# Cancer modeling thinks big with the pig

Large animal models can be important translational steps between basic research in rodents and clinical care in humans. Ever thought about a pig?

Ellen P. Neff

The patient had four legs and a tail. But it wasn't a rat. Surgeon Mark Carlson had some preclinical experience with rodent models, but he had never worked on the animal his colleagues at the University of Nebraska Medical Center (UNMC) were proposing: pigs, part of a study about hemorrhage and hemostasis. "But you know, it's surgery," he recalls thinking. How difficult could it be?

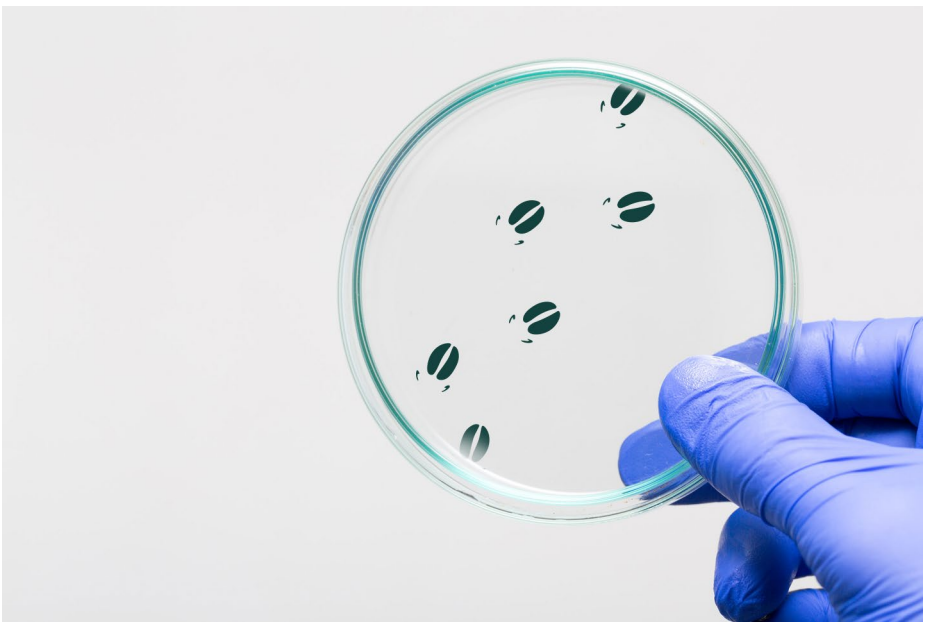
The surgical techniques Carlson used in humans patients were easily adapted to the pig, and he found himself enthusiastic about a larger animal model. "I began to really look around to see what else I could do with pig models," Carlson says. He came to cancer, pancreatic in particular, an area he knew was going to difficult but "but also with a very high potential yield," he says.

UNMC already had an established research program for pancreatic cancer, a disease with a stubbornly poor prognosis if not caught early. "We've kind of hit a wall in terms of improving patient survival," Carlson says, so he started asking other scientists around campus what they thought about introducing the pig as a new preclinical model. "About half of them were very enthusiastic," he says. And the other half? To them, a pig model seemed a bit absurd.

"But the idea was strong enough," he says. Nor was he the only one thinking about porcine models of cancer. "I realized that the field was not empty. Sparsely populated, but not empty."

Scientists and clinicians have valued the pig for its anatomical and physiological similarity to humans for decades. Surgeons often train on the pig before turning to a patient, and the pig is a large animal option for device and pharmaceutical companies to test their new developments before heading to the clinic. Pigs might soon help abate organ shortages too, if the kinks to xenotransplantation can be fully worked out.

But cancer is a relatively more recent frontier. A handful of spontaneous and chemically induced porcine models have been around since the early 2000s, but tumors in those models can be hard to predict and labor-intensive to produce[1]. And the natural occurrence of cancers in pigs is somewhat of a mystery. "They're a food item in western countries, and so they're not allowed to live to a ripe old age like dogs or cats are," Carlson says, "We really don't know what kind of cancer pigs get naturally." Another complication: cancer is often a disease of age, whereas the pigs available are juveniles. "If you want to make them into old pigs, you have to sit around for a few decades and wait for them to get old," he says. But advances in the genetic engineering of swine are opening new doors to cancer research, without the wait.



