared in a conventional setting without need of special containment facilities. The presence of the immune system also allows these models to be candidates for immunotherapeutic testing.

Normal Immunocompetent Pigs

Not unexpectedly, the most reported category of swine model in oncology research thus far are wild-type immunocompetent pigs. These pigs can provide benefit to oncological research for safety and toxicity testing. Such genetically unmodified pigs are easier for researchers to obtain than genetic and SCID pig models, as most genetic and SCID models are localized at specific institutions.

One interesting cancer application that has been described in normal pigs is the transplantation of human gliomas into the brain. Pigs with human gliomas have been developed by direct injection of human tumor cells into the brain with immunosuppression (Selek et al. 2014; Khoshnevis et al. 2017). As the blood-brain barrier poses issues for effective drug delivery to the site of the tumor, methods for direct administration of drugs to the tumor are of interest. These developed porcine glioma models can be used for investigating these methodologies.

Conclusion

In all, there has been a lot of progress made in developing porcine models for cancer research. Existing swine models range from pigs with SCID that can accept human xenografts to genetic models which can develop specific types of cancer. Current popular animal models in oncology are mice, dogs, and primates; all of which either have translational barriers to humans or have ethical concerns. Pig models can help bridge

this gap. As the FDA requires new therapeutics to be tested in non-rodent species, pigs are a viable option for such tests. Continual development of new swine cancer models will provide researchers with another tool to gain additional insight to the physiological responses to new drugs, therapeutics, and devices.

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Conflicts of interest

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References

Adam SJ, Rund LA, Kuzmuk KN, et al. (2007) Genetic induction of tumorigenesis in Swine. Oncogene 26:1038–1045. 10.1038/sj.onc.1209892 [PubMed: 16964292]

Andrews AR, Wang Z, Wilkinson RA, et al. (2019) Development of transplantable B-cell lymphomas in the MHC-defined miniature swine model. Cancer Cell Int 19:236. 10.1186/s12935-019-0954-3 [PubMed: 31516393]

Basel MT, Balivada S, Beck AP, et al. (2012) Human xenografts are not rejected in a naturally occurring immunodeficient porcine line: a human tumor model in pigs. Biores Open Access 1:63–68. 10.1089/biores.2012.9902 [PubMed: 23514746]

Boas FE, Nurili F, Bendet A, et al. (2020) Induction and characterization of pancreatic cancer in a transgenic pig model. PLoS One 15:e0239391. 10.1371/journal.pone.0239391 [PubMed: 32956389]

Boettcher AN, Cino-Ozuna AG, Solanki Y, et al. (2020a) CD3ε(+) Cells in Pigs With Severe Combined Immunodeficiency Due to Defects in ARTEMIS. Front Immunol 11:510. 10.3389/fimmu.2020.00510 [PubMed: 32296428]

Boettcher AN, Kiupel M, Adur MK, et al. (2019) Human Ovarian Cancer Tumor Formation in Severe Combined Immunodeficient (SCID) Pigs. Front. Oncol 9:9 [PubMed: 30723704]

Boettcher AN, Li Y, Ahrens AP, et al. (2020b) Novel Engraftment and T Cell Differentiation of Human Hematopoietic Cells in ART (−/−) IL2RG (-/Y) SCID Pigs. Front Immunol 11:100. 10.3389/fimmu.2020.00100 [PubMed: 32117254]

Boettcher AN, Loving CL, Cunnick JE, Tuggle CK (2018) Development of Severe Combined Immunodeficient (SCID) Pig Models for Translational Cancer Modeling: Future Insights on How Humanized SCID Pigs Can Improve Preclinical Cancer Research. Front. Oncol 8:559 [PubMed: 30560086]

Burkina V, Rasmussen MK, Pilipenko N, Zamaratskaia G (2017) Comparison of xenobiotic-metabolising human, porcine, rodent, and piscine cytochrome P450. Toxicology 375:10–27. 10.1016/j.tox.2016.11.014 [PubMed: 27884721]

Chatzistamou I, Farmaki E, Kaza V, Kiaris H (2018) The Value of Outbred Rodent Models in Cancer Research. Trends in Cancer 4:468–471. 10.1016/j.trecan.2018.05.004 [PubMed: 29937045]

Chiodin D, Cox EM, Edmund AV, et al. (2019) Regulatory Affairs 101: Introduction to Investigational New Drug Applications and Clinical Trial Applications. Clin Transl Sci 12:334–342. 10.1111/cts.12635 [PubMed: 30884199]

