# Ethical and Regulatory Aspects Regarding Xenotransplantation

Von der Naturwissenschaftlichen Fakultät der
Gottfried Wilhelm Leibniz Universität Hannover

zur Erlangung des Grades

**Doktorin der Naturwissenschaften**
**(Dr. rer. nat.)**

genehmigte Dissertation von

**Koko Reyna Kwisda, Mag.rer.nat. Mag.phil.**

2023

**Referent:** Prof. Dr. rer. nat. Thomas Scheper

**Korreferenten:** Prof. Dr. Thomas Reydon
Prof. Dr. med. Stefan Jockenhövel

**Tag der Promotion:** 13.09.2023

## Abstract

Organ transplantation has become a standardised procedure in medicine. Nonetheless, shortage of organs suitable for transplantation has remained a worldwide issue. Thus, animals, and the domestic pig in particular, have arisen as potential suitable organ donors for humans. Two strategies are currently being pursued to generate immunocompatible porcine organs: induced pluripotent stem cells of the human recipient to form the organ within the pig, which serves as bioreactor for the growing organ comprising of human cells and thus effectively constitutes a human-animal chimera. The second approach involves heavy genetic editing of the porcine genome to generate a multitransgenic pig.

Novel technologies are often accompanied by heated debates regarding their ethical implications. These are often focused on the most sensational concepts and thus rarely discussed in a holistic manner. Also, breakthrough technologies are very rarely preceded by specific regulations.

This manuscript sets the basis for the continuing ethical as well as the regulatory discussion regarding xenotransplantation: First, the systematic evaluation of the ethical challenges associated with human-animal chimeras provides an overview and thus hopefully prohibits continued fragmentation of the debate. Furthermore, the characterisation of normative issues pertaining to the generation of chimeric and/or multitransgenic pigs as well as the identification of specific unanswered regulatory questions demonstrate that existing laws fall short of addressing all associated issues.

This thesis thus lays the groundwork for standardising the ethical debate and also guides the regulatory discussion towards specific unresolved problems. This will hopefully facilitate the harmonisation of international regulation of xenotransplantation.

**Key words:** *xenotransplantation, bioethics, chimeras, biotechnology, genetically modified organisms*

## Kurzzusammenfassung

Organtransplantationen stellen mittlerweile einen standardisierten medizinischen Eingriff dar. Dennoch fehlen weltweit passende Spenderorgane. Daher sind Tiere, insbesondere das Schwein, als potentielle Organspender von besonderem Interesse. Für die Generierung eines immunkompatiblen, porzinen Spenderorgans werden prinzipiell zwei Strategien verfolgt: entweder bringt man induzierte pluripotente Stammzellen des menschlichen Rezipienten, welche dann das zu spendende Organ ausmachen, in ein genetisch editiertes Schwein ein. Hierbei nutzt man das Schwein als Bioreaktor für das aus menschlichen Zellen bestehende, wachsende Organ und stellt im Zuge dessen eine Mensch-Tier-Chimäre her. Andererseits kann man das porzine Genom stark genetisch verändern und so ein multitransgenes Schwein erzeugen, dessen Organe kompatibel mit dem menschlichen Immunsystem sind.

Neue Technologien werden oft von heftigen Debatten bezüglich ihrer ethischen Implikationen begleitet. Diese Debatten fokussieren sich häufig auf die aufsehenerregendsten Aspekte und verhindern dadurch eine holistische Betrachtungsweise. Überdies werden selten im Vorhinein spezifische Gesetze für neue Technologien verabschiedet.

Dieses Manuskript schafft die Grundlage für die weiterführende ethische und rechtliche Debatte hinsichtlich Xenotransplantation: einerseits werden die ethischen Herausforderungen bei Mensch-Tier-Chimären systematisch evaluiert, was hoffentlich eine weitere Fragmentierung der Debatte verhindert. Überdies zeigt die Herausarbeitung der normativen Aspekte im Zusammenhang mit chimärischen und/oder multitransgenen Schweinen sowie auch die Identifizierung von spezifischen, unbeantworteten regulatorischen Fragen in diesem Bereich, dass die existierende Rechtslage nicht alle assoziierten Probleme adressiert.

Diese Doktorarbeit schafft daher die Basis für die Vereinheitlichung der ethischen Debatte und lenkt auch die regulatorische Diskussion auf spezifische ungelöste Probleme. Dies wird hoffentlich die Harmonisierung der internationalen Regulierung von Xenotransplantation erleichtern.

**Key words:** *Xenotransplantation, Bioethik, Chimären, Biotechnologie, genetisch modifizierte Organismen*

## Table of Contents

# I. Introduction

Organ shortage is a huge, global problem. Lack of suitable deceased donors constitutes a massive bottleneck for waitlisted patients with terminal organ failure. According to the US Government Information on Organ Donation and Transplantation, more than 108.000 candidates were on the transplant waiting list, while only roughly 39.000 transplants were performed in 2020 [1]. Therefore, animals as a source of suitable organs for human recipients have emerged as a hot topic. This concept, called xenotransplantation (gr. Xenos = foreign), has received much attention all through the 20$^{th}$ century, with several attempts of transplantation of organs from nun-human primates into humans until the 1990s [2-4]. However, non-human primates were eventually deemed unsuitable donors due to several concerns like cross-species infections, organ size disparities and, not least, ethical concerns [5]. Therefore, genetically modified pigs are now deemed the most promising source for organs and cells for humans: They have several advantages over non-human primates, such as large number of off-spring, physiological similarity to humans and thus comparably sized organs.

Recent publications suggest that the most promising scenarios involves either the introduction of human induced pluripotent stem cells (iPSCs) into an organogenesis-disabled pig, which constitutes a bioreactor providing the ecosystem for natural organ development (reviewed in [6]); and/or the heavy editing of the porcine genome to provide metabolically functional organs in human recipients (reviewed in [7-9]). First results from pre-clinical animal models, the transplantation of a pig kidney into a human, brain-dead host as well

as the most recent pig-to-human heart transplantation demonstrate that clinical trials involving humans are around the corner [10].

It is clear that numerous ethical and regulatory concerns need to be addressed, though, before such an approach can be translated into a routine product.

**Scope of This Thesis**

This manuscript is organised around multiple peer-reviewed publications stemming from the research performed to map the legal and ethical landscape surrounding genetic modification of animals as well as the creation of human-animal chimeras for the purpose of xenotransplantation.

**Chapter 2** recaps the current scientific status regarding the various approaches to generate multitransgenic and chimeric pigs and progress regarding xenotransplantation into non-human primates as well as humans.

In **chapter 3**, we dive into the ethical minefield surrounding the mixing of human and animal material. We will provide a systematic overview of ethical arguments appearing in academic publications on human-animal chimera research by means of a systematic review of the available literature.

**Chapter 4** will be dedicated to the fragmented regulatory landscape surrounding this technology. We will first horizon-scan developing regulation regarding current progress in the field of xenotransplantation, and will ultimately assert that existing regulation in this area is generally not fit for purpose, especially regarding international harmonisation of such regulations.

Moving away from general considerations, we will then analyse the normative aspects of xenotransplantation on the basis of a fictitious case study and showcase how it might play out in a specific jurisdiction in Europe.

In **chapter 5,** we conclude this manuscript with a discussion of the results achieved within this work and its impact on current and future debates regarding genetic modification of animals and creating human-animal mixtures for the purpose of xenotransplantation.

## 1.1 Publications

This thesis is based on the following original publications:

**1.** K. Kwisda**,** L. White, D. Hübner. Ethical arguments concerning human-animal chimera research: a systematic review. *BMC Medical Ethics,* 2020. 21:24.

***Contribution:*** I conceived this study, drafted the initial framework for analysis and worked on its refinement, carried out the literature search, analysed all included publications, and drafted the manuscript. L.W. participated in literature searching, data analysis and extensive redrafting of the manuscript. D.H. participated in establishing the framework for analysis, data analysis and redrafting of the manuscript.

**2.** K. Kwisda**,** T. Cantz, N. Hoppe. Regulatory and intellectual property conundrums surrounding xenotransplantation. *Nature Biotechnology*, 2021. 39(7):796-798.

***Contribution*:** I had the idea for the publication, conducted research regarding legal and ethical issues pertaining to xenotransplantation, identified the normative issues associated with the steps for creating the multitransgenic/chimeric animal, and drafted the manuscript. T.C. refined the steps involved in the creation of the multitransgenic/chimeric animal and helped redraft the manuscript. N.H. supported with the identification of normative issues, conducted legal research pertaining to xenotransplantation and drafted parts of the manuscripts.

**3.** K. Kwisda**,** T. Cantz, N. Hoppe. Reply to 'Clarifying US regulations on xenotransplantation' and 'International standards and guidelines for xenotransplantation'. *Nature Biotechnology*, 2021. 39(12):1503.

***Contribution:*** Together with N.H. and T.C. I wrote the manuscript.

**4.** K. Kwisda. Unaddressed Regulatory Issues in Xenotransplantation: a Fictional Example. *Frontiers in Transplantation 2023*; 2:1222031.

***Contribution:*** I conducted the legal research, evaluated the data and wrote the manuscript.

## II. Current State of Research Regarding Xenotransplantation

### 1. Challenges to Overcome

End-stage human organ failure can only be combatted by allotransplantation. However, shortage of suitable donor organs is a common problem in all countries, as evidenced by a total of 1296 patients dying while on the waiting list for an organ in Eurotransplant-participating countries in 2021 alone [11]. These statistics do not take the patients into account whose organ failure has not progressed far enough to be eligible for the transplantation waiting list. Therefore, the genetic modification of animals in order to turn them into organ donors for human recipients has received much attention. The pig has emerged as the most suitable source of xeno-organs as it has many genetic, anatomical and physiological similarities to humans; furthermore, the possibility to genetically modify pigs increases their potential as organ donors [7, 9].

In the context of providing immuno-compatible organs from pigs one needs to identify mechanisms that eliminate the problem of immunological reactions and ensure immediate and sustained compatibility with the human host metabolism without transferring infectious disease agents into the recipient (reviewed in [9, 12, 13]). In the following, i. the various barriers shall be outlined, ii. the technologies to create immuno-compatible donor pigs shall be described and iii. the current status of pig to non-human primate and human xenotransplantation shall be discussed.

#### 1.1 Immunological Barrier

A wild-type porcine organ transplanted into a non-human primate or a human is immunologically rejected by three successive processes, namely hyperacute xenograft rejection, acute humoral xenograft rejection and cellular rejection [13].

### 1.1.1 Hyperacute Rejection and Acute Humoral Xenograft Rejection

Hyperacute rejection describes the process by which antibodies present in the recipient lead to rejection of the xenograft within minutes or hours after transplantation [14]. Humans have natural antibodies against pig carbohydrates, which are expressed on the surface of all pig cells [15]. The majority of these human anti-pig antibodies are directed against galactose-α1,3-galactose (α-Gal) [16, 17]. αGal is a product of the enzyme alpha-1,3-galactosyltransferase (GGTA1) which is encoded by the porcine GGTA1 gene [18]. Alpha-1,3-galactosyltransferase is present in most mammals, including pigs, but not in humans and other primates [19]. GGTA1 is expressed by many gut bacteria though, which is why IgG antibodies directed against the α-Gal epitopes are induced during the neonatal period and make up roughly 1% of all circulating antibodies in the blood [19, 20].

The transplantation of wild-type pig organs therefore elicits various immunological rejection responses, like hyperacute rejection (HAR), which is mediated by those pre-existing anti-α-Gal antibodies binding to the porcine epitope and thus results in the activation of proteins of the complement system and formation of the membrane attack complex. This entails endothelial injury due to cell lysis, destruction of the graft vasculature and, eventually, graft failure within minutes [21, 22]. HAR can be overcome by i) inactivation of C3b and C4b as well as blocking the formation of the membrane attack complex through insertion of human transgenes and ii) genetically knocking out the porcine GGTA1 gene [23, 24]. However, this does not inhibit another non-gal antibody mediated mechanism called acute vascular rejection (AVR) or acute humoral xenograft rejection occurring within a few days or weeks after transplantation

[25, 26]. The underlying mechanism involves pro-coagulatory and pro-inflammatory activation of endothelial cells and a combination of humoral and cellular immune response [27] and entails necrosis, interstitial haemorrhage and infarction, amongst others [28], thus displaying a similar histopathology as HAR [29]. So far, two non-Gal epitopes have been found to be involved: N-glycolylneuraminic acid (Neu5Gc) and the SDa/Cad blood group [30, 31].

Neu5Gc is a product of cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMAH) encoded by the porcine CMAH gene. Humans cannot express Neu5Gc due to the presence of a mutation in the human CMAH gene, which prevents the synthesis of functional CMAH [32], but pigs as well as monkeys are able to synthesise it [33]. Humans thus produce antibodies against porcine Neu5Gc, which is probably triggered after the consumption of pork meat [34].