**Trotting on:** Biomedical researchers are picking up the pig and creating porcine models of a variety of cancers. Credit: (design) E. Dewalt/SpringerNature; (Hoofprints) MeggSt/Getty; (Petri Dish) luchschen/Getty

When the genome of the domestic pig, *Sus scrofa*, was sequenced in 2012, researchers saw that the animals have genetic as well as anatomical and physiological similarities to humans that they could take advantage of too. Investigators like Lawrence Schook, a professor of animal sciences and radiology at the University of Illinois who was involved in the sequencing effort, started exploring whether a new model was possible. "We asked a very, very fundamental question," says Schook: if the pig really was so similar to humans, would the same driver mutations found in cancer patients also cause cancer in pigs? Mutations to genes like *P53* and *KRAS*, two of the most commonly mutated oncogenes founds in human cancers.

The answer that he, and others that are picking up the pig, are discovering, is 'Yes."

## A pig walks into the clinic…

Like many biomedical researchers, Schook started small, studying genetic resistance to diseases like cancer in the little workhouse that is the lab mouse. Genetic approaches were advancing in the mouse and you could

"I've always viewed pigs as a big mouse," says Lawrence Schook.

Credit: D. Hamerman

**Box 1 | The pig among other large options**

Size is an advantage in cancer research, though pigs aren't the only large animal out there. Those who work with pigs point out a number of advantages over other options. Nonhuman primates are the most closely related animal to humans, but they're expensive to keep and, given those similarities to people, come with added ethical constraints. "If you're talking about giving a nonhuman primate liver cancer, lung cancer, it's just not going to happen," says Schook. From a practical perspective, they take a long time to mature—two to three years—and will only have one or two offspring at a time. Ruminants such as sheep and goats are additional large-animal livestock options, but they too have a small reproductive capacity. Pigs meanwhile can produce a lot of piglets—up to 20 per year, which mature within about eight months. That means cohorts of animals of the same age can be quickly produced, an important consideration for things like pharmaceutical testing where 40 or 50 animals might be needed at time to complete the study, says Swart.

An animal that is likely to feature prominently in cancer research are dogs. Companion canines grow old and naturally develop a variety of cancers, so there are opportunities to test different clinical interventions in a larger animal than a mouse. But their cancers are spontaneous, says Schook. Plus, pets are patients, there for a treatment—experimentally manipulations aren't feasible in the same way they are in a research animal, like a pig.

do a lot with them, he says, but the mouse can only take you so far. "The mouse is a great animal to do all the logistics on mutation and things like that," he says. "But when you get into the therapeutic side, the translation side, that's where the weakness comes. Because you can't use instruments, you can't use radiation. When you talk about mice, it just is different. A different animal." His career took a decidedly larger turn towards an animal more often found down on the farm: the pig. "I've always viewed pigs as a big mouse," says Schook.

The size of the pig is a big advantage (Box 1). While domestic agricultural animals can top out near 800 pounds, the "miniature" strains developed in recent years for biomedical research can be kept, with the right diet, at a more manageable 150–200 pounds. That puts them into a similar weight class to the organism that animal models are intended to model: the human. The mouse, by comparison, is tiny: an adult will weigh in around just 20 to 30 grams. A small animal means small anatomy.

Adrienne Watson is a scientist at Recombinetics who has been involved in the development of a porcine model of Neurofibromatosis Type I (NF1), a rare pediatric disease that causes nervous tissue tumors. Many NF1 patients will develop optic pathway gliomas—tumors on the nerves of the eye. Mouse models of NF1 will develop these, but their optic nerve presents a challenge: it's about the size of a piece of dental floss. "To image it on an MRI machine, to go in and perform surgery, which is what they do with patients with problematic optic pathway gliomas, is nearly impossible," says Watson. The pig is the right scale.

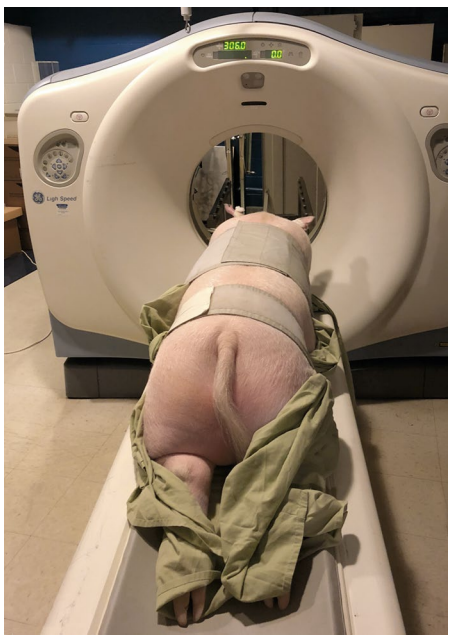
"The pig is large enough to do things like imaging, so you can put a pig into a human MRI machine, a human CT machine, a human PET scanner. You can do radiation therapy; you can do surgery, all these things that are really key in patient care," Watson says. That means care, and treatment, can more closely replicate what occurs in the clinic. As they developed their NF1 model, Watson's collaborator Christopher Moertel, a clinician at the University of Minnesota, would give the full assessment intended for humans to the pigs. "They were pretty much treated just like if a patient with NF1 walked into a clinic," says Watson, with similar biopsy, MRI, and CT protocols.