Choi WJ, Moon J-H, Min JS, et al. (2018) Real-time detection system for tumor localization during minimally invasive surgery for gastric and colon cancer removal: In vivo feasibility study in a swine model. J Surg Oncol 117:699–706. 10.1002/jso.24922 [PubMed: 29193095]

Choi Y-J, Kim E, Reza AMMT, et al. (2017) Recombination activating gene-2(null) severe combined immunodeficient pigs and mice engraft human induced pluripotent stem cells differently. Oncotarget 8:69398–69407. 10.18632/oncotarget.20626 [PubMed: 29050212]

Cizkova J, Sinkorova Z, Strnadova K, et al. (2019) The role of αβ T-cells in spontaneous regression of melanoma tumors in swine. Dev Comp Immunol 92:60–68. 10.1016/j.dci.2018.10.001 [PubMed: 30308209]

Conlon J, Burdette DL, Sharma S, et al. (2013) Mouse, but not human STING, binds and signals in response to the vascular disrupting agent 5,6-dimethylxanthenone-4-acetic acid. J Immunol 190:5216–5225. 10.4049/jimmunol.1300097 [PubMed: 23585680]

Dawson HD, Loveland JE, Pascal G, et al. (2013) Structural and functional annotation of the porcine immunome. BMC Genomics 14:332. 10.1186/1471-2164-14-332 [PubMed: 23676093]

Dežman R, Čemažar M, Serša G, et al. (2020) Safety and Feasibility of Electrochemotherapy of the Pancreas in a Porcine Model. Pancreas 49:1168–1173. 10.1097/MPA.0000000000001642 [PubMed: 32898000]

Donninger H, Hobbing K, Schmidt ML, et al. (2015) A porcine model system of BRCA1 driven breast cancer. Front Genet 6:269. 10.3389/fgene.2015.00269 [PubMed: 26379698]

Duran-Struuck R, Cho PS, Teague AGS, et al. (2010) Myelogenous leukemia in adult inbred MHC-defined miniature swine: a model for human myeloid leukemias. Vet Immunol Immunopathol 135:243–256. 10.1016/j.vetimm.2009.12.005 [PubMed: 20079939]

FDA (2018) Immunomagnetic Circulating Cancer Cell Selection and Enumeration System - Class II Special Controls Guidance Document for Industry and FDA Staff

FDA (2017) Medical Devices; Gastroenterology-Urology Devices; Classification of the High Intensity Ultrasound System for Prostate Tissue Ablation

Flisikowska T, Merkl C, Landmann M, et al. (2012) A porcine model of familial adenomatous polyposis. Gastroenterology 143:1173–1175.e7. 10.1053/j.gastro.2012.07.110 [PubMed: 22864254]

Flisikowska T, Stachowiak M, Xu H, et al. (2017) Porcine familial adenomatous polyposis model enables systematic analysis of early events in adenoma progression. Sci Rep 7:6613. 10.1038/s41598-017-06741-8 [PubMed: 28747659]

Fogel DB (2018) Factors associated with clinical trials that fail and opportunities for improving the likelihood of success: A review. Contemp Clin trials Commun 11:156–164. 10.1016/j.conctc.2018.08.001 [PubMed: 30112460]

Gaba RC, Elkhadragy L, Boas FE, et al. (2020) Development and comprehensive characterization of porcine hepatocellular carcinoma for translational liver cancer investigation. Oncotarget 11:2686–2701. 10.18632/oncotarget.27647 [PubMed: 32733642]

Gardner HL, Fenger JM, London CA (2016) Dogs as a Model for Cancer. Annu Rev Anim Biosci 4:199–222. 10.1146/annurev-animal-022114-110911 [PubMed: 26566160]

Groenen MAM, Archibald AL, Uenishi H, et al. (2012) Analyses of pig genomes provide insight into porcine demography and evolution. Nature 491:393–398. 10.1038/nature11622 [PubMed: 23151582]

Hara H, Shibata H, Nakano K, et al. (2018) Production and rearing of germ-free X-SCID pigs. Exp Anim 67:139–146. 10.1538/expanim.17-0095 [PubMed: 29162766]

Helke KL, Swindle MM (2013) Animal models of toxicology testing: the role of pigs. Expert Opin Drug Metab Toxicol 9:127–139. 10.1517/17425255.2013.739607 [PubMed: 23216131]

