Genetically Modified Pig Kidneys in Humans: Medical, Ethical, Financial and Social Perspectives

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Abstract

This paper seeks to analyze the xenotransplantation of pig kidneys into humans from medical, ethical, financial and social perspectives. Currently, about 100,000 individuals in the United States alone need a live saving kidney transplant and the truth is that most will die before ever receiving one. The paper addresses the possibility of pig kidneys being the solution to the organ shortage crisis. The medical team addresses how this process is carried out and the barriers both in the genetic modification of the pigs and the transplant procedure itself followed by immunosuppression. To evaluate the ethics of this procedure and its consequences, the principles of respect for persons, beneficence, nonmaleficence and justice are applied. Further, the social and financial implications are parsed out by explaining how xenotransplantation stacks up against human-human kidney donation. In conclusion, some innovative action must be taken to reduce the number of people on the kidney waitlist. If pig kidneys can serve as an unlimited supply of life saving organs then it may be the answer to the crisis but it first must be scrutinized from all perspectives.

INTRODUCTION:

Researchers in the realm of organ-transplantation and kidney disease care, must think outside of the box for a solution to the organ shortage crisis as more individuals suffering from chronic kidney disease are added to the organ waitlist each day. Although many get onto the waitlist, it remains that transplantation, the premier treatment for the condition, is simply not a feasible option for the vast majority of the nearly 800,000 individuals in the United States alone living with kidney failure.[1] Although dialysis can sustain life for these individuals, a new kidney provides a far greater quality of life as well as life expectancy. Further, dialysis has proven effective in sustaining life but has limitations of its own, seen by the 240 individuals who die each day awaiting a kidney from a suitable donor. This gap between those in need and those receiving transplants indicates that something more is needed. Current researchers in nephrology have proposed xenotransplantation as a viable option with the potential to provide enough life saving organs to resolve the organ shortage crisis altogether.[2]

With a surplus of viable kidneys, the world's potential transplantation recipients could be effectively cured of their condition. On the surface, this seems like the door to eliminating deaths from kidney failure. The kidneys are essentially personalized as the pigs can be bred en masse and their organs can be genetically altered to match the recipient to minimize rejection. Ethically speaking, as long as pigs are eaten for food, there should be no objection to using them as donor kidney incubators. This proposed procedure may sound like the golden ticket, but it is not without its medical, ethical, financial, and social challenges including immunosuppression, organ rejection and functionality, animal rights, bodily autonomy, etc. Although the elimination of deaths by chronic kidney disease is the ideal situation for researchers in the field, the world as we know it is not a utopia, thus xenotransplantation of porcine kidneys into human subjects must be further analyzed, diving in to what is necessary and what is sufficient for xenotransplantation to become a clinical practice. The following paper discusses in detail the ethics, challenges, history, and medical background of porcine nontransplant and further recommendations.

University of Alabama Case:

The current wait time to receive a human kidney from a deceased donor is approximately 10 years. In 2021, 240 people died each day on dialysis while waiting on a kidney transplant[2]. Fortunately, progress made in life-extending pig-to-human kidney xenotransplantation is promising. The University of Alabama at Birmingham (UAB) Marnix E. Heersink School of Medicine made history in the realm of organ transplantation early in 2022 by transplanting kidneys from a genetically modified pig into a brain-dead human, who was sustained on a ventilator while doctors monitored urine output, renal function, and rejection. The pig, whose organs were harvested, was bred in a pathogen-free facility designed for the sole purpose of raising pigs who may be a source of organs for human donations. The pig used at UAB had 10 genetic modifications which were crucial for making the organs suitable for transplantation to a human subject. The operation went precisely as planned, resulting in functioning kidneys that filter blood, produce urine, and most importantly, showed no signs of rejection for 77 hours after the operation until the study was concluded. Despite the organs showing all signs of proper function, some skeptical researchers question the significance of the experiment, arguing that living human clinical trials are required to determine if genetically modified kidneys from pigs will help to alleviate the human organ shortage. Although there are critics, this is a revolutionary moment in the history of medicine marking a paradigm shift towards xenotransplantation, which is "arguably the best solution to the organ shortage crisis" said Jayme Locke, M.D., the lead surgeon for the study at the UAB's Department of Surgery.[27] The work done at UAB marks a tremendous step forward in the direction of developing the safety and feasibility knowledge necessary to begin clinical trials in living humans with end-stage renal disease. As a result of their efforts, attention may now be directed toward the development of clinical trials

XENOTRANSPLANTATION

History

In the 1960s the first-ever xeno-heart transplant occurred using the heart of a primate. Unfortunately, the heart transplant was deemed unsuccessful and the patient died within a few hours as the heart could not support the patient's circulatory system.[3] Most of the early xenotransplantations followed a similar trend of not being prepared to account for the immunological or physiological challenges to xenotransplantation and were deemed unsuccessful and incompatible with the recipient.

Kidney transplants from differing animal species date back to the early 1900s. In 1905, a child received slices from a rabbit's kidney in France. Unfortunately, the child only survived for 16 days after the procedure.[4] Another example occurred in the 1960s, when chimpanzee kidneys were transplanted into multiple patients, which primarily resulted in only a few days of survival, primarily due to autoimmune rejection.[4] However, one recipient survived up to nine months until dying from electrolyte imbalances resulting from the aforementioned kidney transplantation There are other documented cases in which the kidney transplantation lasted only several months after transplantation, such as in 1966 when 19-year-old Raffaello Cortesini received a kidney from a chimpanzee and survived 31 days after the procedure.[4] Historically, kidney xenotransplantation has been of common interest amongst medical personnel and has undergone multiple attempts with varying degrees of success due to its potential to provide an limitless supply of donor organs.

In more contemporary procedures, xenotransplantation remains relatively successful. To this day insulin derived and produced from cows and pigs are still somewhat commonly used as indicated by the FDA's website.[6] An attempt was even made in 1993 to use pig islet cells in an attempt to assist diabetic patients. The results of the procedure were deemed to have no known medical benefit.[5] Today, much of the success within xenotransplants remains on the cellular level than with organ transplantation. Common cells transplanted from pigs to humans are islet cells and neuronal cells as a way to possibly prevent Parkinson's disease and assist diabetic patients.[7] Since the challenges of xenotransplantations in the 20th century, progress has been made regarding transplantation in general, but specifically in the case of kidney transplantation, as seen by the UAB case. As recently as 2022 a man received a genetically modified pig heart in Maryland and survived up to two months before dying potentially due to infection from a pig virus complication unrelated to any heart conditions.[8] The field of medicine's commitment to advancements in xenotransplantation is manifesting a potential solution to the organ shortage crisis.

Process

Xenotransplantation has the greatest potential of all treatments for renal disease because of the limitless,

renewable supply of life saving organs. *S. domesticus*, also known as the pig, has been accepted as the most compatible donor species for xenotransplantation into humans.[9] Much progress has been made in the modification of the pig genome to minimize immunologic barriers and potential incompatibilities between pigs and humans.[7] Due to the immune system similarities of nonhuman primates (NHP) to humans, the NHP is the preferred model for exploring the response to pig organ or cell transplantation. The pig-to-NHP model has become the gold standard for testing the primate immune response to organs and cells from pigs with GMOs and/or the effect of novel immunosuppressive regimens.[10] The initial modification to the pig genome to prepare it for human transplantation was the deletion of the gene that codes for a protein called alpha-1,3-galactosyltransferase (Gal). The pig's Gal triggers the human immune system, leading to the rejection that has been common in the xenotransplantation of the past [11]. Along with the kidney, the researchers also transplanted a pig thymus, an organ that produces immune cells and helps the body accept the foreign organs. The formation of the "thymokidney" is a novel method for the mitigation of the host's immune response.[8]

The extent to which xenotransplants can help the recipient is immense, yet the procedure needs to avoid causing significant harm to the patients in order to be considered a success. The procedures revolving around xenotransplantation are constantly being altered to better address the major concerns with xenotransplantation. One major concern with xenotransplantation is the risk of transmitting xenozoonosis to the patients. The safety procedures in place to limit the spread of these diseases include controlling the animal donor and the individual animal itself, while using strict sterile techniques during organ harvesting and donor autopsy.[12] Rejection of the transplanted organs is an incredibly dangerous possibility in both allotransplantation but even more so in xenotransplantation. Immunosuppressant drugs such as rituximab, basiliximab, tacrolimus, immunoglobulin, and corticosteroid have been shown to effectively integrate pigs' corneas into their human recipients. [13] Using pigs for xenotransplantation is incredibly feasible as a way to alleviate the need for organs. Using genetically modified pigs in xenotransplantation as a pure substitute for allotransplantation seems like a very possible alternative given the success xenotransplantation has had in bridging. [12] The pigs' organs would keep the patients alive until a human organ match would become available.