The SDa blood group is produced by beta-1,4-N-acetyl-galactosaminyltransferase 2 (β4GALNT2) encoded by the porcine β4GalNT2 gene [31]. While the human genome does contain a homologous enzyme, only a low level of anti-SDa IgM antibodies is detectable in most people. Studies inactivating the porcine β4GalNT2 gene show reduced binding of human IgM and IgG to blood cells of pigs, suggesting the presence of human antibodies which bind to the antigen synthesised by the porcine β4GalNT2 enzyme [14]. Therefore, these two non-Gal epitopes are important targets as well.

### 1.1.2 Cellular Xenograft Rejection

In addition to HAR and AVR, cellular xenograft rejection (also called acute cellular rejection) can lead to transplant rejection weeks after transplantation

[35]. Both the innate and adaptive immune system can cause it through their respective components Natural Killer (NK) cells, macrophages, T- and B-cells, dendritic cells and neutrophils [36]. Due to the vast array of involved players, numerous targets need to be genetically modified in the pig donor to achieve the following (reviewed in [37, 38]:

- Interrupting T-cell activation and inducing T-cell apoptosis to avoid T-cell-mediated cytotoxicity against the transplanted porcine vascular endothelium
- Regulation of macrophage activation to avoid toxic effects due to secretion of pro-inflammatory cytokines as well as phagocytosis
- Preventing interaction of NK receptors with ligands on pig endothelial cells and thus avoid NK cell mediated cytotoxicity
- Modify class I swine major histocompatibility complex (swine leucocyte antigens, SLA) molecules to reduce xenoantigen expression and thus reduce the recipient's immune response.

In summary, to prevent hyperacute, vascular, and cellular rejection it is necessary to combine several genetic modifications of the porcine genome.

### 1.2 Inflammatory Response and Coagulation System Dysregulation

Even when HAR, AVR and cellular xenograft rejection are successfully prevented, xenotransplantation also gives rise to inflammatory reactions, which in turn foster abnormal coagulation processes within the vessels of the transplant, ultimately leading to graft failure [39]. The process is initiated with the destruction of the porcine endothelial cells by antibodies and complement activation, which releases tissue factor into the blood stream and thus triggers

the coagulation cascade via the extrinsic pathway [40]. This pathway dovetails with inflammation and innate immunity and eventually results in thrombotic microangiopathies in the porcine graft and systemic coagulopathies in the transplant recipient [41]. This condition called systemic inflammatory response is a major hurdle in pig-to-human xenotransplantation [39]. To overcome this, inflammation and apoptosis need to be inhibited; the imbalance between procoagulant and anticoagulant activity needs to be corrected; and adhesion, aggregation, and phagocytosis of recipient platelets exposed to xenograft need to be reduced.

### 1.3 Physiological Incompatibility

Apart from immunological incompatibility, there is some significant difference with regards to physiological properties of human/NHP and pig cells. The pig thus needs to be genetically altered in a way to prevent overgrowth of the xenograft in the recipient. Additionally, there are some concerns specific for certain organs: regarding kidney xenotransplantation, post-transplant proteinuria is of concern [42]; and the liver in particular plays a central role in the production of roughly 2000 proteins, and it seems unlikely that all of those produced by a transplanted porcine liver would function properly in humans [43]. In the case of liver xenotransplantation, pig donors would thus need to be genetically altered in a way to produce functional human proteins.

### 1.4 Infectious Disease Barrier

Another issue concerns biosecurity, i.e., the risks associated with potentially introducing animal-based infectious agents into humans, resulting in disease. These so-called zoonoses could be the result of the transfer of pathogenic

organisms like porcine cytomegalovirus, porcine lymphotropic herpes virus and hepatitis E virus from the pig to the recipient. To prevent them, careful screening of donor pigs for potential zoonotic microorganisms in advance, breeding them in a sterile environment, caesarean birth and weaning piglets off early should be able to minimise that risk [44, 45]. These measures, however, are not able to address porcine endogenous retroviruses (PERVs), which are part of the pig's genome and are produced as infectious virions from the pig's cells. They are feared to cause zoonoses in humans after transplantation [46]. One approach to circumvent these involves PERV elimination from the porcine genome, e.g., by using the CRISPR-Cas9 technology.

## 2.Technologies to Create Immuno-Compatible Pigs

Given the challenges outlined above, it is clear that pigs need to be heavily genetically altered before becoming suitable donors. In general, there are two possible scenarios that could ensure compatibility with the recipient and overcome the challenges outlined above. The first involves using human cells to comprise the later-to-be-transplanted organ; the second involves creation of multitransgenic pigs by inserting human transgenes as well as deleting porcine genes.

### 2.1 Using Human Cells to Create a Chimera

The ideal donor organ would genetically match the recipient's own cells. Attempts to grow such organs from stem cells in vitro have failed so far, although organoids grown from adult stem cells now have a variety of applications in *in vitro* disease modelling and regenerative medicine (reviewed in [47]). Therefore, in vivo organogenesis in a suitable host, i.e., the domestic pig, is the next best

thing. This approach has the advantage of providing an in vivo reactor infrastructure that also provides nutrition and oxygen to the growing organ. First, the pig blastocyst needs to be genetically modified by knocking out the essential genes for the development of any given organ and thus thwart development of the respective organ. The created genetic niche can subsequently be filled with induced pluripotent stem cells (iPSCs) from a human patient in need of the particular organ. The process would ideally lead to a human-animal chimera with rescued organogenesis, in which the target organ consists mostly of injected human cells. Proof of principle was demonstrated by Matsunari *et al.* [48], who succeeded in rescuing pancreas development by introducing blastomeres from another pig at the morula stage. The concept of interspecies blastocyst complementation (IBC) has been successfully applied to create rat-mouse chimeras with rescued thymus and pancreas formations [49, 50]. However, there are still some limitations particularly relating to human-animal interspecies chimerism, for instance that the resultant organs are not purely made up of donor cells but do contain some host cells and that IBC does not work for all organs in terms of generating viable chimeric animals [51]. These limitations shall be discussed in more detail below.

### 2.1.1 Chimera Competency of Human PSCs

In theory, any cell type with the potential to differentiate could contribute to a chimera. Nevertheless, when trying to mix stem cells from different species in the blastocyst one needs to ensure that they are in the same state, i.e., possess the same development potential or potency. However, there seem to be differences between species with regards to the potency of each state: mouse

embryonic stem cells (mESCs) possess self-renewing as well as indefinite proliferation capabilities and can also generate chimeras when inserted into blastocysts [52, 53]. On the other hand, rhesus macaque ESCs were not able to generate chimeras after being injected into unrelated rhesus blastocysts, suggesting that they – and, potentially, also human embryonic stem cells (hESCs) – are not chimeric-competent [54]. The isolation of another type of stem cell, the epiblast stem cell (EpiSC), offered an explanation in so far as there seem to be at least two different pluripotent states during embryonic development, namely the "naïve" and the "primed" state [55], which have different chimeric competencies: naïve cells, e.g. mouse ESCs, can generate germline-competent chimeras, whereas primed mouse EpiSCs or primate ESCs do not [56]. One possible explanation is that only naïve ESCs can produce chimeras with blastocysts [54] and primed ESCs can do so only if transferred into epiblasts of post-implantation embryos [51], i.e., if transferred into an environment of cells with the same potency.

Human PSCs (hPSCs) are considered to be in a primed state and failed to integrate into mouse-blastocysts and could thus not efficiently contribute to human–mouse chimeric embryos [57]. Efforts have therefore focused on converting primed hPSCs into naïve or naïve-like states, and success has been reported for human-mice chimeras in which the converted hPSCs contributed to embryonic tissue [58, 59]; and human iPSCs survived and contributed at least to some extent to interspecies chimerism in a pig [51, 60]. As the human cell contribution in these respective chimeras is still not very high, other barriers apart form the status of the hPSCs need to be addressed as well.

### 2.1.2 Apoptosis

When primed PSCs like cultured epiblast stem cells are injected into pre-implantation embryos they disappear within 24 hours [61]. The underlying theory is that the injected cells undergo apoptosis because the embryo eliminates developmentally unmatched cells. Indeed, Masaki *et al*. showed that prevention of apoptosis by expression of the anti-apoptotic BCL2 gene in rat epiblast stem cells enabled them to synchronise their developmental state with that of the mouse blastocysts they had been injected into [62]. Naïve human PSCs with inhibited apoptosis have been shown to contribute to interspecies chimerism in mouse, rabbit and pig [63].

### 2.1.3 Developmental Niche Discrepancy

Even though the process of mammalian development is conserved across species, there are certain limitations. So far there are no successful reports involving T-cells, hematopoietic stem cells or liver to have been created through interspecies chimerism between rat and mouse, let alone humans. To overcome the biological differences between animals and humans it appears necessary to "humanise" the recipient animals through knock-in or gene overexpression crucial for human cell differentiation and development. This concept has been applied in mice, with overexpression of membrane-bound human stem cell factor and knock-in of human thrombopoietin to enhance long-term human hematopoietic stem cell engraftment and thus increase the contribution of human blood cells in the host mice [64-66].

### 2.1.4 Contribution to Germ Line or Central Nervous System

One of the main issues regarding insertion of human cells into an animal host is not an actual scientific hurdle but rather an ethical concern, namely, the potential contribution of these human cells to the central nervous system or the germ line. For instance, transplantation of human glial progenitor cells into mice brain lead to the formation of human astrocytes and enhanced learning ability of mice [67]. Therefore, human-animal chimeras might potentially have more human-like characteristics than their wild-type animal counterparts. In terms of germ line contribution, the introduction of rat embryonic stem cells into mouse embryos resulted in the formation of rat-spermatozoa in the chimera [49]. Unarguably, the species barrier between humans and pigs is greater than between rats and mice; to avoid any risk, though, deletion of critical genes in the inserted human iPSCs could effectively prevent contribution to germ line or central nervous system [68]. Another suggested safeguard is in utero injection of the iPSCs localised to the site of organogenesis to avoid systemic chimerism [69].

### 2.1.5 Xenobarrier

Another problem with interspecies chimeras relates to the amount of chimerism: the amount of contribution of xenogenic cells in the resulting chimeric animal is directly proportional to the risk of abortion or malformation [50]. For example, only 35% of goat embryos transplanted with human hematopietic stem cells in utero were born alive [70, 71]. While addressing potency and apoptosis issues described above has certainly helped, immunologic defences by the host remains a problem.

### 2.1.6 Immunological Rejection

To create pigs that do not reject the human cells or tissues as described above, the pig must have immune tolerance to accept the human cells and not recognise them as antigen for rejection. Classical immunosuppression regimes have been applied successfully [72, 73] but recent efforts have also focused on the generation immunocompromised animals. This can be achieved through various strategies:

- **Thymectomy:** the first successful suppression of the porcine immune system by completely removing the thymus was reported in 1972 [74]. Proof of principle of survival of human cells in these pigs was reported after grafting human hepatocytes into the liver of such an athymic pig and being able to measure human albumin in the peripheral blood for several weeks. Consequently, these athymic minipigs are now commercially available [75].
- **Spleen and thymus removal**: surgical removal of spleen and thymus in piglets and additional feeding of immunosuppressants produces "operational" severe combined immune deficient (SCID) pigs, which were able to accommodate artificial human vascular tubes and maintain blood flow within [76].
- **Injecting human antigen:** here, human iPSCs are injected into the pig thymus to induce immune tolerance as the thymus has immunoisolating capabilities. However, human cells later grafted into these pigs were still rejected [6].
- **Actively acquired tolerance/creation of chimeras**: in utero transplantation of human cells and materials allow later engraftment of these human cells in the pig recipient. In utero transplanted human

hepatocytes remained viable and functional for several weeks and produced human albumin [77].

- **Severe combined immunodeficiency (SCID) pigs**: the ideal model would lack innate as well as adaptive immune functions. Indeed, double ARTEMIS or RAG1/2 and IL2RG knock-out pigs lacking B-, T- and Natural Killer cells have been created [78, 79]. However, challenges remain regarding rearing of these pigs since their viability does not exceed 12 weeks as yet [80, 81].

### 2.2 Creation of Multitransgenic Pigs

In essence, genetic modification of pigs must be able to overcome the issues outlined in section 1, namely the immunological barrier, inflammatory response and coagulation system dysregulation, the physiological incompatibility, and the infectious disease barrier. The advent of gene-editing tools like the CRISPR/Cas9 system has remarkably facilitated genetic modification and has made the production of pigs with multiple edited genes easy and cheap. In principle, a two-pronged, combined approach is being pursued: deletion of expression of certain xenoantigens (knock-out) and transgenic expression of human genes (knock-in). In the following, the current status of multitransgenic pigs for xenotransplantation shall be summarised.