Hendricks-Wenger A, Aycock KN, Nagai-Singer MA, et al. (2021) Establishing an immunocompromised porcine model of human cancer for novel therapy development with pancreatic adenocarcinoma and irreversible electroporation. Sci Rep 11:7584. 10.1038/s41598-021-87228-5 [PubMed: 33828203]

Horák V, Fortýn K, Hruban V, Klaudy J (1999) Hereditary melanoblastoma in miniature pigs and its successful therapy by devitalization technique. Cell Mol Biol (Noisy-le-grand) 45:1119–1129 [PubMed: 10644016]

Huang G, Ye X, Yang X, et al. (2019) Experimental study in vivo ablation of swine pancreas using high-intensity focused ultrasound. J Cancer Res Ther 15:286–290. 10.4103/jcrt.JCRT_986_17 [PubMed: 30964099]

Huang J, Guo X, Fan N, et al. (2014) RAG1/2 knockout pigs with severe combined immunodeficiency. J Immunol 193:1496–1503. 10.4049/jimmunol.1400915 [PubMed: 24973446]

Ito T, Sendai Y, Yamazaki S, et al. (2014) Generation of recombination activating gene-1-deficient neonatal piglets: a model of T and B cell deficient severe combined immune deficiency. PLoS One 9:e113833. 10.1371/journal.pone.0113833 [PubMed: 25437445]

Jin J (2014) FDA Authorization of Medical Devices. JAMA 311:435. 10.1001/jama.2013.286274 [PubMed: 24449330]

Kang J-T, Cho B, Ryu J, et al. (2016) Biallelic modification of IL2RG leads to severe combined immunodeficiency in pigs. Reprod Biol Endocrinol 14:74. 10.1186/s12958-016-0206-5 [PubMed: 27809915]

Kapetanovic R, Fairbairn L, Beraldi D, et al. (2012) Pig bone marrow-derived macrophages resemble human macrophages in their response to bacterial lipopolysaccharide. J Immunol 188:3382–3394. 10.4049/jimmunol.1102649 [PubMed: 22393154]

Käser T (2021) Swine as biomedical animal model for T-cell research—Success and potential for transmittable and non-transmittable human diseases. Mol Immunol 135:95–115. 10.1016/j.molimm.2021.04.004 [PubMed: 33873098]

Khoshnevis M, Carozzo C, Bonnefont-Rebeix C, et al. (2017) Development of induced glioblastoma by implantation of a human xenograft in Yucatan minipig as a large animal model. J Neurosci Methods 282:61–68. 10.1016/j.jneumeth.2017.03.007 [PubMed: 28284687]

Kratkiewicz K, Manwar R, Rajabi-Estarabadi A, et al. (2019) Photoacoustic/Ultrasound/Optical Coherence Tomography Evaluation of Melanoma Lesion and Healthy Skin in a Swine Model. Sensors (Basel) 19:2815. 10.3390/s19122815

Kurihara T, Matsuda S, Nakamura Y, et al. (2019) Establishment of a model of sentinel lymph node metastasis using immunodeficient swine. Sci Rep 9:7923. 10.1038/s41598-019-44171-w [PubMed: 31138877]

Lara PNJ, Douillard J-Y, Nakagawa K, et al. (2011) Randomized phase III placebo-controlled trial of carboplatin and paclitaxel with or without the vascular disrupting agent vadimezan (ASA404) in advanced non-small-cell lung cancer. J Clin Oncol Off J Am Soc Clin Oncol 29:2965–2971. 10.1200/JCO.2011.35.0660

Lawrence J, Cameron D, Argyle D (2015) Species differences in tumour responses to cancer chemotherapy. Philos Trans R Soc Lond B Biol Sci 370:20140233. 10.1098/rstb.2014.0233 [PubMed: 26056373]

League-Pascual JC, Lester-McCully CM, Shandilya S, et al. (2017) Plasma and cerebrospinal fluid pharmacokinetics of select chemotherapeutic agents following intranasal delivery in a non-human primate model. J Neurooncol 132:401–407. 10.1007/s11060-017-2388-x [PubMed: 28290002]

Lee K, Kwon D-N, Ezashi T, et al. (2014) Engraftment of human iPS cells and allogeneic porcine cells into pigs with inactivated RAG2 and accompanying severe combined immunodeficiency. Proc Natl Acad Sci U S A 111:7260–7265. 10.1073/pnas.1406376111 [PubMed: 24799706]

Lei S, Ryu J, Wen K, et al. (2016) Increased and prolonged human norovirus infection in RAG2/IL2RG deficient gnotobiotic pigs with severe combined immunodeficiency. Sci Rep 6:25222. 10.1038/srep25222 [PubMed: 27118081]