Obstacles

The use of pigs as a source of organs for xenotransplantation undoubtedly has the potential to reverse the current organ shortage crisis. However, there are still an array of barriers which need to be experimentally overcome before this is a clinical practice, including immunological barriers, physiological barriers and risk factors of disease. The most prevalent barrier to xenotransplantation is the human immune system's rejection of porcine tissues, which happens through multiple stages: (I) hyperacute rejection (HAR), (II) acute vascular rejection (AVR), (III) cellular rejection, and (IV) chronic rejection. In comparison, allotransplantation is associated with only (III) cellular and (IV) chronic rejection.[14] The significance of the University of Alabama's transplant in early 2022 was that there was no sign of any rejection due to the genetic modifications of the pig whose organs were harvested. Although the success at UAB was a leap in the right direction, more research is needed relating humans to porcine on the levels of anatomical makeup, metabolic processes, endocrine systems and circulatory systems before the true test may be performed: a clinical trial in a living patient. [15] The overarching challenge for researchers in the past decade has been the derivation of a combination of donor genome modifications and immunosuppressive drugs to effectively overcome the multifaceted obstacles to xenotransplantation.[16] The results of the trial in the brain-dead individual at UAB indicate that the day that clinical xenotransplantation will end the organ shortage crisis is closer now than ever before.

As well as the obvious immunologic and physiologic barriers, there also exists social and ethical barriers which must be dealt with. The idea of using animals, specifically pigs as a means to an end is no new phenomenon as the industrial food chain takes full advantage of the species to maximize economic output. The real issues arise surrounding the initial clinical trials. When is it deemed ethical to use a living human being as a guinea pig for xenotransplantation? How many human stem cells can be grown inside a pig until it is no longer a pig but rather a new species somewhere in between the human being and pig? These are just a few questions stemming from a much larger body of ethical and social concerns.

A large concern after organ transplantation is infectious disease due to the immunosuppressive agents given to

prevent graft rejection.[17] The risk of infection may be even greater in xenotransplantation, where immunosuppression may be more intense than in allotransplantation. Pathogens may appear from the recipient's bacterial flora, the environment, or from the donated organ.[18] However, the scarcity of data from clinical trials involving xenotransplantation preclude our full knowledge of infectious risks, and instead compel us to speculate based on experience with immunosuppressed human allograft recipients and studies of immunosuppressed swine xenograft recipients.[19]

Immunosuppression, graft rejection, reperfusion injury, and inflammatory cytokines can increase viral replication. In xenotransplantation, these factors may give way to replication of viruses such as porcine endogenous retrovirus (PERV), porcine cytomegalovirus (PCMV), porcine lymphotropic herpesvirus (PLHV-1, -2, -3), porcine circovirus (PCV), and adenovirus. [20]

Perhaps the most prevalent of these viral concerns are porcine endogenous retroviruses (PERVs). 3 subtypes of porcine endogenous retroviruses (PERV-A, -B, and -C) have been identified. While PERV-A and -B have been shown to infect human and pig cells in vitro, PERV-C infects only pig cells, although it is not present in all pigs.[21-22] The risk in a PERV infection lies in the virus's similarity to gammaretroviruses, which may induce severe immunodeficiencies and tumors in the infected host.[22] Furthermore, there exists the potential for silent transmission with retroviral insertional effects, which may prelude altered gene regulation or oncogenesis. [20]

Whereas transformed human cells lacking some intracellular restriction factors such as ABOPEC can be infected easily, high-titer PERV-A and recombinant PERV-A/C had increased infectivity in adapted human primary cells with increased transcription factor binding sites. No transmission of PERVs has yet been observed in clinical trials with over 200 patients involving xenotransplantations of pig cells/tissues. However, most of the patients in these trials were not exposed for a long time to the xenotransplant, and minimal immunosuppression was applied. Transmission of PERV was likewise not observed in pig to non-human primate (NHP) xenotransplantations. However, NHPs carry a mutated receptor for PERV resulting in low efficiency infections, and are therefore an unsuitable model to study transmission.[22] More research must be done to find out whether PERVs are transmitted in xenotransplantations for human recipients.

Nonviral agents may also cause infections if present in transplanted tissues. Pigs can be seropositive for *T. gondii* parasites or mycobacterial species. To avoid infection, it is recommended that the donor pig for xenotransplantation be free of PERV-C, and bred and raised in protected environments—where viral, bacterial, fungal, or parasitic infection is avoided.[18-19, 21]

Concerns - "Yuck" Factor

Much of the concerns in regards to xenotransplantation can be heavily attributed to the increase in vegan, vegetarian, and pescatarian eating habits over the past several decades. In the United States alone around 5 percent of Americans are vegetarian with younger generations having a higher percentage of around 8 percent in 2018.[23] The significance of the statistic is that as the rise of non-meat eaters increases so does the concern for the same individuals willing to receive an organ from an animal. Though, it is important to mention that the concern mentioned is not supported by any statistical evidence as no known survey has been conducted and it is unknown how many non-meat eaters would be willing to receive a xenotransplant.

One particularly famous case regarding the concerned populace was the case of Baby Fae. In 1984 an infant with a fatal heart condition (hypoplastic left heart syndrome) received a heart transplant from a baboon. The case of "Stephanie's Heart" received international attention from wildly different perspectives; many animal rights groups protested the transplant deeming it unethical and cruel.[24] Many people were shocked and arguably horrified by the prospect of xenotransplantation from the "Baby Fae" case. After 21 months the infant died due to complications from the transplant; the resulting case left the idea of xenotransplants largely abandoned and unexplored for many years.[24] The case of Stephanie's Heart left many people in disgust and horror as well as placing many animal rights activists in protests. The possibility of the same reaction occurring from future efforts of xenotransplantation may be a possible issue to face the field of research.

MEDICAL ANALYSIS:

Surgical procedure:

Research has shown that pig organs are typically considered to be more suitable for xenograft. One of the reasons is that many of the pig organs are similar to that of humans. In this

section, we will highlight factors to consider when initiating a xenograft transplant. We'll also review the pre-transplant, transplant, and post-transplant phases involved in a xenograft transplant.

Pre-transplant phase:

The initial state of the pre-transplant phase involves genetically modifying a pig's kidney to be a close match to the human kidney. This involves genetically altering the pig's tissue so it does not prompt an aggressive human rejection response. Ten genes have been changed in the donor pig. Four are disabled pig genes, also known as knockouts. Six are human genes that have been cloned into pigs, also known as knock-ins.[26-27]

The modified pigs also lack genes that are known to code for specific carbohydrates which are known to set off immune reactions in the human body, as well as genes for specific growth hormone receptors, which will prevent the pig's kidneys from continuous growth once transplanted.[27]

Once the genetically modified kidney is obtained, factors such as safety, size, and resemblance are considered, along with a crossmatch to determine if the pig's modified tissue is now suitable for transplant. There are some differences noted like the pig's kidneys are softer and have slightly thinner capsules compared to the human kidneys. The ureters of the pig's kidneys were slightly larger than the human ureters. These are factors to consider before transplant and adjustments made for these differences. The kidneys were surgically removed from the pig and transported for transplant. The recipient received immunosuppressive drugs to further reduce the risk of organ rejection.[1, 25]

Transplant Phase:

The recipient, a brain-dead patient's kidneys were removed. The pig's kidneys were placed in the same anatomical position as human kidney donors are placed with the renal artery and vein along with the ureter attached[27]