#### 2.2.1 Knock-Out of Xenoreactive Antigens

As outlined above, inactivation of porcine GGTA1, CMAH and β4GalNT2 genes is a key in removing barriers related to hyperacute rejection and acute humoral rejection. The first success was reported in 2003 with homozygous knock-out of GGTA1 (GTKO) [82]. When hearts and kidneys from these GTKO pigs were

transplanted into non-human primates, HAR was successfully prevented [83, 84]. Subsequently, GGTA1/CMAH or GGTA1/ B4GalNT2 double-knockout, and finally, GGTA1/CMAH/B4GalNT2 triple knockout (TKO) pigs were created [85-87]. Blood monocytes and red blood cells from these pigs showed reduced binding to human IgG and IgM antibodies and reduced consumption of human thrombocytes in the liver model [88-90]. These results suggest that the gene knockout approach reduces antigenicity of certain porcine cells, which will hopefully be applicable to other pig tissues and thus help prevent HAR and AVR in humans. However, clinical studies in non-human primates are complicated by the fact that all old-world monkeys, including baboons, express antibodies against TKO pig cells, which can be cytotoxic for the porcine xenograft [91-93].

### 2.2.2 Expression of Human Complement Regulatory Proteins

Even though porcine complement regulatory proteins (CRPs) are similar to those of humans, they cannot prevent human complement-mediated injury of pig xenografts. Therefore, introduction of human CRPs (hCRPs), namely hCD46 (membrane cofactor protein), hCD55 (also called human decay-accelerating factor, hDAF) and hCD59 (MAC inhibitory protein) into pigs was suggested to prevent complement-mediated graft injury and, thus, hyperacute xenograft reaction [94, 95]. Today, many diverse pigs with hCRPs are available (reviewed in [96]) and their expression has been demonstrated to prolong xenograft survival time [97], particularly so when more than one hCRP is being expressed [98, 99]. The effect is even more pronounced when combined with GTKO as xenotransplants from GTKO/hCRP pigs last longer than either GTKO or hCRP

alone and survive for days or even weeks before exhibiting delayed xenograft rejection [28, 100-104].

### 2.2.3 Inhibition of Cellular Xenograft Rejection

Due to the many intricate components of cell-mediated rejection, several targets need to be modified, mostly through insertion of human transgenes.

An important inhibitory pathway for *macrophages* is the regulatory protein α (SIRP-α) - CD47 signalling pathway. Pigs express CD47 but even though human SIRP-α can bind to it, it does not initiate the inhibitory pathway and thus prevent macrophage activation [105]. Inserting human CD47 as transgene has reduced phagocytosis of porcine cells by human macrophages in vitro and prolonged survival of porcine skin xenografts in baboons [105, 106].

A promising route to suppress *T-cell activity* involves blocking the cytotoxic T-lymphocyte-associated antigen (CTLA4) through expression of CTLA4-immunoglobulin (Ig). Indeed, insertion of human CTLA4-Ig into the porcine genome extended survival of porcine skin xenografts in rats and neural xenografts into non-human primates [107, 108]. Additionally, T-cell response can be regulated by knocking out SLA class I or knocking in human class II transactivator genes, which both curtails porcine antigen presentation [109, 110]; or by introducing human transgenes that induce T-cell apoptosis [38].

Regarding *natural killer (NK) cells*, the goal is to inhibit their direct cytotoxicity as well as antibody-dependent cellular cytotoxicity mechanisms. While the latter can be achieved through knock-out of the α-Gal epitopes, the former needs a more sophisticated approach as the activating and inhibiting signal pathways in NK cells are tightly regulated [111]. Proposed solutions involve a mixture of

inserting human transgenes, like human Fas ligand or leukocyte antigen E, as well as knocking out porcine genes like β-2-microglobulin (reviewed in [38]). However, in vivo studies in NHPs to fully elucidate the role of NK cells in cellular rejection are still missing.

### 2.2.4 Expression of Human Coagulation Regulatory Proteins

Coagulation dysregulation is a problem insofar as the vascular endothelial cells of the porcine xenograft are in a procoagulant state, which cannot be regulated by porcine anticoagulant factors and thus results in coagulopathies. Strategies to tackle these problems have so far included the expression of human coagulation regulation proteins like thrombomodulin (TBM) to delay blood clotting [112, 113], endothelial cell protein C receptor (EPCR) to reduce platelet aggregation [113], human tissue factor pathway inhibitor to bind human thrombin and thus activate protein C [41], and CD39 to catalyse extracellular ATP/ADP/AMP and inhibit thrombus formation [114]. Indeed, insertion of these human transgenes have shown promising results in various in vitro studies and have also prolonged graft survival time in pig-to-baboon cardiac transplantations [113-116].

Additionally, knock-out of various porcine coagulation regulation proteins is also being pursued, like inactivation of the porcine von Willebrand factor to decrease platelet aggregation [117]; and inactivation of porcine asialoglycoprotein receptor to avoid platelet phagocytosis and ensuing thrombocytopenia that could result in xenograft rejection [85, 118]. Indeed, inactivation of porcine von Willebrand Factor prolonged lung graft survival time in non-human primates and could also mitigate platelet consumption [117, 119].

### 2.2.5 Expression of Human Anti-Inflammatory Proteins

The insertion of human anti-inflammatory and/or anti-apoptotic transgenes into pigs has been considered in order to overcome systemic inflammatory response. For instance, kidneys from pigs that express human hemeoxygenase-1 (hHO1) were protected from xenograft rejection after ex vivo perfusion with human blood [120], but it is unclear whether such a modification would be sufficient to address the systemic response. It is important to note that the immune rejection of a xenograft does not happen in isolation and multiple genes would need to be altered in pigs. Indeed, transgenic pigs have been created that contain multiple regulators of inflammation, like human hemeoxygenase-1 and human A20, as well as hCD47 [121]. The evaluation of these pigs as suitable donors in NHP models is still outstanding, though.

### 2.2.6 Overcoming Physiological Incompatibility

It is important to overcome the physiological differences between pigs and primate recipients of xenoorgans and thus regulate the porcine intrinsic growth properties. Knock out of the porcine growth hormone receptor has been proposed as potential solution to prevent xenograft overgrowth and this indeed prevented transplanted heart xenografts from overgrowth in NHP recipients [122].

### 2.2.7 Preventing Cross-Species Infections

The potential risk of infection with porcine viruses is of major concern. The U.S. Food and Drug Administration (FDA) has therefore written a guideline regarding breeding and rearing of potential donor animals as well as recommendations for screening for infectious agents pre-transplantation [123]. The importance of this

was recently highlighted when the first human patient to receive a porcine heart transplant died presumably due to an infection with a porcine virus [124].

Particular attention is being given to PERVs, even though their infectious potential remains unclear as transmission to human cells was only shown in *in vitro* studies [125, 126] and to date no transmission in preclinical pig-to-NHP models have been reported [127]. Still, to be on the safe side, PERV-inactivation in potential pig donors was pursued and achieved in 2017 [125]. A few issues remain, however, namely i) whether these PERV-KO pigs can be reinfected with PERVs [13], ii) frequent karyotype anomalies and possibly associated genomic changes [128], and iii) and whether human-tropic PERV infections will be manageable with conventional anti-retroviral drugs [129]. Despite these unanswered questions, PERV-inactivated pigs with additional gene modifications have been created, with some pigs even carrying PERV inactivation, knockout of three main xenoantigens and nine effective human transgenes (abbreviated to PERVKO·3KO·9TG) [130].

## 3. Pig Organ Graft Survival in Non-Human Primates and Humans

Xenotransplantation is an incredibly complex endeavour, with all the hurdles of normal organ transplantation – health of the recipient, organ storage, blood groups, immunosuppression protocols, skill of the surgeon – exacerbated by additional complications due to the animal donor. In recent years, immense progress has been made regarding genetic engineering of pigs and sophistication of immunosuppressive regimes, which has dramatically increased xenograft survival in NHPs, which are the preferred surrogates for humans due to similarities regarding the immune system. In the following, the most recent data

regarding solid organ transplantation from pigs to NHPs as well as the first attempts in humans shall be summarised.

### 3.1 Kidney

Due to the comparatively simple surgical procedure and inherent physiological functions, kidneys were the first solid organs to be xeno-transplanted into NHPs, albeit with moderate survival rates for the longest time (reviewed in [131]. The breakthrough of xenotransplant survival past 125 days came in 2015, with GTKO/hCD55 pig donors and recipient rhesus macaques that had been T-cell depleted and received anti-CD154 monoclonal antibody (mAB) as immunosuppressant [132]. Later, more heavily genetically modified pigs and different immunosuppression regimes were able to prolong survival [87], with 499 days being the longest reported duration and functionality of a transplanted kidney into a rhesus monkey [133]. The first xenotransplantation attempt into brain-dead humans took place in 2021: the first had a kidney from a 10-GE pig (TKO/hDAF/hCD46/hTBM/hEPCR/hCD47/hHO1) attached to the upper leg blood vessel for 54 hours without hyperacute rejection and apparently preserved functionality [134]; the second human xenotransplant, also from a 10-GE pig into a brain-dead human host, were two kidneys. Again, no hyperacute rejection or intraoperative complications were observed and the kidneys remained viable until termination after 74 hours [135].

### 3.2 Heart

Cardiac xenotransplantation has always been pursued through two different procedures: the more common ones, heterotopic transplantations, mean transplantation into the abdomen and are therefore nonlife-supporting. The

longest survival in NHPs has been achieved with a GalT-KO/hCD46/hTBM pigs as donor and an anti-CD40 mAb immune-modulatory regime for 945 days without coagulopathy or thrombocytopenia [115]. Orthotopic, i.e., life-saving transplantation proved more difficult, with survival rates in NHPs up to 57 days until 2018 [136]. Due to their long experience with heterotopic heart transplantation, Längin *et al.* modified the procedure twofold: they used non-ischemic porcine heart preservation as opposed to cold static storage; and controlled post-transplantation porcine organ overgrowth, extending survival of recipient baboons to 195 days [137]. Importantly, the immunosuppressive protocol was well tolerated by the baboons as indicated by lack of immunosuppression-related infection. This paved the way for the first pig to human heart xenotransplantation in 2022 [138]. The patient, who had end-stage heart disease and was ineligible for conventional heart transplantation, received a heart from a 10-GE pig. After initially doing well he died after two months, possibly due to an infection with porcine cytomegalovirus [124].

### 3.3 Liver

Liver xenotransplantation poses to be more difficult than kidney or heart transplantation, as evidenced by more severe coagulation dysregulation, pronounced thrombocytopenia, and systemic consumptive coagulopathy in the recipient [139]. Despite using GTKO/hCD46 pigs as donors, baboon recipient survival was limited to 9 days for the longest time [140]. A small breakthrough was achieved in 2017, when baboons received livers from GTKO pigs as well as anti-CD40 mAb and continuous infusions of human prothrombin concentrate complex. These provisions could extend baboon survival to 29 days [141]. This

relative short duration in comparison to kidney and heart transplantations can be explained by the more intricate role the liver plays in the organism, which likely exacerbates any species incompatibilities in functional proteins.

### 3.4 Lung

Lung xenotransplantation shows even more issues than liver xenotransplantation, mostly due to severe coagulation dysregulation as well as inflammatory processes, which has limited xenograft survival to mere days (reviewed in [142]). A breakthrough was achieved in 2020 with prolonged survival time of 31 days of baboon recipients by using multimodified GTKO/ β4GalNT2-KO/hCD46/hCD47/hEPCR/hTM/hHO-1 pigs [143]. Therefore, as with liver xenotransplantation, genetic engineering and immunosuppression strategies need to be refined before considering potential application in humans.

## III. Ethical Issues Regarding Xenotransplantation

Ethical issues regarding xenotransplantation have been discussed for many years [144-149]. There are several different aspects that can be divided into three broad buckets: the first relates to the biotechnology industry or science itself in terms of the belief that certain scientific experiments should simply not be undertaken; the second refers to potential dangers or unforeseen consequences, e.g., when tampering with the genetic make-up of a living organism or regarding pandemics due to zoonoses; and the third relates to animal welfare, i.e., the suffering of the involved animals. While xenotransplantation provides a textbook example to discuss all these issues, none of them are new or even unique to xenotransplantation: heated discussions about the permissibility of cloning of the sheep dolly, or, potentially, humans [150-152] and regarding embryonic stem cell research [153-155] are two prominent examples for the first issue. The second issue regarding gene modifications was debated regarding gene-edited crops [156] and animals [157], and the pandemics due to zoonoses was discussed in the context of HIV/AIDS [158], and, more recently, Covid-19 [159]. Animal welfare in the context of biomedical research has also been debated for a long time [160-162] and continues today [163-165].

