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News & Highlights

First Pig-to-Live Human Xenotransplant Produces Mixed Results

Sarah C.P. Williams

Senior Technology Writer

On 7 January 2022, a 57-year-old man with terminal heart disease became the first living human to receive a genetically modified pig organ—a heart—transplanted into his chest during a 8 h surgery at the University of Maryland Medical Center in Baltimore, MD, USA (Fig. 1) [1]. The pig heart, provided by the biotechnology company Revivicor (Blacksburg, VA, USA), had ten gene modifications intended to boost the chances of a successful pig-to-human transplant. The patient, David Bennett Sr., initially did well, with the transplanted heart functioning and showing no signs of organ rejection. However, his condition began to deteriorate, and he died about two months later. In a webinar on 20 April 2022, transplant surgeon Bartley Griffith said the heart seemed to have been contaminated with a pig cytomegalovirus which likely infected and killed Bennett [2].

Despite the patient's limited survival time, experts still considered the surgery a leap forward for the field of xenotransplantation—transplantation using organs of another species. Ultimately, transplant surgeons hope that genetically modified pigs can provide a new source of organs for saving human lives.

"For many years, there's been a discrepancy between the demand for transplantation and the availability of donor organs," said David Klassen, the chief medical officer of the United Network for Organ Sharing, located in Richmond, VA, USA. "Xenotransplantation offers a way forward to solve the supply issue for organs for transplantation."

With short gestation periods, large litters, and a similar body size to humans, pigs are considered an ideal source of organs for xenotransplantation. However, pig organs also pose a risk of immune rejection. Pigs have a number of animal proteins against which the human immune system commonly reacts. Moreover, their genomes contain endogenous retroviruses that could theoretically become activated in the bodies of recipients [3].

With the advent of CRISPR/Cas9 gene engineering [4], researchers gained a new tool to modify pig genomes for overcoming these risks. In recent years, more than 30 different genes have been modified in donor pigs—alone or in combination—to prevent immune rejection, reduce the risk of transmitting pathogens, or control the size of organs [5]. Some of these genetic alterations remove porcine genes, such as the sugar molecule galactose-α-1,3-galactose (alpha-gal), which is found in pigs but not humans. Others involve the addition of human immune genes, the inactivation of pig viruses or the removal of a pig growth hormone receptor [6]—the latter aiming to block the growth of pig organs after transplantation.

Fig. 1. On 7 January 2022, surgeons at University of Maryland Medical Center (Baltimore, MD, USA) received a genetically engineered pig heart and prepared to transplant it into a 57-year-old man with heart disease. The surgery marked the first xenotransplant of a pig organ into a living person. Credit: University of Maryland Medical Center (public domain).

The pig heart transplanted into Bennett in January 2022 had many of the genetic alterations which have been extensively studied for years in pig-to-non-human primate transplants. The operation was considered a last resort. The patient was connected to a heart–lung bypass machine to stay alive, was deemed ineligible for an artificial heart pump, and was rejected from waiting lists for a human donor heart. His surgeons received emergency authorization for the xenotransplantation from the United States Food and Drug Administration (FDA) under its expanded access (compassionate use) provision [1]. "This field is highly regulated, and this surgery did not happen overnight," said Klassen. "This has been a very long time coming and was approached thoughtfully."

In addition to acting as a test of the genetic modifications, the heart xenotransplantation also used an experimental antibody drug, KPL-404, made by Kiniksa Pharmaceuticals (Lexington, MA, USA), to suppress the activity of the immune system's B cells after the surgery [7]. In 2016, the University of Maryland transplant

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team reported that this drug was critical to keeping engineered pig hearts alive in baboons for more than two years [8].

"The ten [altered] genes help, but the antibody, which had been my main focus throughout my career, I think is the game changer," Muhammad Mohiuddin, director of the University of Maryland's Cardiac Xenotransplantation Program told the journal *Science* in January [6].

The heart surgery came on the heels of other tests, in late 2021, transplanting genetically modified pig kidneys into brain-dead human recipients. In one procedure, performed at the University of Alabama at Birmingham (Birmingham, AL, USA), surgeons transplanted two porcine kidneys from Revivicor, with the same ten genetic modifications as the xenotransplanted heart, into a 57-year-old man maintained on a ventilator after his natural death. The results showed that, over the course of 77 h, the kidneys remained viable, filtered blood, produced urine, and were not immediately rejected [9].