After a successful transplant, with no hyperacute rejection, transplanted kidneys were monitored for about 48 hours.[27] It was noted that the patient's body did not produce an immune response against the kidneys. Pre-transplant, the pig donor tested negative for porcine endogenous retroviruses, a virus present in the genome of all pigs, which infect certain human cells and therefore pose a special risk for xenotransplantation using pig cells, tissues, and organs.[27] There were no signs of this virus in the patient post-transplant.[25]

Post-Transplant phase:

Some of the questions asked and obstacles faced in the post-transplant phase is getting the transplanted organ to function in the human body. A hyperacute reaction which occurs within minutes of transplant is the first concern for most surgeons, but once it is confirmed that the human recipient did not reject the kidney, the next concern is, will the pig kidney function in the human body? Blood pressure is another obstacle that is of concern with xenograft transplant, as pigs have a lower blood pressure than humans do.[25] There was no specific way to confirm that the vascular integrity would not be compromised as there was no preclinical model to test this out. The hemodynamic stability strongly depended on the reperfusion of the kidneys.[25]

Production of urine is the next hurdle, and it was noted that the right kidney had a significant amount of urine production, compared to the left kidney.[26-27] It is unclear the reason for this difference, though authors suggest differences in procurement and room temperature. Perhaps additional research needs to be done to understand the peculiarities in such differences. Crossing the urine production phase, functionality is the next step. Can the transplanted kidneys filter waste from the body, as the human kidney does? It was noted by the team that the kidneys did not excrete creatinine, and the serum creatinine levels remained elevated. Noting that the xenograft recipient was brain dead, it's unclear if these abnormalities are due to damaged kidneys or resulted from physiological changes to the brain-dead patient.[27]

Safety/Efficacy

Essential to a fair ethical adjudication of pig-to-human kidney transplantation is having a thorough understanding of the safety concerns and their frequency, both intraoperatively and post-procedural. Our medical team, composed of residents, medical students, and supervised by Dr. Fazle Noor, a Kidney Transplant Surgeon, sought to compile and provide here the data from publications, articles, and experimental trials to date, looking back even as far as the 1950's. A Tulane University surgeon transplanted chimpanzee kidneys into 13 end-stage kidney disease patients in 1963-64. Those experimental recipients had little alternative other than death because chronic dialysis was not then available. Despite the close evolutionary relationship between chimpanzees and humans, no patient survived this desperation surgery for more than nine months, and nearly

all died within weeks, with acute transplant rejection being the most likely culprit of failure and subsequent death.

The paramount safety concern for any organ transplantation, even more so for xenotransplantation, is hyperacute rejection. Hyperacute rejection occurs within minutes to hours of transplantation, and is associated with a very high likelihood of failure of the transplanted organ, but more notably is associated with a very high frequency of mortality.[28] It occurs when pre-existing recipient antibodies react to donor antigens (type II hypersensitivity reaction) causing activation of the complement cascade. It results in widespread thrombosis of the graft vessels; thus, the graft must be removed.[29] It may be obvious to our reader that hyperacute rejection is both a safety concern and a consideration of efficacy assessment, as a transplant that fails cannot still remain effective. However, for the sake of our discussion, we will consider Hyperacute Rejection a component of transplant safety. The advancement of GMO NHP models, allowing for knock-out of antigens causative of thrombosis and complement activation, and the knock-in of other anti-inflammatory genes and anticoagulatory proteins, gives strong reason to suspect that, after a prospective compatible crossmatch between the GMO pig and human recipient is performed, experimenters may have much more success preventing hyperacute reaction from occurring.[30] Research over the past several years has demonstrated strong in-vivo evidence that GMO Pig kidneys containing a selection of tailored knocked-out and knocked-in genes would prevent rapid hyperacute rejection from occurring (11-13** below) These data encouraged the NYU surgical team and University of Alabama, Birmingham's research teams to test these GMO pig kidney in-vitro, by anastomosing and fully transplanting the kidneys (respectively) into brain dead recipients.[30-31] In both studies, performed 5 days apart from one another in September of 2021, the pig xenografts maintained vascular integrity with stable reperfusion and preserved good color and turgor. The metric used in the University of Alabama's study was gross appearance. They utilized doppler ultrasound to assess for maintenance of adequate reperfusion and performed post-transplantation biopsies of the kidneys each day post-transplantation and after the trial was complete. Biopsies of the right kidney (with the ureter anastomosed to the bladder) read "consistent with thrombotic microangiopathy, with diffuse glomerular capillary congestion, swollen endothelial cells, and near complete obliteration of the peripheral capillary lumina along with the presence of fibrin thrombi". Biopsy on day 3 read "evidence of progressive tubular injury with extensive acute tubular necrosis". The left kidney (urostomy) biopsy reports were listed as "mild-mod ATI, normal glomeruli". The lack of hyperacute rejection in these two trials, as well as the success of the first pig-to-human GMO TKO heart transplant performed in January 2022 in a living patient (who died several months later), provide strong optimism that hyperacute rejection can be avoided with these GMO pig organs containing tailored knock-out and knock-in genes. A limitation to consider for a potential future Phase 1 clinical trial is the short duration (approximately 2-3 days) of xenograft monitoring post-transplantation. While apparently successful in preventing hyperacute rejection, acute and chronic rejection of the xenografts are still valid safety considerations to be assessed.

Another acute, perioperative safety concern in these types of transplantations is the hemodynamic stability of the human recipient after transplant. The subjects of these two novel pig kidney studies were all brain-dead, ventilated patients. While observation of the subjects vitals was certainly telling, the physiology, including the blood pH, metabolic and hormonal homeostatic mechanisms (electrolyte levels, RAAS reactivity, etc) of a brain dead subject is potentially quite different from that of a living patient who is expected to recover to full functional status. However, the lack of cardiovascular collapse in the transplant subjects of these studies indicates that the washout of inflammatory mediators from the xenograft during reperfusion should not be a significant concern during the operation.[30] There has been a long-standing concern that a pig kidney could not withstand the increase in MAP (average arterial blood pressure) that is a normal level for a human, yet is at an "elevated level" for a pig. Resulting damage to the vasculature would be a safety consideration important to assess in future long-term transplant studies.

The second most important safety concern, alongside failure due to rejection, is the risk of side effects due to immunosuppressive medications required to prevent rejection. Around the late 1990's, concern around xenotransplantation grew as it was observed that a porcine kidney cell line could transmit C-type porcine endogenous retroviruses (PERVs) to human cell lines in vitro. Since the dawn of this concern, many measures have been constructed to address this theoretical risk. Sensitive PCR assays have been developed with the capability of detecting as few as 100 copies of PERV-C. [32] As research on the potential for human infection by PERVs furthered, it was realized that human cells are very difficult to infect due to the presence of

APOBEC restriction factors, and that it was primarily PERC A-C recombinants that infected cell lines in vitro.[31] As expected, no detection of PERV transmission to humans was present in the University of Alabama, Birmingham renal transplant study. The authors, however, recognized the need for a next generation sequencing technology with increased sensitivity and specificity in detecting PERV-A/C recombinant virus, which could provide the foundation for a robust Phase 1 clinical trial aimed at demonstrating microbiological safety of renal pig-to-human xenotransplantation.[30] To date, PERV infection has not been detected in any of over 200 patients who were exposed to porcine cells or xenografts in recent decades. Additionally, hundreds of non-human primates receiving porcine xenografts have also failed to show evidence of PERV infection. [35] A pair of studies in New Zealand demonstrated no transmission of zoonotic disease after human subjects were transplanted with encapsulated porcine islets and monitored for 7 years. [36, 32] However, it has also been reported that the risk that PERV transmission during xenotransplantation poses to human recipients is not well specified, as many trials hitherto have not involved full immunosuppression or involved encapsulated xenografts which carry less of an infectious threat.[37] Another safeguard utilized by the University of Alabama, Birmingham researchers was a rigorous, tightly-controlled housing and breeding protocol for preventing PERV existence among the selected pigs. All porcine renal xenografts were procured from genetically engineered pigs provided by Revivicor, Inc, one of the two manufacturers of genetically engineered, PERV-free pigs to be utilized for xenotransplantation. In their study, under the care of veterinarians, the donor pigs were tested every 3 months for porcine viruses, and housed in isolated facilities. Rigorous standard operating procedures for herd husbandry were also utilized. All of this considered, the risk of pig-to-human transmission of porcine viruses appears quite low, though a Phase 1 trial further demonstrating the safety of this xenotransplantation is lacking.