As a cancer model, a pig's absolute size has a relative advantage too, says Dhanansayan Shanmuganayagam, a scientist at the University of Wisconsin-Madison who has worked with pigs for the past twenty years, initially for cardiac research. In rodent models of cancer, tumors often outgrow the relative size observed in a human patient. "Thus, physiology is completely different," he says. "You're no longer really looking at these tumor environments in the same way." There are also opportunities to measure co-morbidities and variables as well, such as age, diet, and metabolic syndrome, in the pig. "Cancer really doesn't exist in a vacuum," says Shanmuganayagam.

Tumors in a human-sized animal such as the pig can therefore follow a more human-like trajectory, and the tumor burden doesn't cause a welfare issue as quickly as in the smaller rodents, which even without those concerns have a much shorter natural lifespan. "The pigs live longer, so that the tumors can fully metastasize," says John Swart, president of porcine model and service provider Exemplar Genetics. "The pig will survive, whereas a mouse won't."

Genetically engineered pigs, engineered to express mutations found in human cancers, will also have a competent immune system (an area being extensively studied by xenotransplantation scientists). In the past, pharmaceutical testing performed in pigs was mostly for safety purposes because the animals themselves were healthy, says Watson. "Now, we can actually replicate a human disease, test the drug, and determine both safety and efficacy."

Beyond treatments, tools and techniques developed to diagnose and monitor cancer in the pig might also be adapted for clinical care, says Jill Weimer from the Sanford Research Institute. Weimer isn't a cancer biologist by training but rather, interested in cortical brain development and rare pediatric diseases, which brought her to NF1—the neurofibromas can cause cognitive and behavioral issues that are notably absent in mouse models, so she's also developed an NF1 pig model, with Exemplar and other academic collaborators (Box 2). For example, her collaborators at the University of Iowa are assessing whether imaging tools and approaches developed to detect tumors on



**Pig in an MRI:** Working with a human-sized animal means researchers can use human-sized equipment, like MRI machines to recreate how cancer in human patients is assessed and treated. Credit: L. Schook

**Box 2 | Swine surrogates**

In 2015, a litter of piglets was born at Exemplar. They were a strain known as the Yucatan Minipig, but these piglets had spots. Cognitive issues and neurofibromas followed, phenotypes characteristic of NF1 in children. Mouse models of the disease just don't recapitulate it in the same way, says Weimer. The Yucatans aren't alone. Ossabaw NF1 minipigs developed at Recombinetics, with collaborators at the University of Minnesota and the National Cancer Institute, have nerve tumors and Café au Lait spots too.

Under normal circumstances, two projects affiliated with two separate companies with similar business models might be quite competitive. But they were both funded by the Children's Tumor Foundation through a scheme the foundation calls "Synodos." Both teams are expected to work together and to share much their data along the way, with each other and eventually in a public database. "It's actually been great," says Weimer. Working with a new animal often means starting from scratch, but the two groups have shared protocols and tools, she says. The pigs have slightly different mutations and resulting phenotypes[10,11], but the side-by-side comparison they offer is unique. "We're able to show very similar but some unique differences in our pig models. And so I think that gave the field a lot of confidence in the model as well because it was verified by two independent groups," says Watson. Drug trials in the pigs are under way.

NF1 pigs in a strain known as the Wisconsin Miniature have been born at the University of Wisconsin as well. Academic researchers there are engineering not one just genetic variant but several, with the intent to model mutations found in individual patients, says Shanmuganayagam. They plan to follow the pigs for several years in longitudinal studies that will help them better understand the progression of NF1, he says.

In these cases, the swine are effectively acting as surrogates. As gene editing technologies continue to improve, the specific mutations that occur in a human can be ever more precisely captured in animal models. That can be especially important in rare cancers, like NF1.