Leuchs S, Saalfrank A, Merkl C, et al. (2012) Inactivation and Inducible Oncogenic Mutation of p53 in Gene Targeted Pigs. PLoS One 7:e43323 [PubMed: 23071491]

Li S, Edlinger M, Saalfrank A, et al. (2015) Viable pigs with a conditionally-activated oncogenic KRAS mutation. Transgenic Res 24:509–517. 10.1007/s11248-015-9866-8 [PubMed: 25716163]

Maxwell AWP, Park WKC, Baird GL, et al. (2019) Effects of a Thermal Accelerant Gel on Microwave Ablation Zone Volumes in Lung: A Porcine Study. Radiology 291:504–510. 10.1148/radiol.2019181652 [PubMed: 30747590]

Meurens F, Summerfield A, Nauwynck H, et al. (2012) The pig: a model for human infectious diseases. Trends Microbiol 20:50–57. 10.1016/j.tim.2011.11.002 [PubMed: 22153753]

Millecam J, De Clerck L, Govaert E, et al. (2018) The Ontogeny of Cytochrome P450 Enzyme Activity and Protein Abundance in Conventional Pigs in Support of Preclinical Pediatric Drug Research. Front. Pharmacol 9:470 [PubMed: 29867477]

Mishima K, Itano O, Matsuda S, et al. (2021) Development of human hepatocellular carcinoma in X-linked severe combined immunodeficient pigs: An orthotopic xenograft model. PLoS One 16:e0248352. 10.1371/journal.pone.0248352 [PubMed: 33750947]

Misra SK, Ghoshal G, Gartia MR, et al. (2015) Trimodal Therapy: Combining Hyperthermia with Repurposed Bexarotene and Ultrasound for Treating Liver Cancer. ACS Nano 9:10695–10718. 10.1021/acsnano.5b05974 [PubMed: 26435333]

Musther H, Olivares-Morales A, Hatley OJD, et al. (2014) Animal versus human oral drug bioavailability: do they correlate? Eur J Pharm Sci Off J Eur Fed Pharm Sci 57:280–291. 10.1016/j.ejps.2013.08.018

Nedelcovych MT, Tenora L, Kim B-H, et al. (2017) N-(Pivaloyloxy)alkoxy-carbonyl Prodrugs of the Glutamine Antagonist 6-Diazo-5-oxo-l-norleucine (DON) as a Potential Treatment for HIV Associated Neurocognitive Disorders. J Med Chem 60:7186–7198. 10.1021/acs.jmedchem.7b00966 [PubMed: 28759224]

Nguyen F, Peña L, Ibisch C, et al. (2018) Canine invasive mammary carcinomas as models of human breast cancer. Part 1: natural history and prognostic factors. Breast Cancer Res Treat 167:635–648. 10.1007/s10549-017-4548-2 [PubMed: 29086231]

Nurili F, Monette S, Michel AO, et al. (2021) Transarterial Embolization of Liver Cancer in a Transgenic Pig Model. J Vasc Interv Radiol 32:510–517.e3. 10.1016/j.jvir.2020.09.011 [PubMed: 33500185]

Okada S, Vaeteewoottacharn K, Kariya R (2019) Application of Highly Immunocompromised Mice for the Establishment of Patient-Derived Xenograft (PDX) Models. Cells 8:889. 10.3390/cells8080889

Ortega-Palacios R, Trujillo-Romero CJ, Cepeda Rubio MFJ, et al. (2018) Feasibility of Using a Novel 2.45 GHz Double Short Distance Slot Coaxial Antenna for Minimally Invasive Cancer Breast Microwave Ablation Therapy: Computational Model, Phantom, and In Vivo Swine Experimentation. J Healthc Eng 2018:5806753. 10.1155/2018/5806753 [PubMed: 29854360]

Oxenhandler RW, Adelstein EH, Haigh JP, et al. (1979) Malignant melanoma in the Sinclair miniature swine: an autopsy study of 60 cases. Am J Pathol 96:707–720 [PubMed: 474716]

Paoloni M, Mazcko C, Selting K, et al. (2015) Defining the Pharmacodynamic Profile and Therapeutic Index of NHS-IL12 Immunocytokine in Dogs with Malignant Melanoma. PLoS One 10:e0129954–e0129954. 10.1371/journal.pone.0129954 [PubMed: 26091536]