Xenotransplantation involving the introduction of human iPSCs into animal blastocysts does not, per se, constitute a novel issue either, as the mixture of human and animal material and the possibility of human-animal offspring has been foreseen and discussed in other contexts [166-168]. However, it does add an intriguing level of complexity and several ethical dimensions to the ongoing debate regarding xenotransplantation. The far-reaching nature of the various

controversies involves a large variety of factors debated over a range of different venues (see above). It is therefore difficult to obtain an overview of the breadth and depth of the ethical debate as academics typically focus on one or a few aspects within a paper, e.g., animal welfare or the moral status of the created creatures [169-171]; and the public debate often revolves around murky concepts like, e.g., respect, dignity, (un)naturalness or moral taboos [172-174].

The underlying hypothesis for the subsequent paper was that the advent of mixing of species during blastocyst complementation would re-ignite the ethical debate and rehash well-known arguments in a highly fragmented manner, owing to the many dimensions of the issue. This in turn would undermine a coherent assessment and impede potential consensus on proposed human-animal chimera research. The following systematic review of reasons therefore had the goal to carve out central concerns, identify potential argumentative gaps and straw man arguments and provide structure to the debate. The idea was thus to provide a ground zero for the continued discussion.

The systematic review was based on the research question *"What ethical reasons have been given for or against conducting human-animal chimera research, and how have these reasons been treated in the ongoing debate?".* Several databases were screened using controlled vocabulary and specific search strings to retrieve peer-reviewed publications. The publications were selected based on pre-defined inclusion criteria, the most important one being that they include at least one argument for or against chimera research. The identified reasons were then assorted into five categories. To accurately depict the discussion, we analysed whether a reason was rejected or endorsed, i.e., arguments were put forth for or

against it. Moreover, we distinguished whether a reason was merely mentioned, i.e., reiterated without argumentative support. This was of particular importance as we assumed that this approach would allow the identification of straw man arguments, i.e., the illusion of a reason without an argumentative base which then can be attacked to strengthen one´s own claim.

**Added Value of This Publication**

This systematic review was the first of its kind within the ethical debate concerning human-animal chimera research. It takes a purely descriptive approach to the debate and thus does not allow any conclusion regarding the validity of the arguments put forth. Still, the five broad categories identified - positive reasons (P), and negative reasons pertaining to chimera creation (A), chimera treatment (B), chimera existence (C), and downstream effects (D) – constitute a simple overview of the discussion. Furthermore, the illustration of the 12 broad and 31 narrow types of reasons along with their frequencies of mentions, rejections and endorsement reveal the broad and diverse nature of the arguments discussed. Also, as suspected, some noteworthy trends and interesting patterns could be identified, for instance that positive reasons for chimera research only make out a small fraction of all discussed reasons, presumably because one needs to assume whether certain scientific advancements will happen. Another peculiarity pertains to the category with reasons regarding the existence of a chimera (C), including reasons like that the creation of chimeras might violate moral taboos, meet with instinctive repugnance, corrupt the natural order, or amount to playing God. These reasons

are rejected three times more than they are endorsed, which suggests that these arguments are, largely, targeted at straw man.

All in all, this systematic review gives orientation in a complex and fragmented discussion and thus hopefully facilitates further academic debate and helps inform policy decisions.

Kwisda *et al. BMC Medical Ethics* (2020) 21:24
https://doi.org/10.1186/s12910-020-00465-7

BMC Medical Ethics

RESEARCH ARTICLE Open Access

# Ethical arguments concerning human-animal chimera research: a systematic review

Koko Kwisda[1*], Lucie White[2] and Dietmar Hübner[2]

## Abstract

**Background:** The burgeoning field of biomedical research involving the mixture of human and animal materials has attracted significant ethical controversy. Due to the many dimensions of potential ethical conflict involved in this type of research, and the wide variety of research projects under discussion, it is difficult to obtain an overview of the ethical debate. This paper attempts to remedy this by providing a systematic review of ethical reasons in academic publications on human-animal chimera research.

**Methods:** We conducted a systematic review of the ethical literature concerning human-animal chimeras based on the research question: "What ethical reasons have been given for or against conducting human-animal chimera research, and how have these reasons been treated in the ongoing debate?" Our search extends until the end of the year 2017, including MEDLINE, Embase, PhilPapers and EthxWeb databases, restricted to peer-reviewed journal publications in English. Papers containing ethical reasons were analyzed, and the reasons were coded according to whether they were endorsed, mentioned or rejected.

**Results:** Four hundred thirty-one articles were retrieved by our search, and 88 were ultimately included and analyzed. Within these articles, we found 464 passages containing reasons for and against conducting human-animal chimera research. We classified these reasons into five categories and, within these, identified 12 broad and 31 narrow reason types.
15% of the retrieved passages contained reasons in favor of conducting chimera research (Category P), while 85% of the passages contained reasons against it. The reasons against conducting chimera research fell into four further categories: reasons concerning the creation of a chimera (Category A), its treatment (Category B), reasons referring to metaphysical or social issues resulting from its existence (Category C) and to potential downstream effects of chimera research (Category D). A significant proportion of identified passages (46%) fell under Category C.

**Conclusions:** We hope that our results, in revealing the conceptual and argumentative structure of the debate and highlighting some its most notable tendencies and prominent positions, will facilitate continued discussion and provide a basis for the development of relevant policy and legislation.

**Keywords:** Human-animal chimeras, Chimera research, Ethics, Systematic review

* Correspondence: koko.kwisda@cells.uni-hannover.de
[1]CELLS – Centre for Ethics and Law in the Life Sciences, Leibniz University Hannover, Otto-Brenner-Strasse 1, 30159 Hannover, Germany
Full list of author information is available at the end of the article

## Background

Research involving the mixture of human and animal materials has been controversial from its inception. Proposed research projects, particularly geared towards the production of human organs for transplantation in an animal host [1–7], involving the implantation of human brain stem cells into other animals [8–10], or aiming at the creation of human-animal admixed embryos [11–14], have spurred this debate, generating a wide spectrum of arguments both for and against such research in public and academic discourse. The far-reaching nature of these controversies, involving a large variety of factors debated over a range of different venues, makes it particularly difficult, and important, to obtain a general overview of the debate.

This paper provides a systematic review of ethical arguments contained in academic publications on human-animal chimera[1] research. Systematic reviews involve searching databases in a methodical and reproducible way, retrieving literature according to predefined inclusion criteria, and analyzing this literature in order to answer a specific research question. Originally a tool of the social sciences, their use was extended to medical contexts, providing comprehensive information on research findings and clinical results in order to facilitate decision-making. More recently, this method has been extended to philosophical bioethics, taking as its focus the argument-based literature found in this field [15, 16].

The research question underlying this systematic review is: *"What ethical reasons have been given for or against conducting human-animal chimera research, and how have these reasons been treated in the ongoing debate?"* In order to adequately answer this question, we produced a detailed, multilayered classification system of reasons, which elucidates the basic conceptual structure of the debate. We provide quantitative information on how often reasons have been endorsed, rejected, or merely mentioned, to give a thorough account of positions, tendencies and camps within the literature. Finally, we comment on the nature of our findings in the discussion section, giving an indication of the factors that might explain certain notable patterns in the results. By providing structure to the debate, drawing attention to central concerns, and uncovering certain specific features of the current dispute, including potential argumentative gaps and straw man arguments, we aim to establish a basis for continued discussion and to facilitate the development of relevant policy and legislation.

[1]"Chimera" is the most frequently used term in discussions on this topic, typically encompassing any creature arising from a mixture of human and animal material, including hybrids and cybrids.

## Methods

### Literature search and eligibility criteria

To minimize potential bias and ensure an exhaustive retrieval, several databases were screened, namely MEDLINE, Embase, PhilPapers and EthxWeb (see Fig. 1).

Databases were searched up to 31 December 2017. Database-specific controlled vocabulary and search strings applied are summarized in Table 1. Respective search results were fused with a bibliography software (Thomson Reuters EndNote®) and duplicate references removed. All 88 included publications are listed alphabetically in Table 2.

We restricted our search to English literature, due to the proficiencies of the authors and the availability of sources. We also focused exclusively on original, academic publications in international, peer-reviewed journals, excluding reports, surveys, encyclopedia entries, handbook articles, guidelines, opinions, editorials, reviews, monographs, anthologies, letters, web-posts and newspaper articles.

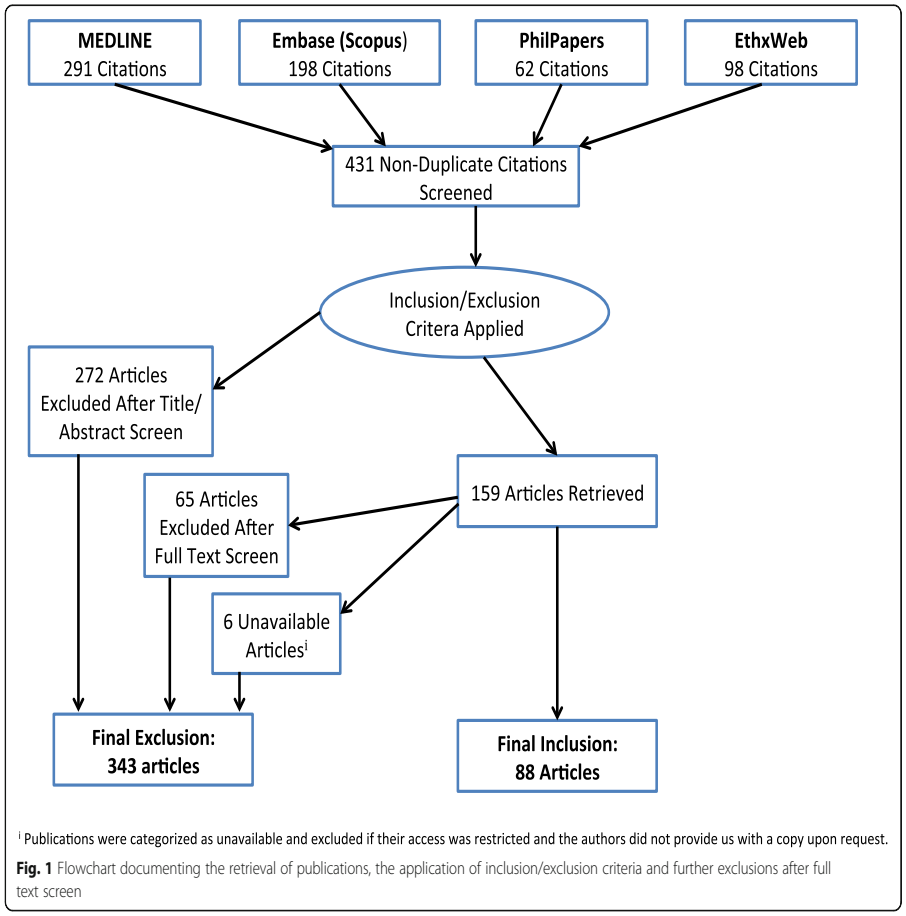
A publication was included only if it addressed at least one ethical reason concerning why human-animal chimera research should or should not be pursued.[2] Decisions concerning whether articles should be included were based on the publications' abstracts, or, if these were inconclusive, on a close reading of the full text. All 88 included publications are listed in Table 2.

### Extraction and categorization of reasons

For the development of the coding system for reasons, we followed the methodology suggested by Strech and Sofaer (2012) [15]. To adequately mirror the ongoing discussion and provide in-depth analysis, we distinguished between three stances taken regarding a reason:

- Mere mentioning of a reason (i.e., reiteration or consideration of a reason without unequivocal rejection or endorsement). This includes statements such as "X constitutes a reason against chimera research unless measures ABC are taken", or "X does not constitute a reason against chimera research as long as measures ABC are taken".
- Rejection of a reason.
- Endorsement of a reason OR development of own reason.

[2]This means that some prominent literature on chimera research was excluded: For example, Monika Piotrowska's "Transferring Morality to Human-Nonhuman Chimeras" [94] was excluded due to the fact that it does not discuss reasons for or against conducting chimera research, but rather puts forward a potential classification system that might allow us to better determine how to treat human-nonhuman chimeras. By contrast, two peer commentaries on this article that do contain discussion of reasons for or against chimera research were included in our survey [21, 29].




[i] Publications were categorized as unavailable and excluded if their access was restricted and the authors did not provide us with a copy upon request.