"These patient populations have a unique problem in that there's really a lack of patients to go into clinical trials," says Watson. In the face of competing trials, "families are going to want to know that this is a surefire thing before they hedge their bet on trial one versus trial two," says Weimer. The ability to demonstrate the safety and efficacy of a treatment not just in a mouse but another animal that's more closely related to humans is a powerful feature, she says.

The NF1 pigs are very much like NF1 patients, capturing even subtle phenotypes that aren't particularly prominent in the clinical literature. For example, the Wisconsin group has observed gastrointestinal issues in their animals. That might not be the most pressing priority when treating a patient, says Shanmuganayagam, but it's a daily inconvenience that families experience and where the pig could be of additional use. Weimer ran into a complication when the usually enticing marshmallows weren't doing the trick to motivate their animals. "My technician had to spend two days figuring out what treat every single pig wanted so they could do the behavioral testing," Weimer says. When she discussed that observation with the clinicians on her team, they shared that NF1 children often have issues with swallowing different food textures.

"What the pig work has really forced me to do is work as a collaborative team," Weimer says. Working closely with patients and their families and doctors can force some new perspectives for scientists. In Wisconsin, Shanmuganayagam and his team interact with patients and their families regularly and will even invite them to visit the animals. He notes that their research timeline is often very personal—the son of his collaborator, Charles Konsitzke, was born with NF1. He and the researchers they work with recognize that observations that are academically interesting might not be the most translationally relevant to helping patients. "The focus is definitely on finding a treatment and finding a cure for the disease," says Watson. There's inherently a basic research element involved, but she echoes a duty to the patients. "We have to do translational work."

nerves and arteries in their pigs could help interpret and diagnose the same tumors in human patients. With inducible genetic models, where tumors can be 'turned on' at specific times in specific tissues, there are also opportunities to look at other diagnostic approaches, such as early biomarkers, says Schook. "We know what time zero is." Tumor progression and other biomarkers can be tracked from there.

With a pig model, you can recreate key elements of clinical care and test new treatments, says Adrienne Watson, pictured with two Ossabaw NF1 piglets.

Credit: Recombinetics

## Not your average pig pen

Last year, Carlson received his grant—an R01 from the National Cancer Institute to develop a porcine model of pancreatic cancer. In the end, he approached the application, and convincing his colleagues of the project's merits, in a practical way. "We said, 'we're going to build this model and we're going to use it to develop a specific technique that requires a human-sized specimen,'" says Carlson. The technique in question is fluorescence-guided surgery, in which a tumor is tagged with a fluorescent marker to assist surgeons in resecting it. Over the past year, he's been building the facilities and capacity at UNMC and the Omaha VA Medical Center to house and handle genetically engineered pigs, derived from Schook's 'oncopig' line, and they're currently testing a handful of different ways to best induce pancreatic tumors in juvenile pigs.

Picking up the pig as a potential animal model can be a big undertaking. "They're expensive, they're large animals, you have to have a facility that can handle them," says Watson. And it's important to remember that "miniature" is a relative term—Weimer notes surprise in many researchers who've reached out to her since their NF1 paper was published who had animals considerably smaller than they actually are in mind. "They think that these are going to be like tea cup pigs, or like 25 pounds, and I'm like, "No no, they get up to 200 pounds," she laughs. Mind the males too—they grow tusks when they hit puberty.

Big animals need big space. The minimum area recommended for a mouse in the United States is just under 100 square centimeters; for a rat, about 450. A ~100 kilogram pig should be given about 2 square meters per animal, instructs the *Guide for the Care and Use of Laboratory Animals*. In Wisconsin, for example, they house about 1500 pigs in a 40,000 square foot, pathogen-free barrier facility; two additional facilities

The ability to demonstrate the safety and efficacy of a treatment in an animal that's more closely related to humans than rodents is a powerful feature of the pig, says Jill Weimer.

Credit: Sanford Health

can house up to 200 animals and have dedicated imaging and surgery rooms. That's not to say that pigs, in smaller numbers, aren't popping up in populated places like Chicago, Philadelphia, and even New York City, but "sometimes it makes more sense to come this way," says Swart. Weimer's animals are housed at Exemplar's expansion facility in Iowa; both Exemplar and Recombinetics offer research services in addition to animals. Shanmuganayagam notes that Wisconsin can provide the physical infrastructure for housing and manipulating animals for collaborators to conduct their research there without needing to set up their own facility. Options are out there for those interested in the pig that might not have the right capacity at their own institutions.