Immunosuppression

The first publication to report transplantation of pig kidneys into humans came from the University of Alabama. Their study aimed to examine the following goals in regards to immunosuppression: Is genetic engineering sufficient to prevent hyperacute rejection? Is negative prospective crossmatch sufficient to prevent hyperacute rejection? The answer to these questions were measured intraoperatively by visually inspecting the gross appearance of the kidneys. Are porcine derived products detectable in human blood? This was measured post-operatively by PERV-C transmission and presence of porcine proteins ubiquitous to all cells (i.e., ribosomal components).[1]

The first porcine kidney xenotransplant utilized kidney from genetically engineered pigs produced by company Revivicor, which were housed in a pathogen free animal facility close to the transplant center and tested every 3 months. The pigs were tested for infectious agents including hepatitis E, herpes virus gamma, influenza A, myoplasm hyopneumoniae, porcine circovirus [2,3,] porcine cytomegalovirus, porcine endogenous retrovirus A,B, and C, porcine epidemic diarrhea virus (S gene), porcine deltacoronavirus, transmissible gastroenteritis virus, and porcine reproductive and respiratory syndrome (PRRSV) virus (North American and European).[1] Results of the pathogen screening were negative except for Porcine endogenous retrovirus A and B which tested positive on August 19, 2021.[34] The University of Minnesota Veterinary Diagnostic laboratory was utilized for all testing. The decedent's blood after xenotransplant was tested daily for porcine endogenous retroviruses and remained negative. Chimerism, or presence of porcine large ribosomal protein (pRPL4), was not observed.

There were 10 genetic modifications made to the pig genomes, hence the name 10-GE pigs. These modifications included two human complement inhibitor genes (decay accelerating factor - hDAF and membrane cofactor protein-hCD46), two human anticoagulant genes (hTBM and hEPCR), and two immunomodulatory genes (hCD47 and hHO1).[1] They also had deletions (Knockout) of three pig carbohydrate antigens and the pig growth hormone receptor gene. Knockout of the genes for alpha-1,3-galactosyltransferase (GGTA1) is responsible for preventing synthesis of Gal. Knockout of the genes for beta-1,4-N-acetyl galactosyltransferase (B4GalNT2) was responsible for preventing synthesis of SDa. Knockout of genes encoding enzyme CMP-N-acetylneuraminic acid hydroxylase (CMAH) is responsible for preventing synthesis of Neu5Gc.[34] The new phenotypes GGTA1KO, B4GALNT2KO, and CMAHKO were confirmed in the 10-GE pigs utilizing flow cytometry of PBMC stained with IB4 lection, DBA lectin, and anti-Neu5Gc to show the absence of xenogeneic carbohydrate residues.1 GHRKO phenotype was determined by showing reduced serum IGF-1 levels and body weight. These 10-GE pigs no longer express their

original pig red blood cell antigens so they can be used as a universal donor when considering blood type.[26] Expression of the individual transgenes was confirmed by kidney biopsy of donor pig kidney after transplantation by western blot and immunohistochemistry.[1]

Testing between the donor pig and human decedent (recipient) was completed prior to transplantation to determine histocompatibility. The 10-GE pig donor lymphocytes were targeted by flow cytometry crossmatch with pre-xenotransplant decedent serum. The negative control used pooled human male AB serum (Decedent's blood type AB+) and the positive control serum was human serum containing IgG known to react with porcine cells.[1]

For induction immunosuppression, daily methylprednisolone taper, anti-thymocyte globulin (4mg/kg), and anti-CD 20 (Rituximab)were used. The methylprednisolone and anti-thymocyte globulin were administered immediately prior to xenotransplant. Mycophenolate mofetil, tacrolimus, and prednisone were used for maintenance immunosuppression. There was effective depletion of lymphocytes.[26] On POD 0, the decedent received 175mg anti-thymocyte globulin, 1800mg Rituximab, 1mg Tacrolimus (PM), 2000mg Mycophenolate mofetil (PM), and 500mg Methylprednisolone. On POD 1, 175mg anti-thymocyte globulin, 1mg Tacrolimus twice daily, 1000mg Mycophenolate mofetil twice a day, and 250mg Methylprednisolone were administered.[32] On POD 2, 1 mg Tacrolimus in the morning and 2mg Tacrolimus in the evening, 1000mg Mycophenolate mofetil twice a day, and 125mg Methylprednisolone were administered. On POD 3, 2 mg Tacrolimus daily, 1000mg Mycophenolate mofetil daily, and 90mg Methylprednisolone were administered.[1]

Hyperacute rejection was not observed in this study, so this provides some evidence than knockout of the genes that encodes the carbohydrate xenoantigens may be sufficient to prevent the reaction in humans[1] Also, it showed there were no preformed antibody against either the major histocompatibility complex in pigs (swine leukocyte antigen; SLA) or other unknown minor antigens using a novel flow crossmatch assay to predict that hyperacute rejection would not occur. This novel flow crossmatch assay will need additional research as the sensitivity and specificity is unknown. Additional research is needed to aid in interpretation of positive crossmatches, and to identify other antigenic targets.[26]

Histologic analysis was done and on post-operative day 1 and showed diffuse glomerular capillary congestion, swollen epithelial cells, and almost complete obliteration of peripheral capillary lumina and presence of fibrin thrombi. These findings were concerning thrombotic microangiopathy. On postoperative day 3, histology showed progressive tubular injury with extensive acute tubular necrosis. On day 3, wedge biopsy did not show evidence one would expect of TMA progression to cortical necrosis or interstitial hemorrhage due to antibody mediated damage.[1] There was expression of the human transgenes within the parenchyma of the porcine kidney. The xenograft biopsies were negative for IgM, IgG, C4d, C1q, and C3, so the TMA observed was not mediated by complement or antibody in the xenografts.[32] The mechanism of molecular incompatibility causing the TMA is unknown. The observed TMA could be the result of complement-mediated cytotoxicity, as the alternative complement pathway does not require antibody or C4 to trigger formation of the membrane attack complex (MAC; C5-9). Even though the 10-GE xenograft was genetically modified to contain complement inhibitor genes DAF and CD46 to address histologic findings associated with acute humoral xenograft rejection, the proteins only slow MAC formation and do not necessarily prevent it. The study presents that additional genetic or pharmacologic interventions may be necessary to improve graft survival and function.[1] A C5-antibody is available and is utilized for treatment of severe antibody-mediated rejection in human allotransplantation, and prevention of MAC formation with anti-C5 antibody was shown to improve xenograft survival in non-human primates' model.

Questions:

What pig genetics are necessary?

What immunosuppressive/anti-inflammatory/adjunctive therapeutic regimen is optimal to offer success?

Did the genetic modifications of the pegs alter the overall structure and integrity of the renal parenchyma? And do these structural changes impact renal function and recovery?

Did brain death affect poor renal recovery by complement activation and hemodynamic decompensation requiring vasoactive agents?

What caused the microvascular injury and how did it affect the renal dysfunction?

Since the xenograft biopsies were negative for IgM, IgG,

C4d, C1q, and C3, was TMA mediated but some other unknown mechanism or molecular incompatibility or by complement-mediated cytotoxicity through the alternative complement pathway?[34] Does Anti-C5 antibody have any role in xenotransplantation for the treatment of severe anti-body mediated rejection and prevention of MAC formation given improved xenograft survival in a non-human primate model?