**Fig. 1** Flowchart documenting the retrieval of publications, the application of inclusion/exclusion criteria and further exclusions after full text screen

**Table 1** List of databases screened with respective search strings used

| Database | Search string[a] |
|---|---|
| MEDLINE | ((((“Chimera”[Mesh])) OR (chimera))) AND ((((((“Ethics”[Mesh])) OR (ethics)) OR (ethical)) OR (bioethics)) OR (bioethical)) |
| Embase (Scopus) | Bioethic* OR ethic* AND chimer* (restricted to “Articles” and “Articles in Press”) |
| PhilPapers | (ethic* bioeth*) (chimer*) Fuzzy filter advanced |
| EthxWeb | Chimer+ AND (ethic+ OR bioethic+) restricted to journal articles |
| Updates via email update on various databases | As above |

[a]Words refer to controlled vocabulary of respective databases

We coded each reason once per publication. For instance, if a reason was first mentioned but then ultimately rejected, this was only counted once as a rejection. Alternatively, if a reason was, for example, rejected multiple times within the same paper, perhaps on different grounds, only one passage was coded as a rejection. Note that an author may endorse a certain reason for one type of chimera, but, in the same article or in another publication, reject this very same reason with regard to another type of chimera.

In our categorization of types of reasons, we differentiated between broad types (e.g. A.2 “Human beings/human material might be mistreated/misused”) and narrow types (e.g. A.2.i “Human embryo protection may be neglected”, or A.2.ii “Undue forms of human egg donation may occur”), with each narrow type falling under one broad type. Each broad reason type, in turn, was collected under one of five main reason categories (see below).

The extraction and categorization of reasons unavoidably involves interpretation. To produce a stable coding

**Table 2** Included and analyzed publications in alphabetical order


[17] Abelman M, O'Rourke PP, Sonntag KC (2012) Part-human animal research: the imperative to move beyond a philosophical debate. Am J Bioeth **12** (9):26–8

[18] Ankeny RA (2003) No real categories, only chimeras and illusions: the interplay between morality and science in debates over embryonic chimeras. Am J Bioeth **3** (3):31–3

[19] Anton R (2016) On recent advances in human engineering Provocative trends in embryology, genetics, and regenerative medicine. Politics Life Sci **35** (2):54–68

[20] Austriaco NP (2006) How to navigate boundaries: a reply to The American Journal of Bioethics. Natl Cathol Bioeth Q **6** (1):61–71

[21] Badura-Lotter G, Fangerau H (2014) Human-animal chimeras: not only cell origin matters. Am J Bioeth **14** (2):21–2

[12] Bahadur G, Iqbal M, Malik S et al. (2008) Admixed human embryos and stem cells: legislative, ethical and scientific advances. Reprod Biomed Online **17** (Suppl 1):25–32

[22] Baylis F (2008) Animal eggs for stem cell research: a path not worth taking. Am J Bioeth **8** (12):18–32

[8] Baylis F, Fenton, A. (2007) Chimera research and stem cell therapies for human neurodegenerative disorders. Camb Q Healthc Ethics **16** (2):195–208

[23] Baylis F, Robert JS (2007) Part-human chimeras: worrying the facts, probing the ethics. Am J Bioeth **7** (5):41–5

[24] Benham B, Haber M (2008) Moral confusion and developmental essentialism in part-human hybrid research. Am J Bioeth **8** (12):42–4

[25] Bok H (2003) What's wrong with confusion? Am J Bioeth **3** (3):25–6

[4] Bourret R, Martinez E, Vialla F et al. (2016) Human-animal chimeras: ethical issues about farming chimeric animals bearing human organs. Stem Cell Res Ther **7** (1):87

[26] Cabrera Trujillo LY, Engel-Glatter S (2014) Human-Animal Chimera: A Neuro Driven Discussion? Comparison of Three Leading European Research Countries. Sci Eng Ethics

[13] Camporesi S, Boniolo G (2008) Fearing a non-existing Minotaur? The ethical challenges of research on cytoplasmic hybrid embryos. J Med Ethics **34** (11):821–5

[27] Capps B (2017) Do Chimeras Have Minds? Camb Q Healthc Ethics **26** (4):577–591

[28] Castle D (2003) Hopes against hopeful monsters. Am J Bioeth **3** (3):28–30

[29] Chan S (2014) Hidden anthropocentrism and the "benefit of the doubt": problems with the "origins" approach to moral status. Am J Bioeth **14** (2):18–20

[30] Chapman A, Hiskes AL (2008) Unscrambling the eggs: cybrid research through an Embryonic Stem Cell Research Oversight Committee (ESCRO) lens. Am J Bioeth **8** (12):44–6

[31] Charland LC (2003) Are there answers? Am J Bioeth **3** (3):1–2

[32] Cheshire WP, Jr. (2007) The moral musings of a murine chimera. Am J Bioeth **7** (5):49–50

[33] Cohen CB (2003) Creating human-nonhuman chimeras: of mice and men. Am J Bioeth **3** (3):3–5

[34] Cooley DR (2008) Genetically Engineering Human-Animal Chimeras and Lives Worth Living. Between The Species **8**:1–19

[35] Coors ME (2006) Considering chimeras: the confluence of genetic engineering and ethics. Natl Cathol Bioeth Q **6** (1):75–87

[36] de Melo-Martin I (2008) Chimeras and human dignity. Kennedy Inst Ethics J **18** (4):331–46

[37] deGrazia D (2007) Human-animal chimeras: human dignity, moral status, and species prejudice. Metaphilosophy **38** (2–3):310–329

[38] DiSilvestro R (2012) The two-essence problem that wasn't. Am J Bioeth **12** (9):34–5

[39] Eberl JT (2007) Creating non-human persons: might it be worth the risk? Am J Bioeth **7** (5):52–4

[40] Eberl JT (2012) Ontological kinds versus biological species. Am J Bioeth **12** (9):32–4

[41] Eberl JT, Ballard RA (2008) Exercising restraint in the creation of animal-human chimeras. Am J Bioeth **8** (6):45–6

[42] Eberl JT, Ballard RA (2009) Metaphysical and ethical perspectives on creating animal-human chimeras. J Med Philos **34** (5):470–86

[43] Franklin S (2003) Drawing the line at not-fully-human: what we already know. Am J Bioeth **3** (3):25–27

[44] Gerrek ML (2008) Who really causes the lady to vanish? Am J Bioeth **8** (12):46–7

[45] Greely HT (2003) Defining chimeras...and chimeric concerns. Am J Bioeth **3** (3):17–20

[9] Greely HT, Cho MK, Hogle LF et al. (2007) Thinking about the human neuron mouse. Am J Bioeth **7** (5):27–40

[46] Greene M, Schill K, Takahashi S et al. (2005) Ethics: Moral issues of human-non-human primate neural grafting. Science **309** (5733):385–6

[47] Haber MH, Benham B (2012) Reframing the ethical issues in part-human animal research: the unbearable ontology of inexorable moral confusion. Am J Bioeth **12** (9):17–25

[48] Heathcotte B, Robert JS (2006) The strange case of the humanzee patent quest. Natl Cathol Bioeth Q **6** (1):51–9

[49] Hermeren G (2015) Ethical considerations in chimera research. Development **142** (1):3–5

**Table 2** Included and analyzed publications in alphabetical order *(Continued)*


[50] Hyun I (2015) From naive pluripotency to chimeras: a new ethical challenge? Development **142** (1):6–8

[51] Hyun I (2016) What's Wrong with Human/Nonhuman Chimera Research? PLoS Biol **14** (8):e1002535

[52] Irvine R, Degeling C, Kerridge I (2012) Uncanny animals: thinking differently about ethics and the animal-human relationship. Am J Bioeth **12** (9):30–2

[53] Johnston J, Eliot C (2003) Chimeras and "human dignity". Am J Bioeth **3** (3):W6-w8

[14] Jones DA (2010) Is the creation of admixed embryos "an offense against human dignity"? Hum Reprod Genet Ethics **16** (1):87–114

[54] Jones DA (2012) The ethics of creating chimeras and other admixed organisms. Ethics and Medicine **28** (3):81–93

[55] Karpowicz P (2003) In defense of stem cell chimeras: a response to "Crossing species boundaries". Am J Bioeth **3** (3):17–19

[11] Karpowicz P, Cohen CB, van der Kooy D (2004) It is ethical to transplant human stem cells into nonhuman embryos. Nat Med **10** (4):331–5

[56] Karpowicz P, Cohen CB, van der Kooy D (2005) Developing human-nonhuman chimeras in human stem cell research: ethical issues and boundaries. Kennedy Inst Ethics J **15** (2):107–34

[57] Knoppers BMJ, Yann (2007) Our social genome? Trends Biotechnol **25** (7):284–288

[58] Kobayashi NR (2003) A scientist crossing a boundary: a step into the bioethical issues surrounding stem cell research. Am J Bioeth **3** (3):15–16

[59] Lavieri RR (2007) The ethical mouse: be not like Icarus. Am J Bioeth **7** (5):57–8

[60] Levine S, Grabel L (2017) The contribution of human/non-human animal chimeras to stem cell research. Stem Cell Res **24**:128–134

[61] Masaki H, Nakauchi H (2017) Interspecies chimeras for human stem cell research. Development **144** (14):2544–2547

[62] McGee DB (2003) Moral ambiguity? Yes. Moral confusion? No. Am J Bioeth **3** (3):11–12

[63] Mirkes R (2006) Is it ethical to generate human-animal chimeras? Natl Cathol Bioeth Q **6** (1):109–30

[6] Munsie M, Hyun I, Sugarman J (2017) Ethical issues in human organoid and gastruloid research. Development **144** (6):942–945

[64] Murphy TF (2008) When is an objection to hybrid stem cell research a moral objection? Am J Bioeth **8** (12):47–9

[65] Nelson JL (2008) Respecting boundaries, disparaging values. Am J Bioeth **8** (12):33–4

[66] Palacios-Gonzalez C (2015) Ethical aspects of creating human-nonhuman chimeras capable of human gamete production and human pregnancy. Monash Bioeth Rev. **33** (2–3):181–202

[5] Palacios-Gonzalez C (2016) The ethics of killing human/great-ape chimeras for their organs: a reply to Shaw et al. Med Health Care Philos **19** (2):215–25

[67] Palacios-Gonzalez C (2017) Chimeras intended for human gamete production: an ethical alternative? Reprod Biomed Online **35** (4):387–390

[68] Palacios-González C (2015) Human dignity and the creation of human–nonhuman chimeras. Medicine, Health Care and Philosophy **18** (4):487–499

[69] Piotrowska M (2012) Who are my parents? Why assigning moral categories to genealogical relations leads to more confusion. Am J Bioeth **12** (9):28–30

[70] Pusch AF (2015) Splices: When Science Catches Up with Science Fiction. NanoEthics **9** (1):55–73

[71] Ravelingien A, Braeckman, J., Legge, M. (2006) On the moral status of humanized chimeras and the concept of human dignity. Between the Species **6**:1–22

[72] Robert JS (2006) The science and ethics of making part-human animals in stem cell biology. Faseb j **20** (7):838–45

[73] Robert JS, Baylis F (2003) Crossing species boundaries. Am J Bioeth **3** (3):1–13

[74] Robert JS, Baylis F (2003) A response to commentators on "Crossing species boundaries". Am J Bioeth **3** (3):W-c6

[75] Robertson JA (2003) A response to "Crossing species boundaries" by Jason Scott Robert and Francoise Baylis. Am J Bioeth **3** (3):W-c5

[76] Rollin BE (2007) Of mice and men. Am J Bioeth **7** (5):55–7

[77] Rollin BE (2007) On chimeras. Zygon **42** (3):643–648

[78] Sagoff M (2003) Transgenic chimeras. Am J Bioeth **3** (3):30–1

[10] Sagoff M (2007) Further thoughts about the human neuron mouse. Am J Bioeth **7** (5):51–2

[79] Salter B, Harvey A (2014) Creating problems in the governance of science: Bioethics and human/animal chimeras. Science and Public Policy **41** (5):685–696

[80] Saniotis A (2013) Remaking homo: Ethical issues on future human enhancement. Ethics in Science and Environmental Politics **13** (1):15–21

[81] Savulescu J (2003) Human-animal transgenesis and chimeras might be an expression of our humanity. Am J Bioeth **3** (3):22–5

[82] Savulescu J, Skene L (2008) The kingdom of genes: why genes from animals and plants will make better humans. Am J Bioeth **8** (12):35–8

[83] Schaub DJ (2006) Chimeras: from poetry to science. Natl Cathol Bioeth Q **6** (1):29–35

[84] Seyfer TL (2006) An overview of chimeras and hybrids. Natl Cathol Bioeth Q **6** (1):37–49

**Table 2** Included and analyzed publications in alphabetical order *(Continued)*


[2] Shaw D, Dondorp W, de Wert G (2014) Using non-human primates to benefit humans: research and organ transplantation. Medicine, Health Care and Philosophy **17** (4):573–578