Going *to* the pigs can help avoid some complications with the **Food** and Drug Administration (emphasis on "food" added). "The pig is legally defined as a food animal," says Schook, and "the transportation of a genetically modified food across state lines is regulated." In short, lots of security is involved and special exemptions from the agency are needed. Obtainable, but an added step.

Wherever the animals may physically be, they get a lot of attention from Day 1. "We feed them a lot of marshmallows," says Watson. They're handled regularly so they're used to interacting with people, which means the pigs can go through routine examinations without sedation.

They're smart and social animals, often with unique personalities, notes Shanmuganayagam. That makes them well-suited for studying cognition as well as social interactions, complications of neurological cancers such as NF1 but also side effects from a number of established cancer treatments, he says. Testing those attributes though means coming up with new assays—and physical infrastructure. The long arm of the T-maze Weimer and her colleagues built for their NF1 pigs measured 15 feet wide[10], a bit bigger than a mouse version. Wisconsin has been working to automate spatial, memory, and neurobehavioral tests for their animals.

The actual gene editing process to create a cancer model isn't necessarily new, says Shanmuganayagam, though it is getting faster and more efficient, particularly in light of CRISPR/Cas9. Producing a cohort of genetically engineered pigs can be an orchestrated event though. He walked *Lab Animal* through the process: To engineer a new pig model, you need eggs. To get those eggs, you need to know when the female is ovulating. There are hormone regimens to help there, but no way to know precisely when the animals are ovulating besides sheer manpower. Females need to see a boar, and then one-by-one, staff must physically check each donor animal to see whether she is in the appropriate phase of her ovulation cycle. When ready, she must be artificially inseminate; fertilized eggs then need to be removed, ferried to another facility to be edited within four short hours, and then surgically implanted into waiting sows that are also ovulating, just on a schedule that's about twelve hours reproductively behind that of the donor animals. "So it's quite a bit of an undertaking," Shanmuganayagam says, compared to say a mouse where everything can happen more or less in the same room.

Pigs are amenable to a variety of different editing techniques—TALENS, transposons, and CRISPRs, as well as Cre-Lox systems for inducible expression of mutated alleles, have all been demonstrated in the pig[2,3]. In the published literature, pigs have been produced that develop soft tissue sarcoma[4], (used recently to test a potential treatment too[5]) hepatocellular carcinoma[6] and pancreatic cancer[7], melanoma[8], lymphoma[9] and osteosarcoma[9], and neurofibromatosis type I[10,11], to name a few recent examples.

## That's (not) all folks!

The larger community of pig researchers is getting ready to convene again soon: the next "Swine in Biomedical Research Conference," first held by Schook in 1995, will take place at the University of Wisconsin-Madison in summer 2020. The meeting will cover current research and technology, but also provide hands-on training to the next generation of interested pig researchers. And the organizers hope to garner more interest in the animals, says Shanmuganayagam, among swine scientists, biomedical researchers, and human clinicians, as well as the biomedical industry, regulatory agencies, and funding bodies. Getting those different stakeholders in sync will be essential to building an integrated pipeline that can accelerate translational research in an efficient and cost-effective manner, he says.

Developing new animal models and fully characterizing them takes time, but the academic and commercial providers that *Lab Animal* talked with all indicated that interest is growing in porcine models of cancer. Schook for example makes his 'onco-pigs' available as close to cost as he can, partnered with the NIH-funded National Swine Resource and Research Center at the University of Missouri to develop the model for tissue-specific cancers. Commercial suppliers such as Exemplar and Recombinetics also have a number of porcine models of cancer available, with more in the works.

The pig, says surgeon Mark Carlson, "is the penultimate step before going into the clinic."

Credit: University of Nebraska Medical Center

At the end of the day, the pig will not usurp the position of the mouse in cancer research, nor is it necessarily intended to. "It's not a replacement, it's a transition," says Schook.

The pig won't be the starting point for basic cancer research, nor a place to screen potential therapeutic compounds but rather, it will be a model to check those results in a more translationally relevant animal before looking to the risk and the expensive of a human trial, says Carlson. He views the pig as "the penultimate step before going into the clinic."

The idea is mouse to man, with pig filling in gaps in between. "The challenge moving forward is going to be to identify the specific questions that you can't answer in the mouse, and you can't answer in the patient, and really apply those to the pig," says Watson. Ready to go whole hog? ❐

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