Dr. Seth Karp noted in ASN Kidney News notes "Pig organs may have initial difficulty with any of these issues. Even so, the transplant community supports a deep culture of scientific investigation and innovation. Combined with major advances in genetic manipulation, it is reasonable to assume that these hurdles will be overcome over time. Concerns about transplantation-mediated zoonosis (e.g., pig viruses) will remain, and these will need to be assiduously monitored. When recent studies were reported in major media outlets, comment sections were filled with objections to using pigs as organ farms from an animal rights perspective. These concerns will similarly need to be addressed". Dr. Karp also highlighted immunologic questions that need to be investigated going forward: "What immunologic issues accompany the use of pig organs? What are the optimal immunosuppressive regimens? What is the nature of rejection, and how is it treated? Are these organs subject to chronic rejection, and can this be avoided? What additional genetic modifications could improve immunocompatibility".[36] He also posed questions about function: "Do pig organs faithfully reproduce the function of the corresponding human organ? In particular for the kidneys, will the higher human blood pressure be an issue in the short or long terms? Will filtering and other kidney functions be appropriate". [36]

FINANCIAL ANALYSIS

Although this paper thoroughly examines the efficacy and safety of GMO kidney xenotransplantation through the conceptual frameworks of medicine, ethics, and societal expectations, the scope of its analysis is still fundamentally limited. The standard of healthcare outcomes must be considered by its economical implications just as much as the clinical and humanistic contributions. Therefore the “true value of healthcare interventions, programs, and policy can be assessed only if all three dimensions are measured and considered”.[37] The discipline of pharmacoeconomics, defined as the cost analysis of therapeutic treatments incurred by the health care systems, by the patients, and by society, enables one to compare the absolute repercussions of multiple treatment modalities.[26] The primary methodologies utilized to compare numerous treatment options include cost-benefit, cost-effectiveness, cost-minimization, and cost-utility analyses. Unfortunately, because GMO kidney xenography is in its infancy, many of the metrics, including costs and quality adjusted life years (QALY) gained, are currently variable and subject to drastic change as the procedure is augmented, it is not currently possible to accurately evaluate the procedure utilizing pharmacoeconomic methodologies. Therefore, I will provide a brief conceptual framework, cost-benefit and cost-effectiveness analyses, that can be used to evaluate this treatment option compared to current treatment once cost and outcomes become less volatile.

The two primary methodologies that should be employed to evaluate the economic implications of GMO xenographic kidney transplantations compared to a typical kidney allotransplantation are cost-minimization and cost-utility analysis. A cost-minimization analysis is an analytical tool that compares the costs of treatment options that possess similar patient outcomes.[28] The first step in a cost-minimization analysis is to approximate all costs associated with each surgical procedure. This includes direct costs, indirect costs, and productivity costs. Direct medical costs include “a physician’s fees, hospital costs and physiotherapy cost”.[38] Indirect medical costs include out-of-pocket costs that patients are expected to pay following a surgery. For example, pain pills and immunosuppressant medications. Productivity costs are a subtype of indirect costs including the loss of wages incurred by patients and caregivers following a medical procedure. The second step of a cost-minimization analysis is to take the summation of all costs associated with each procedure. If both procedures possess similar patient health outcomes, then the procedure with less total costs is the more economical option for both the patient, health system, and society. Therefore, this pharmacoeconomic methodology should only be employed if GMO xenographic kidney transplantations eventually yield similar patient health outcomes. If there are patient health outcome discrepancies between traditional allographic kidney transplantations and GMO xenographic kidney transplantations, then a cost-utility analysis should be utilized. This methodology compares the costs incurred in each treatment to the QALYs gained from the procedure. A QALY is a unit measurement that quantifies the quality of life experienced by an individual in a single life year. Values of a QALY range from 0 to 1, where a QALY value of 1 is equivalent to a year of perfect health, typically free from any

ailments.[39] Reciprocally, an individual who is suffering from significant physical or emotional pathologies would possess a QALY value that is closer to 0. The first step of a cost-utility analysis is to determine the QALYs gained from a surgical intervention. The next step is to calculate the total costs associated incurred from the procedure. This step is identical to cost calculation utilized in the aforementioned cost-minimization methodology. The third step is to divide the total costs of the procedure by the total QALYs gained to calculate the cost per QALY. Most health policy experts believed that a medical treatment with a cost per QALY above $50,000 was not economically justified.[30] Therefore, if the GMO xenographic kidney transplantation possesses a cost per QALY that is below $50,000 or a cost per QALY that is comparable to the traditional allographic kidney transplantation, then the novel treatment option is viable.

In conclusion, more studies should be conducted on the economic viability of GMO xenographic transplantation of kidneys as the procurement process and surgical techniques become more standardized.

ETHICAL ANALYSIS

The issue of xenotransplantation has raised serious interdisciplinary concerns. Many have called for a continued public debate on the issue of xenotransplantation, especially genetically modified pig kidneys for human transplants, that would examine all aspects of it including the crucial ethical and moral implications. These issues include the safety of the technology, animal welfare issues and the claim by some that genetic engineering is a technology that should not be advanced by humans. However, some have warned that the agenda of the debate, especially the moral debate, should not be wholly framed within the aspirations of the practitioners. All parties need to be consulted—clinicians, recipients and society as a whole. The surgeons involved are still talking in terms of how we should proceed rather than should we proceed. If this is going to be an open debate with all parties participating, then all options must be placed on the table, including the option that we should not proceed with xenotransplantations and in particular, genetically modified pig kidneys in humans. To determine if this procedure is ethical, the principles of respect for persons, beneficence, nonmaleficence and justice will be applied to this procedure and its consequences.

Respect for persons refers to the right of a person to exercise self-determination and to be treated with dignity and respect. Proponents argue that genetically modified pig kidneys have the potential to save hundreds of lives. We know that the current wait time to receive a human kidney from a deceased donor is approximately 10 years and that 240 people die each day on dialysis while waiting for a kidney transplant.[1] The two experiments at the University of Alabama and NYU Langone Health "suggest that major barriers to human xenotransplantation have been surmounted and identify where new knowledge is needed to optimize xenotransplantation outcome in humans."[1] Opponents argue that there is not enough information available for recipients to give informed consent. Safety concerns exist in regards to porcine endogenous retroviruses (PERVs) carried by pigs that could transfer to the recipient and be not only detrimental to the recipient but possibly become a public health crisis, immunosuppressants have serious side effects and genetic modifications pose serious concerns medically and ethically. Proponents argue that respect for persons is protected because any participant in this experimental surgery would give their informed consent and be made well aware of the animal trials and previous human trials that have preceded this surgery and the potential risks, benefits and alternatives. In addition, they would know that the surgery has met the conditions of being ethically justified research by the local Institutional Review Board (IRB). The problem is that in the United States and many European countries a new surgical technique requires no formal regulatory approach and is controlled primarily through surgeons' self-regulation that is sometimes, but not always supplemented by local control over research including peer review and IRB approval of a formal protocol.[40]

The conditions for approval by an IRB are:

1) a reasonable prospect that the research will generate the knowledge that is sought;

2) the necessity of using human subjects;

3) a favorable balance of potential benefits over risks to the subject; and

4) a fair selection of subjects.[41]

Proponents argue that the duty of the IRB is to check that researchers have not overestimated the potential success and underestimated the possible risks. Their duty is also to ensure that the risk-benefit ratio of undergoing this surgery is reasonable.[40] Approval by the IRB would be an added assurance to potential recipients and to society as a whole.

To give valid informed consent to be a subject in an

experimental surgery, two conditions must be met: the consent must be freely obtained from a competent person and the individual must be adequately informed regarding all aspects of the experimental surgery.[42] First, from the recipient's perspective, the option open to patients with severe end stage renal disease (ESRD) is dialysis until a human kidney becomes available for transplant. "Dialysis is a form of renal replacement therapy. It's the clinical process of replacing the filtration functions of the normal kidneys. In hemodialysis, this is done by using a machine to filter the circulating blood in the vessels through an artificial membrane. Metabolites and excess fluid are removed from the blood. The blood is filtered through diffusion across a semipermeable membrane. This works by microscopic openings in the filter and concentration gradient. Concentration gradient means that the concentration of molecules is lower in the dialysate, causing unwanted molecules from the blood to naturally move into the 'less crowded' solution. Which molecules are filtered out of the blood can be controlled by the contents of the dialysate solution. The membrane also filters molecules by size, keeping larger molecules like proteins in the blood. In hemodialysis, the blood vessel can be accessed several ways. In emergent or temporary dialysis (after trauma or acute illness) a dual lumen dialysis catheter is placed into a large vein above the heart. These catheters are easy to place and can be used immediately, but carry risk of infection and clotting. For chronic hemodialysis, an arteriovenous fistula or graft is created, connecting the vein and artery in the forearm, and the vein accessed by two needles. With a fistula, a surgical anastomosis, or connection, is created and must mature before use. In an arteriovenous graft, a synthetic material is implanted connecting the vein and artery. While it can be used immediately, the artificial graft carries risk for infection. The ideal approach depends on urgency and the quality of the patient's vessels."[43] Hemodialysis is done three times a week and usually takes four hours per day. It is relatively painless but there are side effects—low blood pressure, muscle cramps, anemia, hypertension, fluid overload. Patients can survive on dialysis for over 10 years but one's quality of life is lessened. As stated above, 240 people died each day on dialysis while waiting for a kidney transplant.