[85] Shaw D, Dondorp W, Geijsen N et al. (2014) Creating human organs in chimaera pigs: an ethical source of immunocompatible organs? J Med Ethics

[86] Siegel AW (2003) The moral insignificance of crossing species boundaries. Am J Bioeth **3** (3):33–4

[87] Streiffer R (2003) In defense of the moral relevance of species boundaries. Am J Bioeth **3** (3):37–8

[88] Streiffer R (2005) At the edge of humanity: human stem cells, chimeras, and moral status. Kennedy Inst Ethics J **15** (4):347–70

[89] Streiffer R (2010) Chimeras, moral status, and public policy: implications of the abortion debate for public policy on human/nonhuman chimera research. J Law Med Ethics **38** (2):238–50

[90] Thompson PB (2003) Crossing species boundaries is even more controversial than you think. Am J Bioeth **3** (3):14–5

[91] Urie KA, Stanley A, Friedman JD (2003) The humane imperative: a moral opportunity. Am J Bioeth **3** (3):20–21

[92] Watt H (2007) Embryos and pseudoembryos: parthenotes, reprogrammed oocytes and headless clones Journal of Medical Ethics **33** (9):554–556

[93] Zwanziger LL (2003) Crossing perspectival chasms about species. Am J Bioeth **3** (3):9–10


system and ensure intercoder reliability we employed the following procedure: The publications that at initial inspection appeared to be more detailed and comprehensive were grouped together in a first cluster of seven publications. Two authors (D.H. and K.K.) identified and initially categorized text passages independently in this subsample, then discussed whether these passages displayed a reason and how it should be categorized. The result was a first version of the coding system. A second cluster of 20 publications, which still appeared to be relatively comprehensive, was then used to check theoretical saturation of the categorical spectrum, and to revise and fine-tune the categorization of reasons. At this point, the main categories and broad reason types had been established; only minor adjustments within the narrow types of reasons were subsequently necessary. All three authors (K.K., D.H., L.W.) then checked the extraction and categorization of reasons in a random sample of another five publications. Our assignment of reasons was largely consistent, which we took to demonstrate the validity of our category system.

Within the complete set of included articles, each publication was analyzed by at least two authors. In the event of any coding incongruities, concordance was reached through in-depth discussion.

## Results

### Publication characteristics

Our literature research retrieved 431 non-duplicate references, 88 of which were included (see Fig. 1). All articles were published between 2003 and 2017 in peer-reviewed journals. Table 3 characterizes the disciplines of the journals in which the articles were published.

### Categories, types and frequencies of reasons

Within the 88 retrieved publications, we found 464 text passages containing reasons. The latter fall into five main categories, 12 broad types, and 31 narrow types of reasons. Tables documenting the frequency of reason types for each category can be found below. A quotation exemplifying each reason type is contained in Additional file 1.

Of the five main categories, *Category P* (*positive reasons)* contains discussion of reasons in favor of chimera research. This category contains 15% of all identified passages (70 passages), making it the third most debated category. The reasons within Category P were divided into four broad reason types: creating chimeras might lead to advances in basic research (P.1), produce benefits for humans (P.2), prevent direct harm to humans or animals (P.3), or entail other benefits (P.4). 31% of all coded passages in this category are mentions, 7% are rejections, and 61% are endorsements (see Table 4).

The remaining four categories contain discussions of reasons against chimera research.

*Category A* (*chimera creation)* contains reasons pertaining to the process leading to the creation of a chimera. 11% of all identified passages (53 passages) are in this category, making it the second-least debated category. There

**Table 3** Journal disciplines for all included publications

| Journal Disciplines | |
|---|---|
| Bioethics | 46 (52.3%) |
| Science/Medicine | 14 (15.9%) |
| Medical Ethics | 8 (9.1%) |
| Theology | 7 (7.9%) |
| Ethics General | 5 (5.7%) |
| Ethics of Science/Technology | 3 (3.4%) |
| Philosophy of Medicine | 2 (2.3%) |
| Philosophy General | 1 (1.1%) |
| Law | 1 (1.1%) |
| Politics and Life Sciences | 1 (1.1%) |
| **Total** | **88 (100%)** |

**Table 4** Mentions, rejections and endorsements of positive reasons (Category P)

| Positive Reasons (Category P) | |
|---|---|
| P.1: The creation of chimeras may advance basic research | *Mention* [23, 35, 54, 71, 79]<br>*Reject* [8, 72]<br>*Endorse* [9, 12, 13, 17, 41, 42, 59–61, 75, 76, 81, 82] |
| P.2: The creation of chimeras may produce benefits for humans | |
| P.2.i: New therapies might be developed on the basis of chimera research | *Mention* [8, 20, 41, 43, 54, 66, 71, 79, 84]<br>*Reject* [11, 22, 23]<br>*Endorse* [2, 9, 12, 13, 17, 34, 35, 39, 42, 55, 60, 67, 72, 75, 85] |
| P.2.ii: Chimeras might serve as sources of transplantable organs and tissues | *Mention* [5, 63]<br>*Reject* [none]<br>*Endorse* [2, 4, 34, 41, 42, 51, 60, 66, 72, 85] |
| P.2.iii: Chimera research might open ways to human enhancement | *Mention* [80]<br>*Reject* [none]<br>*Endorse* [81] |
| P.3: The creation of chimeras may prevent direct harm to humans or animals[a] | *Mention* [12, 23, 66]<br>*Reject* [none]<br>*Endorse* [13, 41, 42] |
| P.4: The creation of chimeras may have other benefits[b] | *Mention* [46, 80]<br>*Reject* [none]<br>*Endorse* [13] |

[a] E.g. by helping to replace human subjects or laboratory animals in biomedical research
[b] E.g. by fostering the preservation of endangered species, or by allowing animal enhancement

**Table 5** Mentions, rejections and endorsements of reasons concerning chimera creation (Category A)

| Reasons Concerning Chimera Creation (Category A) | |
|---|---|
| A.1: Animals might be mistreated | |
| A.1.i: General animal welfare may be infringed | *Mention* [9, 14, 22, 26, 49, 54, 59, 65, 80, 89]<br>*Reject* [76, 79]<br>*Endorse* [37] |
| A.1.ii: Special protection of higher animals such as primates may be infringed | *Mention* [22, 26, 46]<br>*Reject* [2]<br>*Endorse* [37, 42] |
| A.2: Human beings/human material might be mistreated/misused | |
| A.2.i: Human embryo protection may be neglected | *Mention* [12, 14, 24, 26, 44, 49, 59, 64, 65, 79, 89]<br>*Reject* [9, 66, 76]<br>*Endorse* [63, 92] |
| A.2.ii: Undue forms of human egg donation may occur | *Mention* [12, 14, 24, 54, 65]<br>*Reject* [30, 44, 64, 66, 82]<br>*Endorse* [22] |
| A.2.iii: Other human biological material may be used improperly | *Mention* [54, 59]<br>*Reject* [9, 66, 67, 76]<br>*Endorse* [89] |

are two concerns here, reflected by the two broad reason types: the potential mistreatment of animals (A.1), and the potential mistreatment of human beings or misuse of human material (A.2). 58% of all coded passages in this category are mentions, 28% are rejections, and 13% are endorsements (see Table 5).

Reasons in *Category B (chimera treatment)* focus on how the chimera will be treated once it is brought into existence, holding that either in virtue of its very existence, or owing to the conditions to which it will be subjected, the chimera will not receive a level of protection and care befitting its moral status. 23% of all coded passages (105 passages) fall in this category, making it the second-most debated category. As with the *chimera creation* category, the concerns here fall into two broad reason types, which differ on the moral status attributed to the chimera: B.1 assumes that the chimera will have a moral status akin to an animal, while B.2 imagines that a chimera might have human analogous moral status. 33% of all coded passages in this category are mentions, 27% are rejections, and 40% are endorsements (see Table 6).

*Category C (chimera existence)* contains reasons concerning potential problems resulting from the existence of a chimera. This is the most heavily debated category, containing 46% of all coded passages (215 passages). Again, it contains two broad reason types: C.1 is concerned with the potential metaphysical implications of a human-animal chimera (particularly the breaking down or crossing of certain boundaries), while C.2 focuses on potential social issues (such as moral confusion or slippery slope effects). 49% of all coded passages in this category are mentions, 39% are rejections, and 12% are endorsements (see Table 7).

Finally, *Category D (downstream effects)* is concerned with harms that may result from the application of chimera research, and the resources that must be invested in it. Only 5% of all coded passages (21 passages) fall under this category, making it the least debated group of reasons. Once more, two broad reason types can be distinguished: D.1 focuses on potential harms to individual patients, from, for example, the uncritical translation of research results or the premature transfer of material from chimeras to humans, whereas D.2 focuses on the interests of third parties, which might be impacted by the diversion of research funding, or by biosafety concerns. 52% of all coded passages in this category are mentions, 29% are rejections, and 19% are endorsements (see Table 8).

## Discussion

The frequency of endorsements, rejections and mentions of a reason cannot, on its own, lead us to a conclusion about that reason's cogency, or about the merits of the arguments in which that reason is deployed. Nonetheless, our categorization and documentation of reasons concerning chimera research yields a descriptive account of the current debate, allowing us to highlight noteworthy trends, argumentative clusters and interesting patterns within the discussion.

**Table 6** Mentions, rejections and endorsements of reasons concerning chimera treatment (Category B)

| Reasons Concerning Chimera Treatment (Category B) | |
|---|---|
| B.1: The chimera might be violated in its animal-analogous moral status | |
| B.1.i: Chimera's mere existence might be inconsistent with animal welfare and/or animal non-instrumentalization | *Mention* [9, 26]<br>*Reject* [34, 85]<br>*Endorse* [19, 70] |
| B.1.ii: Chimera's further treatment might be inconsistent with animal welfare and/or animal non-instrumentalization | *Mention* [8, 54, 66]<br>*Reject* [2, 4, 67, 82, 85]<br>*Endorse* [none] |
| B.2: The chimera might be violated in its human-analogous moral status | |
| B.2.i: Chimera's mere production might violate human-analogous respect | *Mention* [36, 49]<br>*Reject* [34, 67, 68]<br>*Endorse* [14, 20, 21, 53, 63] |
| B.2.ii: Chimera's mere existence might be incompatible with human-analogous welfare | *Mention* [66]<br>*Reject* [34, 68]<br>*Endorse* [53] |
| B.2.iii: Chimera's developmental options might not allow for its relevant potential[a] | *Mention* [50, 56]<br>*Reject* [36, 68]<br>*Endorse* [26] |
| B.2.iv: Chimera's early treatment might violate human-analogous embryo protection | *Mention* [22, 41, 54]<br>*Reject* [89]<br>*Endorse* [39, 42, 84] |
| B.2.v: Chimera's later treatment might be incompatible with human-analogous rights[b] | *Mention* [9, 26, 36, 41, 68]<br>*Reject* [4, 8, 59, 66]<br>*Endorse* [5, 10, 11, 19, 27, 37, 42, 56, 81, 88, 89, 92] |
| B.2.vi: Chimera might lack adequate human-like surrounding | *Mention* [46]<br>*Reject* [none]<br>*Endorse* [36, 37] |
| B.2.vii: Chimera might be attributed a questionable role in society[c] | *Mention* [84]<br>*Reject* [none]<br>*Endorse* [10, 14, 81, 82] |
| B.2.viii Chimera might have unclear moral status | *Mention* [21, 26, 60, 68, 71, 76, 77]<br>*Reject* [17, 29, 38, 52, 69]<br>*Endorse* [14, 27, 28, 32, 40, 47, 63] |
| B.2.ix Chimera might have human-like capacities/characteristics[d] | *Mention* [6, 17, 26, 27, 36, 61, 72, 79]<br>*Reject* [9, 10, 50, 51]<br>*Endorse* [11, 19, 34, 35, 89] |

[a] E.g. when a potential for rational behavior is confined to a bodily structure that will not support its development
[b] E.g. when the chimera is experimented on without adequate consent or killed for research purposes
[c] E.g. when the chimera is abused as an inferior member of a slave race
[d] Insinuating that this possibility in itself constitutes an ethical problem