In another test, a team of surgeons at New York University (NYU) Langone Health in New York City, NY, USA transplanted pig kidneys into two recently deceased patients (Fig. 2). This test was more minimalistic in terms of gene alterations, with only a single edited gene, the deletion of alpha-gal. Still, the results were similar: Over the course of 54 h the organs remained alive and functioning, with no signs of rejection [10].

"The main purpose of these tests was to move the field along by demonstrating that what we have seen in the preclinical primate model holds true in humans," said Robert Montgomery, the surgeon who led the NYU team. As for why the NYU team relied on fewer gene edits, Montgomery said that surgeons already have a plethora of drugs and procedures for thwarting the immune rejection of human-to-human organ transplants, and these approaches work just as well when it comes to xenotransplantation. "We feel that less is more—there has been a lot of unnecessary complexity introduced during the decades of preclinical primate studies," he said.

His point fits into the broader challenges of studying and regulating xenotransplantation. Researchers are testing different organs with numerous different combinations of gene alterations, all of which makes it difficult to pinpoint which genes are critical to the success of any given transplantation. In addition, the not-perfectly-efficient nature of CRISPR/Cas9 gene engineering means that the exact levels of edited genes may vary between pigs. So far, researchers are producing newly cloned pigs to generate each new organ, rather than breeding herds of pigs that pass the gene alterations to their offspring. The diversity of approaches likely means that there are still lessons to be learned about the best way to proceed, said Montgomery. "This is a very complicated technology," he said, "It will not be perfect in the beginning."

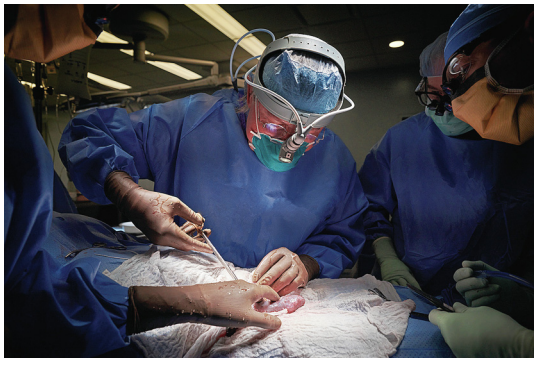

Fig. 2. In recent years, scientists have fine-tuned protocols for creating genetically engineered pig organs, using CRISPR/Cas9 technology to make the organs more compatible with humans. In 2021, surgeon Robert Montgomery transplanted a genetically engineered pig kidney into a recently deceased human to test how well the animal organ would function inside the human body. Credit: NYU Langone Health (public domain).

The heart xenotransplant illustrates the hurdles that remain. Over the days and weeks after Bennett received the pig heart, doctors closely monitored his health with an array of high-tech tests, even sequencing loose strands of DNA in his blood and screening him for hundreds of bacteria and viruses. Twenty days after surgery, Griffith said, that pathogen test alerted doctors to the possible presence of porcine cytomegalovirus [2]. Despite extensive testing of the donor pig, the virus can remain latent in pigs, and only present in deep tissues of the animals' bodies, making it difficult to detect. It is too soon to say for sure whether it was the virus that killed Bennett, but Griffith said it probably contributed. The good news? "If this was an infection, we can likely prevent it in the future," he said [2].

Better testing of donor pigs, or more stringent regulations on how the animals are bred, raised, and isolated before organ donation, could help. But additional gene editing is another possibility. In 2020, Chinese scientist Luhan Yang of Qihan Biotech Co., Ltd. (Hangzhou, China) and colleagues created pigs with more than 50 genomic alterations—most of them using CRISPR/Cas9 to inactivate porcine retroviruses [11].

Much more work will be required before the use of pig hearts and kidneys for human transplants becomes routine, but the short-term success of the first porcine heart xenotransplant is a harbinger of things to come. The heart pumped blood through Bennett's body for two months and was not immediately rejected, a better outcome than the first human-to-human heart transplant conducted in South Africa in 1967, which lasted only 18 d [12]. Organized trials approved by the FDA, or similar regulators in other countries, will be the next step to evaluating the feasibility of pig organs on a larger scale.

"We have reached a point where, from a scientific standpoint, we're able to approach these transplants in a more focused and potentially successful way," said Klassen. "I think we can expect to see some initial clinical trials launch soon."

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