To give informed consent, recipients would have to be made aware of the risks, benefits and alternatives available to them. They would need to understand and comprehend that the pig kidney has ten genetic modifications. Four genes were inactivated, including one that encodes a molecule that causes an aggressive human reaction response. Another gene is genetically tweaked to prevent any donated organs responding to human growth hormones and growing out of control. Another key alteration removes a sugar molecule, called alpha-GAL, which sticks to the surface of pig cells and acts like a gigantic flashing neon sign marking the tissue as absolutely alien. Two other neon signs will be genetically removed and six human ones added in, acting like a camouflage net over the pig cells to help hide them from the immune system. The resulting 10-gene pigs are then raised in sterile conditions so they are suitable for transplant.44 The problem is that besides the risk of rejection, which would mean removal of the kidney, the patient would also have to be on immunosuppressant drugs for the remainder of his or her life, which are expensive and have serious side-effects such as increased chances of serious infections.

This is still an experimental procedure with serious risks, but with additional research, genetically modified pig kidneys for humans has the potential to be lifesaving and to give individuals with ESRD a far better quality of life. Under these circumstances, it is questionable whether the recipient is really free to give consent for such a procedure. Research has shown that whenever a new form of surgery is proposed that patients tend to dwell more on the benefits than the risks. "If potential patients are desperate for a procedure, the question arises whether it is feasible for them to assess if possible improvements in quality of life outweigh the potential morbidity and mortality caused by long-term immunosuppression."[45] It is very difficult to determine if informed consent can be freely obtained with the way the media has sensationalized this surgery and the hype by various surgeons about its potential benefits. The consent may appear to be free but unfortunately, it may be based upon unrealistic expectations. At the present time, what this procedure realistically offers these patients is the possibility of improvement in the quality of their life.

Second, for a patient to give informed consent, he or she must have the necessary information to make such a decision. The basic elements of informed consent are:

1. A fair explanation of the procedures to be followed, including an identification of those which are experimental;
2. A description of the attendant discomforts and risks;
3. A description of the benefits to be expected;
4. A disclosure of appropriate alternative procedures that would be advantageous for the subjects;
5. An offer to answer any inquiries concerning the procedures;
6. An instruction that the subject is free to

> discontinue participation in the project or activity at any time.[46]

In a specific sense, the surgeons who want to transplant genetically modified pig kidneys into humans have an ethical obligation to give an objective, unbiased assessment of all materially relevant information pertaining to the animal studies and the human trials so that the patient can give informed consent. In addition, the rates of rejection, the costs and side-effects of the immunosuppressant drugs, the psycho-social issues, and other risks must be clearly stated and explained to the patient. The surgeons are also responsible to verify, to the best of their ability, that the patient can comprehend and has comprehended the information and has not engaged in "selective hearing." This means, surgeons should explain the risks, benefits and alternatives at a 5th grade level so that all patients can comprehend the information. Under the circumstances, it is not uncommon for patients to engage in "selective hearing," that is, taking in all information about potential benefits and filtering out all information about potential risks. To overcome "selective hearing" the surgeon should invoke the "teach-back method," which means the patient repeats back to the surgeon what he/she heard and understands. In addition to this, surgeons must be vigilant against their influence over subjects, who may unwittingly treat the surgeon with the same deference as they treat their primary care physicians. Dr. Robert Levine, professor of Medicine at Yale University, describes the surgeon/researcher's obligation as one of "forthright disclosure." This includes preliminary evidence and data from animal studies and previous human clinical trials that indicate the risks and benefits as well as the safety and efficacy of these controlled studies. [47] Patients need to have information that a reasonably prudent person would require to make well-reasoned decisions that will protect their personal interest.

The problem is determining what sort of knowledge translates to what degree of risk to patients. This is a value judgment that must be made by the surgeons. The concern is that the judgment of some surgeons may be biased by considerations of career self-interest and even financial gains.[48] "The potential for coercion can be difficult for surgeons. On the one hand, most accept that the final choice for surgery should be left to the patient. On the other hand, surgeons want what they believe to be best for their patients. Therefore, there is ample room for unintentional coercion through selecting information for disclosure that overtly reinforces the surgeon's beliefs."[49] There is also the problem of forming an "innovative alliance." Patients may encourage their surgeons to try any new and promising technique to improve their quality of life or prospects for survival and surgeons also may be eager to apply a promising new technique for the same reasons. It is the duty of the surgeons to decide whether responsible behavior lies in attempting an innovative technique or in concluding that the background research is not sufficient to warrant its use, even when the patient consents.[44] The surgeon has the responsibility to act in the best interest of the patient. The belief that this experimental surgical procedure will not cause too much harm to too many people or that society will benefit at the possible expense of particular individuals violates the duty of the surgeon/researcher to act in the best interest of the patient. To determine whether that duty has been breached, a surgeon/researcher's actions should be measured against the accepted practice as set by professional norms. Those researchers whose treatments fall below the professional standards and cause harm to patients may be held civilly liable for that failure.[50] Various ways have been proposed that ensure individuals going into research protocols are giving informed consent, these include: written and oral forms of consent so that the patient has time to read and reflect on the risks and benefits; someone other than a member of the surgical team obtains the informed consent; obtaining second opinions from other knowledgeable physicians regarding the feasibility of such a procedure; and appointing an objective advocate who would accompany the patient during the decision-making process. These advocates would ensure that the patient is capable of understanding the information and comprehends all the information, that researchers do not overestimate potential benefits and underestimate potential risks, and that all viable options are given, even the option of no transplant. These are not only excellent ideas; they should be implemented with every research protocol.

The complexity of this experimental surgery and its multileveled physical, psychological and social dimensions, make informed consent very complex. Since this surgery has been performed on a limited basis, it would be hard for surgeons to evaluate the potential risks and then adequately inform the patient of them to satisfy informed consent. Therefore, information that is necessary for informed consent is limited at the present time. In fact, the obstacles to informed consent in this situation seem almost insurmountable. In addition to weighing the risks and benefits, we are also asking individuals considering a transplant from a genetically modified pig to weigh just as many psychologically demanding variables. These issues

only highlight the complexity of a patient giving informed consent under the circumstances. However, if the previous mentioned safeguards are enacted, it may be possible for patients to give informed consent, but this must be done objectively, comprehensively and honestly by a team of medical professionals.

Beneficence involves the obligation to prevent and remove harm and to promote the good of the person by minimizing the possible harms or risks and maximizing the potential benefits. Beneficence includes nonmaleficence, which prohibits the infliction of harm, injury, or death upon others. In medical ethics this principle has been closely associated with the maxim Primum non nocere: "Above all do no harm."

Proponents argue that xenotransplantation and in particular, transplant of a genetically modified pig kidney into a human has the potential to save thousands of lives. Proponents contend that the risks are present as they are with any form of transplantation, but that if a patient comprehends the risks, benefits and alternatives and consents freely and knowingly to the surgery, then that individual should be given the right to make that informed decision. To delay the inevitable when the knowledge, technology and skills are available and when patients believe this surgery is in their best interest, is not only standing in the way of scientific advancement but is failing to promote the good of the patient and the good of society as a whole.