**Table 7** Mentions, rejections and endorsements of reasons concerning chimera existence (Category C)

| Reasons Concerning Chimera Existence (Category C) | |
|---|---|
| C.1: Crossing human-animal species boundaries could have detrimental metaphysical effects | |
| C.1.i: Existence of chimeras may threaten human dignity | *Mention* [2, 9, 11, 12, 20, 22, 24, 26, 27, 48, 49, 53, 73, 74, 79, 80]<br>*Reject* [8, 34, 36, 37, 57, 60, 65–68, 71, 72, 81, 82, 85, 88]<br>*Endorse* [14, 42, 54, 56, 63, 92] |
| C.1.ii: Existence of chimeras may blur species identities | *Mention* [9, 12, 20, 23, 24, 26, 36, 37, 41, 44, 45, 48, 53, 64, 70, 71, 74, 76, 88]<br>*Reject* [4, 11, 18, 22, 46, 47, 55, 56, 66, 73, 75, 81, 85]<br>*Endorse* [14, 17, 33, 49, 63, 80, 83, 87] |
| C.1.iii: Existence of chimeras may violate moral taboos[a] | *Mention* [9, 22, 26, 37, 49, 73, 88]<br>*Reject* [11, 55, 56, 66]<br>*Endorse* [83] |
| C.1.iv: Existence of chimeras may evoke instinctive repugnance[b] | *Mention* [9, 10, 12, 14, 22, 26, 34, 54, 59, 73, 93]<br>*Reject* [13, 28, 57, 65, 66, 77, 82, 85, 88]<br>*Endorse* [84] |
| C.1.v: Creation of chimeras may be unnatural | *Mention* [4, 9, 20, 22, 26, 37, 45, 48, 49, 73, 79, 80]<br>*Reject* [11, 13, 46, 56, 66, 81, 83, 87, 88]<br>*Endorse* [none] |
| C.1.vi: Creation of chimeras may amount to playing God | *Mention* [2, 20, 22, 48, 73, 87, 93]<br>*Reject* [65, 82, 85, 88]<br>*Endorse* [none] |
| C.2: Crossing human-animal species boundaries could have detrimental social effects | |
| C.2.i: Existence of chimeras may lead to moral confusion[c] | *Mention* [8, 9, 20, 23, 26, 27, 29, 30, 33–35, 43, 48, 49, 52, 74, 75]<br>*Reject* [22, 24, 25, 28, 31, 38, 40, 47, 55, 58, 62, 65, 66, 78, 81, 82, 86, 87, 90, 91]<br>*Endorse* [17, 69, 73, 93] |
| C.2.ii: Existence of chimeras may have slippery slope effects[d] | *Mention* [12, 22, 65, 88]<br>*Reject* [13, 54, 82]<br>*Endorse* [14, 32] |
| C.2.iii: Creation of chimeras may undermine public support for scientific research | *Mention* [22, 30, 59, 72]<br>*Reject* [76]<br>*Endorse* [9, 14] |
| C.2.iv: Creation of chimeras may result in cross-species pregnancies | *Mention* [6, 12, 23, 34, 35, 60, 61, 84]<br>*Reject* [4, 9, 66, 67]<br>*Endorse* [14, 54, 56] |

[a] Suggesting that these taboos demarcate essential moral borders
[b] Suggesting that this repugnance hints to some relevant moral aberration
[c] Supposing that the existence of chimeras leads to an erosion of important moral differences in the respective treatment of humans and animals
[d] Supposing that the existence of chimeras, once permitted, makes it impossible to argue consistently against clear moral malpractices

### Positive reasons (category P)

***(15% of all coded passages. Distribution within: 31% mentions, 7% rejections, 61% endorsements)***

It is striking that discussions of *positive reasons* (Category P) constitute a rather small fraction of all passages retrieved (15%). Additionally, these *positive reasons* are mostly endorsed (61%) or mentioned (31%), and only rarely rejected (7%). Both phenomena can be accounted for.

The relatively low frequency of passages referring to *positive reasons* might be explained by the fact that engaging with these reasons often involves speculation concerning whether certain states of affairs will obtain. In

**Table 8** Mentions, rejections and endorsements of reasons concerning downstream effects (Category D)

| Reasons Concerning Downstream Effects (Category D) | |
|---|---|
| D.1: Individual medical safety might be infringed | *Mention* [4, 49, 54, 66]<br>*Reject* [82, 85]<br>*Endorse* [8, 19, 35] |
| D.2: Third party interests might be infringed | |
| D.2.i: Findings and substances may threaten general biosafety[a] | *Mention* [14, 26, 27, 54, 85]<br>*Reject* [2, 72]<br>*Endorse* [89] |
| D.2.ii: Funding chimera research may contradict distributive justice[b] | *Mention* [24, 89]<br>*Reject* [13, 82]<br>*Endorse* [none] |

[a] Particularly by spreading new diseases
[b] Particularly by affording more financial resources than would be warranted on objective grounds

particular, endorsing or rejecting these reasons mainly depends on scientific or medical prognosis (will chimera creation lead to advances in basic research or will it not (P.1), will chimera research foster the development of application options or will it not (P.2)?). Additionally, it is largely uncontroversial that these potential advances in basic and applied research are morally desirable and they thus do not form an attractive basis for an in-depth ethical discussion. By contrast, more intricate ethical questions concerning competition and allocation of resources are framed negatively and are thus grouped under *downstream effects* (D.2.ii). Authors of papers retrieved in a survey of ethical arguments are likely to focus on ethically controversial issues that call for discussion and analysis, while, at the same time, they may not be ideally placed to predict in a detailed manner just what benefits we might expect to obtain from chimera research. It is therefore unsurprising that these authors do not engage primarily with *positive reasons*, focusing instead on the more ethically controversial issues in the negative categories.

Concerning the relatively low rejection rate of *positive reasons*, suggestions that chimeras might contribute to basic research or could lead to valuable applications are rather vague, making targeted criticism difficult. Rejections in this field would mainly have to amount to accusations of "overselling". This skepticism, however, is not easy to substantiate. Furthermore, it would require detailed predictions of benefits, which, as noted above, are not likely to form a central focus in the ethical literature.

#### Negative reasons (categories A-D)
#### *(85% of all coded passages. Distribution within: 46% mentions, 34% rejections, 20% endorsements)*

The four remaining negative categories focus on more ethically controversial issues, require more ethical analysis, and involve, on the whole, less detailed empirical conjecture. This is particularly the case for the categories concerning *chimera treatment* (B) and *chimera existence* (C). For example, suggestions that bringing a chimera into existence might violate human-analogous respect (B.2.i) or that the existence of chimeras might threaten human dignity (C.1.i) requires discussion of ethical concepts (just what do human-analogous respect and human dignity amount to, and why would chimera research threaten these standards?). In addition, these discussions do not necessarily have to assume that a very specific type of chimera will exist, as, for example, any chimera with human-associated capacities or with a sufficient amount of human material might invoke concerns of human respect and human dignity (even if the existence of these types of chimeras remains, for the moment, rather visionary). To be sure, some of these issues do involve a certain degree of empirical conjecture (relatively specific capacities will be relevant to some reasons in the *chimera treatment* category (B), such as the contention that certain types of chimeras would seriously suffer (B.1.i), or that ill-treatment will result from the chimera having a human-like consciousness (B.2.ix)). Similarly, other arguments rely on certain psychological or sociological postulates (for example, reasons in the *chimera existence* category (C) assume that there are certain social taboos that the existence of chimeras might violate (C.1.iii), or that important psychological and social barriers will be threatened by the existence of chimeras, leading to moral confusion (C.2.i)). Even in these cases, however, there are hotly debated ethical concepts and issues that require sustained discussion to make a case that we should (or should not) view this as a serious ethical problem (could this suffering be justified in certain circumstances, why should a human-like consciousness be avoided, is there anything wrong with violating taboos, why is moral confusion a problem?).

It should also be noted that some of the ethical issues raised by chimera research are familiar from other bioethical contexts. This is particularly true for reasons concerning *chimera creation* (A), *chimera treatment* (B – particularly B.1, where the chimera is presumed to have animal-analogous moral status) and *downstream effects* (D), which refer to common problems of animal experimentation (A.1, B.1), the use of human biological material (A.2), safety (D.1, D.2.i), and justice (D.2.ii). It is thus unsurprising that, in a survey of academic literature, which is inherently striving for originality and innovation, these reasons are reiterated relatively infrequently (B.1, D), or merely mentioned rather than discussed in a sustained manner (A). In addition, the safety-based concerns in D.1 and D.2.i, like the *positive reasons*, are predicated on specific scenarios coming to pass (will it indeed be the case that the results of chimera research pose a threat to the individual (D.1) or to biosafety in general (D.2.i), and, if so, how significant are the risks?). Although there is a

more controversial ethical issue at the heart of these concerns than within the category of *positive reasons* (which risks would be acceptable?), this question is not an attractive candidate for sustained ethical consideration, due to the fact that it is not specific to chimera research and thus tends to bypass the discussion of novel issues in favor of appeals to general moral standards concerning risk-taking.

Finally, the fact that more articles are concerned with *negative reasons* (A-D) than with *positive reasons* (P) does not imply a negative attitude towards chimera research, as reasons discussed might only be mentioned, or even ultimately rejected rather than endorsed by the author. At the same time, however, the fact that reasons in the negative categories (A-D) exhibit an overall surplus of rejections (34%) over endorsements (20%) does not indicate a positive attitude towards chimera research either, as it is possible to repudiate certain reasons against conducting chimera research without approving of the practice overall.

#### Reasons concerning chimera creation (category a) *(11% of all coded passages. Distribution within: 58% mentions, 28% rejections, 13% endorsements)*

The relatively infrequent appearance of passages within this category (11%) might be attributed to the fact that these reasons rehash well-known arguments concerning the treatment of animals in research (A.1) and the use of human biological material, including human embryos and human eggs (A.2) (see above). As such, arguments dealing with these issues can be found in existing bioethical literature, requiring only minor amendments for application to the cases at hand. There is thus a limited incentive for authors to engage in sustained discussion of reasons pertaining to *chimera creation*. Of course, this by no means precludes their practical relevance.

#### Reasons concerning chimera treatment (category B) *(23% of all coded passages. Distribution within: 33% mentions, 27% rejections, 40% endorsements)*

The *chimera treatment* category is composed of two broad reason types – one based on the assumption that chimeras will have animal-analogous moral status (B.1), while the other proceeds from the assumption that chimeras will have human-analogous moral status (B.2). The relatively high proportion of endorsements (40%) compared to rejections (27%) for both broad reason types might be a result of the fact that challenging these reasons is likely to be based on specific assumptions about the capacities of chimeras (for example, doubting that chimeras would ever attain human-analogous capacities (see B.2.ix)), which, as noted above, may not be the area of expertise of many authors writing ethical papers. It is only in very few cases that it seems possible to challenge these arguments through questioning the moral standards to which they appeal (for example, by arguing that a being with a certain potential is not necessarily owed corresponding developmental options (see B.2.iii)), but generally, the moral standards invoked are largely uncontroversial. Thus, while pointing out problems with *chimera treatment* may involve novel ethical discussion (through highlighting novel dangers of maltreatment, instrumentalization etc. in biomedical practice), the repudiation of these arguments will mostly be a matter of suggesting that these potential scenarios will not ultimately materialize.

Reasons predicated on the idea that chimeras have animal-analogous status (B.1) suffer from the familiarity of animal ethics arguments in general, which could explain their infrequent representation in comparison to reasons which involve speculation that a chimera might have human-analogous moral status (B.2). As above, this does not preclude their importance in practice, particularly as the notion of a chimera with human-analogous traits is rather speculative. Furthermore, it should be noted that concerns with animal protection are distributed between the *chimera treatment* category (B, specifically B.1) and the *chimera creation* category (A, specifically A.1), depending on whether the authors are concerned with harms to animals in the chimera generation process, or to the resulting animal-analogous chimera. Animal issues thus make up a greater proportion of the debate than may be apparent at first glance.

#### Reasons concerning chimera existence (category C) *(46% of all coded passages. Distribution within: 49% mentions, 39% rejections, 12% endorsements)*

Reasons concerning *chimera existence* make up a significant proportion of all retrieved passages (46%). One explanation for this, and particularly for the higher prevalence of discussions concerning *chimera existence* (C) compared to discussions concerning *chimera treatment* (B), is that much discussion of the latter involves scrutiny of specific types of chimeras (the origin of a chimera's cells, or its prospective capacities, etc., are likely to be relevant factors in determining how it should be treated). Reasons concerning *chimera existence*, by contrast, mostly deal with human-animal chimeras in general, invoking the potential metaphysical or social implications of these beings' mere presence.

The overall proportion of rejections (39%) in the *chimera existence* category is quite high compared to endorsements (12%). The particularly low frequency of endorsements of reasons C.1.iii-C.1.vi (stating that the creation of chimeras might violate moral taboos, meet with instinctive repugnance, corrupt the natural order, or amount to playing God), relative to mentions and rejections, may suggest that discussions and refutations of these reasons are, predominantly, targeted at straw men. Alternatively, these reasons could appear, or be perceived to appear, in lay discourse, rather than in scholarly debate.