Park MK, Lee CH, Lee H (2018) Mouse models of breast cancer in preclinical research. Lab Anim Res 34:160–165. 10.5625/lar.2018.34.4.160 [PubMed: 30671101]

Powell EJ, Charley S, Boettcher AN, et al. (2018) Creating effective biocontainment facilities and maintenance protocols for raising specific pathogen-free, severe combined immunodeficient (SCID) pigs. Lab Anim 52:402–412. 10.1177/0023677217750691 [PubMed: 29325489]

Principe DR, Overgaard NH, Park AJ, et al. (2018) KRAS(G12D) and TP53(R167H) Cooperate to Induce Pancreatic Ductal Adenocarcinoma in Sus scrofa Pigs. Sci Rep 8:12548. 10.1038/s41598-018-30916-6 [PubMed: 30135483]

Prouteau A, André C (2019) Canine Melanomas as Models for Human Melanomas: Clinical, Histological, and Genetic Comparison. Genes (Basel) 10:. 10.3390/genes10070501

Rais R, Jančařík A, Tenora L, et al. (2016) Discovery of 6-Diazo-5-oxo-l-norleucine (DON) Prodrugs with Enhanced CSF Delivery in Monkeys: A Potential Treatment for Glioblastoma. J Med Chem 59:8621–8633. 10.1021/acs.jmedchem.6b01069 [PubMed: 27560860]

Rao AD, Shin EJ, Beck SE, et al. (2018) Demonstration of Safety and Feasibility of Hydrogel Marking of the Pancreas-Duodenum Interface for Image Guided Radiation Therapy (IGRT) in a Porcine Model: Implications in IGRT for Pancreatic Cancer Patients. Int J Radiat Oncol Biol Phys 101:640–645. 10.1016/j.ijrobp.2018.02.024 [PubMed: 29680252]

Ren J, Yu D, Fu R, et al. (2020) IL2RG-deficient minipigs generated via CRISPR/Cas9 technology support the growth of human melanoma-derived tumours. Cell Prolif 53:e12863. 10.1111/cpr.12863 [PubMed: 32871045]

Roberts ZJ, Ching L-M, Vogel SN (2008) IFN-beta-dependent inhibition of tumor growth by the vascular disrupting agent 5,6-dimethylxanthenone-4-acetic acid (DMXAA). J Interf cytokine Res Off J Int Soc Interf Cytokine Res 28:133–139. 10.1089/jir.2007.0992

Rowson-Hodel AR, Manjarin R, Trott JF, et al. (2015) Neoplastic transformation of porcine mammary epithelial cells in vitro and tumor formation in vivo. BMC Cancer 15:562. 10.1186/s12885-015-1572-7 [PubMed: 26228788]

Saalfrank A, Janssen K-P, Ravon M, et al. (2016) A porcine model of osteosarcoma. Oncogenesis 5:e210–e210. 10.1038/oncsis.2016.19 [PubMed: 26974205]

Sato K, Oiwa R, Kumita W, et al. (2016) Generation of a Nonhuman Primate Model of Severe Combined Immunodeficiency Using Highly Efficient Genome Editing. Cell Stem Cell 19:127–138. 10.1016/j.stem.2016.06.003 [PubMed: 27374787]

Schachtschneider KM, Liu Y, Mäkeläinen S, et al. (2017a) Oncopig Soft-Tissue Sarcomas Recapitulate Key Transcriptional Features of Human Sarcomas. Sci Rep 7:2624. 10.1038/s41598-017-02912-9 [PubMed: 28572589]

Schachtschneider KM, Schwind RM, Darfour-Oduro KA, et al. (2017b) A validated, transitional and translational porcine model of hepatocellular carcinoma. Oncotarget 8:63620–63634. 10.18632/oncotarget.18872 [PubMed: 28969016]

Schenk M, Matar AJ, Hanekamp I, et al. (2019) Development of a Transplantable GFP+ B-Cell Lymphoma Tumor Cell Line From MHC-Defined Miniature Swine: Potential for a Large Animal Tumor Model. Front. Oncol 9:209 [PubMed: 31001475]

Schook LB, Collares TV, Darfour-Oduro KA, et al. (2015a) Unraveling the swine genome: implications for human health. Annu Rev Anim Biosci 3:219–244. 10.1146/annurev-animal-022114-110815 [PubMed: 25689318]

Schook LB, Collares TV, Hu W, et al. (2015b) A Genetic Porcine Model of Cancer. PLoS One 10:e0128864. 10.1371/journal.pone.0128864 [PubMed: 26132737]