Opponents argue that the safety concerns regarding xenotransplantation are a major factor. The most significant concern is the immunological rejection of the organ by humans. The human system will recognize the organ as foreign and correlatively reject it. To date the 10 genetic modifications have been successful, on a limited clinical trial basis of avoiding the hyper-acute rejection. The use of immune-system-suppressing drugs has also reduced the probability of rejection. The immunosuppressant drugs do have serious side-effects, but the patient would need to weigh the side-effects against the potential of a lifesaving transplant. The costs of the drugs must also be factored into the patient's consent process.

Second, there are safety concerns for endogenous retroviruses carried by pigs, which could be capable of making humans very ill. This is not only a recipient concern but also a public health concern. Porcine endogenous retroviruses (PERVs) could impact the common good of society. Proponents will argue that the pigs used in xenotransplantation are not raised under the traditional husbandry conditions. "Rather they are kept much in the manner of laboratory animals, under confined, sterile conditions that minimize the risk of pathogen proliferation and keep the animals sufficiently healthy to provide a (relatively) safe source for transplantation. Although such conditions are far better than agricultural conditions in terms of animal health, they are equally deficient in accommodating the animal's biological and psychological natures."[51] Most ethicists would argue that the good of humanity would take priority in this regard. Another ethical issue concerns the limited privacy of recipients of a genetically modified pig kidney. These patients would need to be monitored for pathogens dangerous to them and others for extended lengths of time, which in turn would impact on the privacy of the patient but also the patients' family, friends, work associates and others who are in contact with the patient. Due to the experimental nature of these transplants, it would be difficult to protect the anonymity and confidentiality of the recipient and their respective families from the public and the press. These patients may be looked upon as "freaks of nature."[51] Critics fear the large amount of publicity will place unrealistic expectations on the recipient thus creating additional psychological stress and pressure. This could result in discrimination, alienation and exploitation of the patient and his/her family. To imagine or even calculate the psychological impact on the recipient and his or her family seems almost impossible.

The final issue regarding the risk/benefit ratio has to do with the genetic modification of the pigs with human stem cells. To date the process seems very regulated and controlled but some critics raise the issue of the human stem cells migrating to the brain of the pig and creating medical and ethical concerns. This is an issue that has been advanced in regards to all genetic engineering in regard to xenotransplantation. This is a valid concern, but it is not enough of a concern to limit all genetic engineering technology. Researchers are also moral agents and we must respect their integrity in regards to following proper human research protocols. There are also many safeguards in place to verify this integrity.

No one will dispute that balancing the benefits and risks is difficult. Some will say that the value of life will take precedence over potential risks. After reviewing the facts concerning the state of our knowledge regarding xenotransplantation, the effects of immunosuppressant drugs and the inevitable psychological impact on the recipient and

the recipient's family, it seems reasonable to argue that the transplant of a genetically modified pig kidney into a human person could maximize the benefits and minimize the risks incurred by these patients. This is still an experimental surgery and more research in this area will need to be done. Unless more research is done, we will never know conclusively if the benefits outweigh the burdens. Arguably, this form of xenotransplantation has the potential to not only pass the test of beneficence, but also pass the test of nonmaleficence.

Finally, *justice* recognizes that each person should be treated fairly and equitably, and be given his or her due. The principle of justice can be applied to this situation in two ways. First, questions of justice have been raised about whether those patients who are desperate for kidney transplants might be classified as vulnerable individuals and whether this type of experimental surgery is a form of exploitation. No one seems to dispute that surgeons have the skills and techniques needed to perform this experimental xenotransplantation. However, there also seems to be a competition present among the various transplant teams examining this form of xenotransplantation. This debate cannot and must not be framed within the aspirations of the surgeons. There must be equality between the surgeons and the possible recipients. To allow these charismatic surgeons to present this form of transplantation in the media in such a way that seems to trivialize the side-effects and downplays the possibility of rejection and even death, is to exploit these recipients and use them as a means to an end. At the present time the debate among the transplant surgeons is on how they should proceed. Most transplant surgeons see no need for a delay in increasing the surgery because there is nothing to be learned during this time of delay. With this attitude, how objective and unbiased will the information about the surgery and its possible benefits and risks be that will be disclosed to potential recipients? How will surgeons know when the potential vulnerability of some patients is unduly influencing their willingness to consent? These transplant surgeons have suggested ways they believe will ensure informed consent however, since many of these potential recipients have no other viable options, one could say it is unjust to place these vulnerable individuals in this position now. However, if the safeguards to informed consent are enacted, and recipients clearly comprehend the benefits and risks of the xenotransplantation, then it would be just to allow these individuals to become involved in further research protocols.

Second, the issue of justice pertains to genetically modified pig transplants into humans specifically in regards to distributive justice, which concerns the fair and equitable allocation of medical resources. The main issue here is research priorities. Should funds be used to support xenotransplantation surgery now when the potential risks seem unreasonable and even deadly? The amount of money spent on these surgeries could certainly be invested in new ways to tolerate immunosuppressant drugs and primate experimentation to lessen the rejection rates. This would help to minimize the risks and maximize the benefits not only for recipients of xenotransplantation but for all transplant patients. Also, immunosuppressant drugs cost tens of thousands of dollars a year. Will this not limit the individuals who would qualify for this surgery? If so, this now becomes a social justice issue, because those who would have access to this technique would logically be those who are privileged. The poor, the uninsured, the underinsured, and many middle-class individuals would never be viable candidates for this surgery, because they could not afford the cost of a life-time supply of immunosuppressant drugs. As a matter of social justice, who this surgery would benefit and whether it is a fair and equitable allocation of medical resources is an important ethical issue. Medical professionals have an ethical obligation to use available resources fairly and to distribute them equitably.

In conclusion, transplanting genetically modified pig kidneys into humans could be considered ethically and morally acceptable under certain circumstances. If there is a standardization of outcomes and comprehensive inclusion/exclusion criteria for xenotransplantation and guidelines and safeguards for informed consent, then and only then would this surgery be ethically permissible. To make xenotransplantation a reality, physicians have an ethical obligation to perfect the safety and efficacy of this technique. This will entail additional research to overcome the unknown long-term prospects.

RECOMMENDATIONS/CONCLUSIONS

In a world where efforts to prevent kidney disease prove ineffective as the proportion of individuals with diabetes and hypertension increases constantly, there must be an effort focused towards enabling all of those in need of a transplant to receive a lifesaving organ. Xenotransplantation may be the very solution that can alleviate this crisis. With an unlimited supply of kidneys for donation, individuals around the world could go on living, outside of the hospital without

the financial and physical burden of dialysis, the even worse outcome of waiting for an organ that will never come, or the case of never receiving any treatment at all like most individuals in developing nations. The only way to make this ideal a reality is to view this innovation from all angles including medical, social, financial and ethical, which we have parsed out above. Below are recommendations to the xenotransplantation researchers, industry, as well as executives in facilities where these procedures will be taking place in order to ensure the safety and feasibility of this procedure in the future:

1. We recommend beginning clinical trials in living humans. The animal to animal trials give us enough evidence to justify a small, phased trial of pig kidney transplants to humans. Transplants into brain dead patients will not give us enough new insights.
2. Researchers need to examine the question of will these transplants last for many years or just long enough for human organs to become available? Defining whether these are to serve as a bridge to human organ transplantations, or as the solution to the crisis is key to developing a roadmap for xenotransplantation.
3. To ensure patient informed consent there should be the appointment of objective advocates for these patients who would accompany them during the decision-making process.
4. Develop more research into the genetic engineering of the pigs using human stem cells so as to minimize the rejection.
5. The final question we pose to researchers of this industry is must there be assurance that the genetic modification causes no harm, and ideally, as is the case in genetic modification to prevent disease or ameliorate genetic defects, the animals will be better off in virtue of such modification.[51]

The death toll from chronic kidney disease and kidney failure must be addressed and all things considered, xenotransplantation seems to be the most probable solution in the near future. With further research to ensure the safety of xenotransplant recipients, clinical trials in fully living human subjects may begin and the next set of barriers to clinical application will be uncovered. It is only then that the potential of xenotransplantation to save lives can be brought to fruition. Although genetic engineering has eliminated many of the barriers to using xenotransplantation as a clinical practice in the past, there remain issues as we have parsed out that must be resolved. Additional testing and further research into this procedure hopefully will lead to the success of the clinical application of kidney xenotransplantation in the near future.

References

1. Porrett PM, et al. First clinical-grade porcine kidney xenotransplant using a human decedent model. Am J Transplant. 2022 Apr;22(4):1037-1053. doi: 10.1111/ajt.16930. Epub 2022 Jan 20. PMID: 35049121.
2. Greer, T. (n.d.). UAB announces first clinical-grade transplant of gene-edited pig kidneys into brain-dead human. UAB News. Retrieved June 6, 2022, from https://www.uab.edu/news/campus/item/12566-uab-announces-first-clinical-grade-transplant-of-gene-edited-pig-kidneys-into-brain-dead-human
3. Cooper, DKC. Genetically engineered pig kidney transplantation in a brain-dead human subject. Xenotransplantation. 2021; 28:e12718. https://doi.org/10.1111/xen.12718
4. Jack-Yves Deschamps et al., "History of Xenotransplantations," Xenotransplantation 12, no. 2 (2005), https://doi.org/10.1111/j.1399-3089.2004.00199.x, Wiley Online Library.
5. David C. K. Cooper et al., "A Brief History of Clinical Xenotransplantation," United States Department of Health and Human Services, (2015), 5, doi:10.1016/j.ijsu.2015.06.060.History of Clinical Xenotransplantation," 3-4.
6. Center for Drug Evaluation and Research. "Importing Beef or Pork Insulin for Personal Use." U.S. Food and Drug Administration, FDA, https://www.fda.gov/drugs/frequently-asked-questions-popular-topics/questions-and-answers-importing-beef-or-pork-insulin-personal-use.
7. Cooper, David K C, "A brief history of cross-species organ transplantation," Proceedings (Baylor University. Medical Center) vol. 25,1 (2012), 49-57. doi:10.1080/08998280.2012.11928783.
8. Press, T. A. (2022, March 9). A man who got the 1st Pig Heart Transplant has died after 2 months. NPR. Retrieved November 20, 2022, from https://www.npr.org/2022/03/09/1085420836/pig-heart-transplant
9. Montgomery RA, Stern JM, Lonze BE, Tatapudi VS, Mangiola M, Wu M, Weldon E, Lawson N, Deterville C, Dieter RA, Sullivan B, Boulton G, Parent B, Piper G, Sommer P, Cawthon S, Duggan E, Ayares D, Dandro A, Fazio-Kroll A, Kokkinaki M, Burdorf L, Lorber M, Boeke JD, Pass H, Keating B, Griesemer A, AliNM, Mehta SA, Stewart ZA. Results of Two Cases of Pig-to-Human Kidney Xenotransplantation. N Engl J Med. 2022 May 19;386(20):1889-1898. doi: 10.1056/NEJMoa2120238. PMID: 35584156.
10. Cooper, D. K., Satyananda, V., Ekser, B., van der Windt, D. J., Hara, H., Ezzelarab, M. B., & Schuurman, H. J. (2014). Progress in pig-to-non-human primate transplantation models (1998-2013): a comprehensive review of the literature. Xenotransplantation, 21(5), 397–419. https://doi.org/10.1111/xen.12127
11. Reardon, S. (2022). First pig kidneys transplanted into people: What scientists think. Nature, 605(7911), 597–598. https://doi.org/10.1038/d41586-022-01418-3
12. Michler, R. E. (1996). Xenotransplantation: Risks, Clinical Potential, and Future Prospects. Emerging Infectious Diseases, 2(1), 64-70. https://doi.org/10.3201/eid0201.960111.
13. Choi, HJ, Yoon, CH, Hyon, JY, et al. Protocol for the first clinical trial to investigate safety and efficacy of corneal xenotransplantation in patients with corneal opacity, corneal perforation, or impending corneal perforation. Xenotransplantation. 2019; 26:e12446. https://doi.org/10.1111/xen.12446
14. Denner, J., & Tönjes, R. R. (2012). Infection barriers to successful xenotransplantation focusing on porcine

endogenous retroviruses. Clinical Microbiology Reviews, 25(2), 318–343. https://doi.org/10.1128/cmr.05011-11
15. Vanderpool H. Y. (1999). Xenotransplantation: progress and promise. Interview by Clare Thompson. BMJ (Clinical research ed.), 319(7220), 1311. https://doi.org/10.1136/bmj.319.7220.1311
16. Hryhorowicz, M., Zeyland, J., Słomski, R., & Lipi□ski, D. (2017). Genetically Modified Pigs as Organ Donors for Xenotransplantation. Molecular biotechnology, 59(9-10), 435–444. https://doi.org/10.1007/s12033-017-0024-9
17. Fishman J. A. (2000). Infection in xenotransplantation. BMJ (Clinical research ed.), 321(7263), 717–718. https://doi.org/10.1136/bmj.321.7263.717
18. Michaels, Marian G.. (2014). 29 - Xenozoonoses : The Risk of Infection after Xenotransplantation. American College of Laboratory Animal Medicine, , 1371-1379. https://doi.org/10.1016/B978-0-12-409527-4.00029-8
19. Fishman J. A. (2019). Infection in xenotransplantation: opportunities and challenges. Current opinion in organ transplantation, 24(5), 527–534. https://doi.org/10.1097/MOT.0000000000000682
20. Fishman, Jay A.. (2018). Infectious disease risks in xenotransplantation. American Journal of Transplantation, 18(8). https://doi.org/10.1111/ajt.14725
21. Yang, B., Wang, R. & Qin, C. (2020). Xenotransplantation: Current Status in Preclinical Research. Front. Immunol., https://doi.org/10.3389/fimmu.2019.03060
22. Denner, J. & Mueller, Nicolas J.. (2015). Preventing transfer of infectious agents. International Journal of Surgery, 23. https://doi.org/10.1016/j.ijsu.2015.08.032
23. Zach Hrynowski, "What Percentage of Americans are Vegetarian," Gallup, (2018), https://news.gallup.com/poll/267074/percentage-americans-vegetarian.aspx.
24. Larry Kidder, "Stephanie's Heart: The Story of Baby Fae," Loma Linda University Health, September 8, 2016, https://news.llu.edu/patient-care/stephanie-s-heart-story-of-baby-fae.
25. Megan Skyes, MD, David H Sachs, MD. "Transplanting organs from pigs to humans"Transplanting organs from pigs to humans - PMC (nih.gov)
26. David K.C. Cooper, Hidetaka Hara. "You cannot stay in the laboratory forever" Taking pig kidney xenotransplantation from the laboratory to the clinic. https://doi.org/10.1016/j.ebiom.2021.103562
27. Tyler Greer. "UAB announces first clinical-grade transplant of gene-edited pig kidneys into brain dead human."https://www.uab.edu/news/campus/item/12566-uab-announces-first-clinical-grade-transplant-of-gene-edited-pig-kidneys-into-brain-dead-human
28. Treatment of Acute and Chronic Rejection, Imtiazuddin Shaik, ... Pauline W. Chen, in Transplantation of the Liver (Third Edition), 2015
29. USMLE First Aid 2021
30. Wynyard S, Nathu D, Garkavenko O, Denner J, Elliott R. Microbiological safety of the first clinical pig islet xenotransplantation trial in New Zealand. Xenotransplantation. 2014 Jul-Aug;21(4):309-23. doi: 10.1111/xen.12102. Epub 2014 May 7. PMID: 24801820.309-323.; 27.
31. Cooper, DKC. Genetically engineered pig kidney transplantation in a brain-dead human subject. Xenotransplantation. 2021; 28:e12718. https://doi.org/10.1111/xen.12718
32. Kaulitz D, Mihica D, Dorna J, et al. Development of sensitive methods for detection of PERC-C in the genome of pigs. J Virol Methods. 2011. 60-65
33. Skyes M, Sachs D, Transplanting Organs from pigs to humans. Sci Immunol. 2019
34. Matsumoto S, et al. Long-term follow-up for the microbiological safety of clinical microencapsulated neonatal porcine islet transplantation. Xenotransplantation. 2020.

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