Selek L, Seigneuret E, Nugue G, et al. (2014) Imaging and histological characterization of a human brain xenograft in pig: the first induced glioma model in a large animal. J Neurosci Methods 221:159–165. 10.1016/j.jneumeth.2013.10.002 [PubMed: 24126047]

Sieren JC, Meyerholz DK, Wang X-J, et al. (2014) Development and translational imaging of a TP53 porcine tumorigenesis model. J Clin Invest 124:4052–4066. 10.1172/JCI75447 [PubMed: 25105366]

Strauss J, Heery CR, Kim JW, et al. (2019) First-in-Human Phase I Trial of a Tumor-Targeted Cytokine (NHS-IL12) in Subjects with Metastatic Solid Tumors. Clin cancer Res an Off J Am Assoc Cancer Res 25:99–109. 10.1158/1078-0432.CCR-18-1512

Suzuki S, Iwamoto M, Hashimoto M, et al. (2016) Generation and characterization of RAG2 knockout pigs as animal model for severe combined immunodeficiency. Vet Immunol Immunopathol 178:37–49. 10.1016/j.vetimm.2016.06.011 [PubMed: 27496741]

Suzuki S, Iwamoto M, Saito Y, et al. (2012) Il2rg Gene-Targeted Severe Combined Immunodeficiency Pigs. Cell Stem Cell 10:753–758. 10.1016/j.stem.2012.04.021 [PubMed: 22704516]

Tan HL, Kim G, Charles CJ, et al. (2020) Safety, pharmacokinetics and tissue penetration of PIPAC paclitaxel in a swine model. Eur J Surg Oncol J Eur Soc Surg Oncol Br Assoc Surg Oncol. 10.1016/j.ejso.2020.06.031

Tasher D, Dalal I (2012) The genetic basis of severe combined immunodeficiency and its variants. Appl Clin Genet 5:67–80. 10.2147/TACG.S18693 [PubMed: 23776382]

Tora MS, Texakalidis P, Neill S, et al. (2020) Lentiviral Vector Induced Modeling of High-Grade Spinal Cord Glioma in Minipigs. Sci Rep 10:5291. 10.1038/s41598-020-62167-9 [PubMed: 32210315]

Van Norman GA (2019) Limitations of Animal Studies for Predicting Toxicity in Clinical Trials: Is it Time to Rethink Our Current Approach? JACC Basic to Transl Sci 4:845–854. 10.1016/j.jacbts.2019.10.008

Velikyan I, Lindhe Ö (2018) Preparation and evaluation of a (68)Ga-labeled RGD-containing octapeptide for noninvasive imaging of angiogenesis: biodistribution in non-human primate. Am J Nucl Med Mol Imaging 8:15–31 [PubMed: 29531858]

Vozenin M-C, De Fornel P, Petersson K, et al. (2019) The Advantage of FLASH Radiotherapy Confirmed in Mini-pig and Cat-cancer Patients. Clin Cancer Res 25:35 LP–42. 10.1158/1078-0432.CCR-17-3375 [PubMed: 29875213]

Waide EH, Dekkers JCM, Ross JW, et al. (2015) Not All SCID Pigs Are Created Equally: Two Independent Mutations in the Artemis Gene Cause SCID in Pigs. J Immunol 195:3171–3179. 10.4049/jimmunol.1501132 [PubMed: 26320255]

Wong CH, Siah KW, Lo AW (2019) Estimation of clinical trial success rates and related parameters. Biostatistics 20:273–286. 10.1093/biostatistics/kxx069 [PubMed: 29394327]

Yin L, Wang X-J, Chen D-X, et al. (2020) Humanized mouse model: a review on preclinical applications for cancer immunotherapy. Am J Cancer Res 10:4568–4584 [PubMed: 33415020]

Table 1.

Selected FDA Federal Codes of Regulation Directly Relevant to Animal Studies Under Title 21

Title section	Topic
Good Laboratory practice for Nonclinical Laboratory Studies	
58.1	Scope
58.3	Definitions
58.15	Inspection of a testing facility
58.29	Personnel
58.35	Quality assurance unit
58.43	Animal care facilities
58.47	Facilities for handling test and control articles
58.81	Standard operating procedures
58.90	Animal care
Investigational New Drugs	
312.20	Requirement for an IND
312.23	IND content and format
312.32	IND safety reporting
Establishment Registration and Device Listing for Manufacturers and Initial Importers of Devices	
807.92	Content and format of a 510(k) summary
Investigational Device Exemptions	
812.1	Scope
812.3	Definitions
812.27	Report of prior investigations

Note.— Animal studies are also classified as “nonclinical” studies within some of these sections.

Table 2.

Selected IND Sections Relevant to Preclinical Testing

Section	Topic	Content
3.4	Overview of Preclinical Data	Provide brief overview of pharmacology and toxicology data
8.1	Pharmacodynamics	
	8.1.1 Primary pharmacodynamics	Describe mechanism of action and drug activity related to proposed indication
	8.1.2 Secondary pharmacodynamics	Describe any secondary pharmacodynamic activity if there is any
8.2	Safety pharmacology	Describe neurological, cardiovascular, pulmonary, renal, gastrointestinal effects, as well as abuse liability and other related topics
8.3	Pharmacokinetics	
	8.3.1 Absorption	Describe how the drug is absorbed through the body after administration (blood, liver, and other organ systems)
	8.3.2 Distribution	Describe how the drug distributes throughout the body once it is absorbed
	8.3.3 Metabolism	Describe the metabolites that are derived from the parent drug
	8.3.4 Excretion	Provide overview on how metabolites from the drug are excreted (timing, identification of metabolites, etc)
8.6	Toxicology	Summarize toxicology studies that were performed; mention any relevant information from pre-IND meetings with the FDA. This section should include a title, key findings, drug formulations, methods, dosing, observations, results, summary, and conclusions

Table 3.

Overview of Swine Models for Cancer Research

Model or cancer type	Description and Phenotype	Citation
A. Immunocompromised		
ARTEMIS $^{-/-}$	T− B− NK+	(Waide et al. 2015)
ARTEMIS $^{-/-}$ and *IL2RG* $^{-/Y}$	T− B− NK−	(Boettcher et al. 2020b)
RAG1 $^{-/-}$ or *RAG2* $^{-/-}$	T− B− NK+	(Ito et al. 2014; Lee et al. 2014; Huang et al. 2014; Suzuki et al. 2016)
IL2RG $^{-/Y}$	T− B+ NK−	(Suzuki et al. 2012; Kang et al. 2016; Hara et al. 2018; Ren et al. 2020)
RAG2 $^{-/-}$ *IL2RG* $^{-/Y}$	T− B− NK−	(Lei et al. 2016; Hendricks-Wenger et al. 2021)
B. Genetic porcine models		
Cre inducible *KRAS* ^{G12D} *TP53* ^{R167H}	Inducible *KRAS* ^{G12D} and *TP53* ^{R167H} expression upon Cre recombinase localized injection	(Schook et al. 2015b)
Cre inducible *KRAS* ^{G12D}	Inducible *KRAS* ^{G12D} expression upon Cre recombinase localized injection	(Li et al. 2015)
Cre inducible *TP53* ^{R167H}	Inducible *TP53* ^{R167H} expression upon Cre recombinase localized injection	(Leuchs et al. 2012)
TP53 $^{R167H/\ R167H}$	Gene targeted mutation in to introduce R167H missense mutation; *TP53* $^{R167H/\ R167H}$ pigs developed lymphomas, osteogenic, and renal tumors	(Sieren et al. 2014)
B-cell lymphoma	Cell line derived from post-transplant lymphoproliferative disease. Cell line underwent transduction for GFP expression for tracking	(Schenk et al. 2019)
	Spontaneous malignancy; cellular phenotype was $CD3^{-}$ $CD172^{-}$ $CD16^{-}$ $CD25^{+}$ $CD45RA^{+}$ and $CD79\alpha^{+}$ with MHC class I and II expression	(Andrews et al. 2019)
Breast cancer	SV40 LT transfection and miRNA knockdown of *BRCA1* (cellular model)	(Donninger et al. 2015)
	Lentiviral transduction of polyomavirus T antigens	(Rowson-Hodel et al. 2015)
Colorectal cancer	Gene-targeted stop codon; *APC* $^{1311/+}$ pigs develop adenomas in the large intestine	(Flisikowska et al. 2012)
Glioma	Lentiviral induced *PDFG-B*, constitutive *HRAS*, and shRNA-*TP53*	(Tora et al. 2020)
Hepatocellular carcinoma	Inducible *KRAS* ^{G12D} *TP53* ^{R167H}	(Schachtschneider et al. 2017b; Gaba et al. 2020)
Melanoma	Hereditary melanoma; Sinclair miniature swine	(Oxenhandler et al. 1979)
	Hereditary melanoma; melanoma-bearing Libechov minipig.	(Horák et al. 1999)
Myeloid leukemia	Spontaneous development of myelogenous leukemia, shortened chromosome arm identified	(Duran-Struuck et al. 2010)
Osteosarcoma	Spontaneous development of osteosarcoma in pigs with heterozygous knockout of *TP53;* homozygous *TP53* knockout resulted in large tumors in 7–8-month-old animals (~96 cm^3)	(Saalfrank et al. 2016)
Pancreatic cancer	Inducible mutations in *KRAS* ^{G12D} *TP53* ^{R167H}; development of pancreatic ductal adenocarcinoma after adeno-cre injection into pancreatic duct	(Principe et al. 2018)
Soft tissue sarcoma	Inducible *KRAS* ^{G12D} *TP53* ^{R167H}	(Schachtschneider et al. 2017a)
C. Normal immunocompetent pigs		

Model or cancer type	Description and Phenotype	Citation
Glioblastoma	Intracerebral injection of human U87 MG cells with cyclosporin immunosuppression; tumors reached 10,000 mm^3 by 30 days post transplantation	(Selek et al. 2014)
	Injection of human U87 cells within the corpus striatum with cyclosporine immunosuppression; pigs monitored with CT; some tumors grew to 3000 mm^3 by 14 days post injection	(Khoshnevis et al. 2017)

Note.— APC = adenomatous polyposis coli gene, GFP = green fluorescent protein, PDFG-B = platelet growth factor beta

Table 4.

Examples of Preclinical Cancer Studies in Swine

Model	Application	Cancer of interest	Reference
A. Immunocompromised pigs			
RAG2$^{-/-}$ *IL2Rg*$^{-/Y}$	Proof of concept study for irreversible electroporation ablation	Pancreatic	(Hendricks-Wenger et al. 2021)
RAG2 $^{-/-}$	Diagnostic methods and minimally invasive surgeries	Multiple, metastasis	(Kurihara et al. 2019)
B. Genetic porcine models			
Cre inducible *KRAS*^{G12D} *TP53*^{R167H}	Intra-arterial catheterization and angiography	Pancreatic	(Boas et al. 2020)
Melanoma swine model	Photoacoustic, ultrasound, optical coherence tomography	Melanoma	(Kratkiewicz et al. 2019)
Cre inducible *KRAS*^{G12D} *TP53*^{R167H}	Hyperthermia with bexarotone and ultrasound ablation	Hepatocellular carcinoma	(Misra et al. 2015)
Cre inducible *KRAS*^{G12D} *TP53*^{R167H}	Transarterial embolization	Liver cancer	(Nurili et al. 2021)
C. Normal immunocompetent pigs			
Göttingen mini pig	FLASH radiotherapy	Multiple, general	(Vozenin et al. 2019)
Yorkshire	Image guided radiation therapy	Pancreatic	(Rao et al. 2018)
Potbellied Vietnamese minipig	Microwave ablation therapy	Breast	(Ortega-Palacios et al. 2018)
NS	Electrochemotherapy	Pancreatic	(Dežman et al. 2020)
NS	High intensity focused ultrasound	Pancreatic	(Huang et al. 2019)
NS	Surgery	Gastric and colon	(Choi et al. 2018)
NS	Thermal accelerant gel for microwave ablation	Lung	(Maxwell et al. 2019)
Göttingen x Yucutan	Glutamine antagonist drug testing	Glioblastoma	(Rais et al. 2016; Nedelcovych et al. 2017)

Note.— For immunocompromised and genetic porcine models, the genetic background is provided, whereas for normal immunocompetent pigs, the pig strain is provided. NS = not specified

Table 5.

Tumor Models Characterized in SCID pigs

SCID Model	Cancer type	Location	Reference
ARTEMIS $^{-/-}$	Melanoma	Orthotopic	(Basel et al. 2012)
ARTEMIS $^{-/-}$	Pancreatic	Ectopic; subcutaneous	(Basel et al. 2012)
ARTEMIS $^{-/-}$	Ovarian	Ectopic; subcutaneous	(Boettcher et al. 2019)
RAG2 $^{-/-}$	Epithelial carcinoma	Orthotopic; metastases in SLN	(Kurihara et al. 2019)
IL2RG $^{-/Y}$	HCC	Orthotopic	(Mishima et al. 2021)
RAG $^{-/-}$ *IL2RG* $^{-/Y}$	Pancreatic	Ectopic; subcutaneous	(Hendricks-Wenger et al. 2021)

Note.— HCC = hepatocellular carcinoma, SLN = sentinel lymph node