The fact that reason C.2.i (chimeras might generate moral confusion) has so few endorsements and so many mentions and rejections may be an editorial artefact. The first paper to advance this reason was a target article in the American Journal of Bioethics [73] and thus was accompanied by a series of open peer commentaries, which tend to take a critical stance toward the article they address.[3]

#### Reasons concerning downstream effects (category D) *(5% of all coded passages. Distribution within: 52% mentions, 29% rejections, 19% endorsements)*

Reasons concerning *downstream effects* constitute the least debated category (5%). Due to the paucity of data, reliable trends cannot be identified. There are several possible explanations for the infrequent discussion of *downstream effects* within the debate. First, the calculation of *downstream effects* requires making concrete predictions about the results of chimera research (whether, for example, they are likely to present threats to safety). This is compounded by the fact that the concerns discussed in this category often require far-reaching forecasts of consequences in the distant future, which are even more difficult to predict. The relatively far-off nature of these potential consequences also means that they might be viewed as less immediately urgent. Finally, the safety (D.1, D.2.i) and justice-based (D.2.ii) concerns contained in *downstream effects* are not specific to chimera research, but could be invoked in any biomedical context. All of these aspects might contribute to *downstream effects* being less attractive candidates for discussion.

### Limitations

Although we devised the conception and methodology of our work with close regard to its purpose and demands, this study has certain limitations that need to be critically addressed. More precisely, these limitations concern the risks of: (1) data not being comprehensively included in our survey; (2) results not being unanimously extractable from the data; (3) conclusions not being readily inferable from the results.

#### Limitations of data, due to selection criteria and search procedures

As noted above (see Methods), we restricted our review of academic literature to English sources and to articles in international peer-reviewed journals. The restriction to English literature risks overlooking arguments from other cultural spheres. However, because English has become the dominant language for international bioethical discourse, we are confident that our data accurately reflects the scholarly debate at an international level. The restriction to peer-reviewed journal articles might also lead to the inadvertent exclusion of certain arguments. However, the inclusion of non-peer-reviewed literature would make it difficult to consistently exclude lay sources, feature pages, and other public opinion position papers. In addition, reports, surveys, encyclopedia entries, handbook articles etc. often summarize existing debates, and thus may lead to a distortion of data through a double-counting of reasons. The restriction to English [95–98] and peer-reviewed journal articles [95–97, 99, 100] is common in systematic reviews of reasons.

Additionally, it is possible that not all publications conforming to our selection criteria were included, because they do not appear in the databases searched, or because our search strings did not pick them up. It is also possible that, of the articles retrieved, we failed to identify some articles that met our inclusion criteria. We attempted to mitigate the latter limitation by requiring consensus concerning inclusion.

#### Limitations of results, concerning the attribution of text passages to reason types

Because coding of the passages could not be based on a simple search for keywords or catch phrases (the word "dignity", for example, is deployed both to express concerns about the treatment of a chimera and the integrity of the human species), reasons were identified by a close reading and analysis of the texts. This introduces the danger of subjectivity, which we attempted to mitigate by coding passages independently, and eliminating disparities through discussion.

#### Limitations of conclusions

As outlined above (see Discussion), the number of mentions, rejections and endorsements of specific reasons does not allow us to draw any normative conclusions about the quality of the arguments, but rather provides a purely descriptive account of the current debate. Even descriptive conclusions, however, can only be drawn with caution. As outlined in the discussion above, the frequencies of reason mentions, endorsements and rejections might often be explained as a function of the peculiarities of academic bioethical debate. In particular, it is thus possible that our results do not mirror the concerns that bioethicists (even the authors included in our review) would identify as the most pressing. For instance, a bioethicist might publish a paper on a novel issue due to its interesting implications, or to capitalize in a gap on the debate. At the same time, however, she might hold that the most urgent moral problems with chimera research are the more familiar bioethical problems (such as animal suffering or translational risk).

[3] It should be noted that another target article [47] focusing on moral confusion was published in the same journal in 2012 alongside five peer commentaries. Here, however, the results were not as skewed, with two endorsements [17, 69], one mention [52] and two rejections [38, 40] identified in the peer commentaries.

## Conclusion

To the best of our knowledge, this review is the first systematic review of ethical arguments concerning chimera research. We have identified five broad categories of reasons: *positive reasons* (P), and *negative reasons* pertaining to *chimera creation* (A), *chimera treatment* (B), *chimera existence* (C), and *downstream effects* (D). Within these categories, we identified 12 broad and 31 narrow types of reasons, and surveyed the frequencies of their mentions, rejections and endorsements. We hope that the classification into these five broad categories in particular provides an easily accessible overview of the debate, through supplying a systematic classification that reveals the disparate nature of the concerns advanced by various authors across different categories, as well as highlighting the connections between positions taken within the categories.[4]

As an enterprise in descriptive ethics, a systematic review of reasons, as noted above, can yield no immediate normative answers concerning where this debate should move, or which approaches are ethically superior to others. Rather, by outlining the structure of the debate, presenting and interpreting trends, and revealing prominent positions, we have attempted to provide orientation in this complex debate, thus facilitating future academic discussion and policy decisions.

However, some lessons can be drawn from our results. First, we have revealed that ethical stances towards chimera research focus on highly diverse aspects of this scientific endeavor, which invoke a variety of concerns in biomedical ethics (the expected benefits of scientific advances, the ethics of using laboratory animals and human material, the protection of higher organisms, the ontology and sociology of interspecies relations, and the responsibility for more remote research consequences). We suspect that the highly fragmented nature of this debate can undermine coherent assessment of, and ethical consensus concerning the permissibility of, proposed chimera research projects. We hope that our contribution might begin to ameliorate this: by highlighting the different categories of ethical concern, our classification system may help to allow ethicists and policy-makers to get on the same page, and reduce the risk of them talking past each other. Second, our results highlight a potentially fruitful area of further inquiry: work exploring the connections and interdependence of the concerns across the different categories [101]. Ultimately, we need a unified picture of the ethical challenges of human-animal chimera research in order to come to a more integrated assessment of this rapidly developing technology.

[4]It is also important to note that the debate on chimera research is ongoing, and has particularly been fueled by recent developments in chimera research for the purposes of organ transplantation. Because of the end date of our systematic review, some more recent, significant contributions to the debate have not been included [101–112].

## Supplementary information

**Supplementary information** accompanies this paper at https://doi.org/10.1186/s12910-020-00465-7.

**Additional file 1.** Illustrative quotation for each reason type. We have included this file to demonstrate how we assigned reasons to text passages. It contains an exemplary passage for every reason, formulated as endorsement, rejection or mention. This table should help to further understand the classification system of reasons per se, but also to illuminate how mentions demarcate from rejections and/or endorsements.

### Abbreviations

MEDLINE: Medical Literature analysis and retrieval system online (a database); MeSH: Medical subject headings; Embase: Excerpta Medica database (a database)

### Acknowledgements

The authors thank Daniel Strech for his useful comments on methodology and manuscript preparation and Daniel Rasch for his assistance in extracting and compiling the data.

### Authors' contributions

K.K. conceived this study, decided upon the framework for analysis, carried out the literature searching and analysis and drafted the manuscript. L.W. participated in literature searching, data analysis and extensive redrafting of the manuscript. D.H. participated in establishing the framework for analysis, data analysis and redrafting of the manuscript. All authors have read and approved the final version.

### Funding

This project was financially supported by the German Research Foundation (DFG) via the grant "Cluster of Excellence REBIRTH (EXC 62) – From Regenerative Biology to Reconstructive Therapy". The publication of this article was funded by the Open Access fund of Leibniz University Hannover. The funding bodies had no influence on the design of the study, the collection, analysis and interpretation of data, or the writing of the manuscript.

### Availability of data and materials

The authors ensure full transparency of the review process. The full search strategy is presented in Fig. 1 as well as Table 1 of the manuscript, and all databases searched have been listed in the manuscript.
All data generated or analyzed during this study are included in this published article (and its supplementary information files).

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

### Author details

[1]CELLS – Centre for Ethics and Law in the Life Sciences, Leibniz University Hannover, Otto-Brenner-Strasse 1, 30159 Hannover, Germany. [2]Institute of Philosophy, Leibniz University Hannover, Im Moore 21, 30167 Hannover, Germany.

Received: 17 March 2019 Accepted: 6 March 2020
Published online: 23 March 2020

### References

1. Shaw D. Creating chimeras for organs is legal in Switzerland. Bioethica Forum. 2014;14(1):24-6.
2. Shaw D, Dondorp W, de Wert G. Using non-human primates to benefit humans: research and organ transplantation. Med Health Care Philos. 2014; 17(4):573–8.

3. Rashid T, Kobayashi T, Nakauchi H. Revisiting the flight of Icarus: making human organs from PSCs with large animal chimeras. Cell Stem Cell. 2014;15(4):406–9.
4. Bourret R, Martinez E, Vialla F, Giquel C, Thonnat-Marin A, De Vos J. Human-animal chimeras: ethical issues about farming chimeric animals bearing human organs. Stem Cell Res Ther. 2016;7(1):87.
5. Palacios-Gonzalez C. The ethics of killing human/great-ape chimeras for their organs: a reply to Shaw et al. Med Health Care Philos. 2016;19(2):215–25.
6. Munsie M, Hyun I, Sugarman J. Ethical issues in human organoid and gastruloid research. Development. 2017;144(6):942–5.
7. Oldani G, Peloso A, Lacotte S, Meier R, Toso C. Xenogeneic chimera-Generated by blastocyst complementation-As a potential unlimited source of recipient-tailored organs. Xenotransplantation. 2017;24(4). https://doi.org/c10.1111/xen.12327.
8. Baylis F, Fenton A. Chimera research and stem cell therapies for human neurodegenerative disorders. Camb Q Healthc Ethics. 2007;16(2):195–208.
9. Greely HT, Cho MK, Hogle LF, Satz DM. Thinking about the human neuron mouse. Am J Bioeth. 2007;7(5):27–40.
10. Sagoff M. Further thoughts about the human neuron mouse. Am J Bioeth. 2007;7(5):51–2.
11. Karpowicz P, Cohen CB, van der Kooy D. It is ethical to transplant human stem cells into nonhuman embryos. Nat Med. 2004;10(4):331–5.
12. Bahadur G, Iqbal M, Malik S, Sanyal A, Wafa R, Noble R. Admixed human embryos and stem cells: legislative, ethical and scientific advances. Reprod BioMed Online. 2008;17(Suppl 1):25–32.
13. Camporesi S, Boniolo G. Fearing a non-existing minotaur? The ethical challenges of research on cytoplasmic hybrid embryos. J Med Ethics. 2008; 34(11):821–5.
14. Jones DA. Is the creation of admixed embryos "an offense against human dignity"? Hum Reprod Genet Ethics. 2010;16(1):87–114.
15. Strech D, Sofaer N. How to write a systematic review of reasons. J Med Ethics. 2012;38(2):121–6.
16. McCullough LB, Coverdale JH, Chervenak FA. Constructing a systematic review for argument-based clinical ethics literature: the example of concealed medications. J Med Philos. 2007;32(1):65–76.
17. Abelman M, O'Rourke PP, Sonntag KC. Part-human animal research: the imperative to move beyond a philosophical debate. Am J Bioeth. 2012;12(9):26–8.
18. Ankeny RA. No real categories, only chimeras and illusions: the interplay between morality and science in debates over embryonic chimeras. Am J Bioeth. 2003;3(3):31–3.
19. Anton R. On recent advances in human engineering provocative trends in embryology, genetics, and regenerative medicine. Polit Life Sci. 2016;35(2):54–68.
20. Austriaco NP. How to navigate boundaries: a reply to the American journal of bioethics. Natl Cathol Bioeth Q. 2006;6(1):61–71.
21. Badura-Lotter G, Fangerau H. Human-animal chimeras: not only cell origin matters. Am J Bioeth. 2014;14(2):21–2.
22. Baylis F. Animal eggs for stem cell research: a path not worth taking. Am J Bioeth. 2008;8(12):18–32.
23. Baylis F, Robert JS. Part-human chimeras: worrying the facts, probing the ethics. Am J Bioeth. 2007;7(5):41–5.
24. Benham B, Haber M. Moral confusion and developmental essentialism in part-human hybrid research. Am J Bioeth. 2008;8(12):42–4.
25. Bok H. What's wrong with confusion? Am J Bioeth. 2003;3(3):25–6.
26. Cabrera Trujillo LY, Engel-Glatter S. Human-animal chimera: a Neuro driven discussion? Comparison of Three Leading European Research Countries. Sci Eng Ethics. 2015;21(3):595–617.
27. Capps B. Do chimeras have minds? Camb Q Healthc Ethics. 2017;26(4):577–91.
28. Castle D. Hopes against hopeful monsters. Am J Bioeth. 2003;3(3):28–30.
29. Chan S. Hidden anthropocentrism and the "benefit of the doubt": problems with the "origins" approach to moral status. Am J Bioeth. 2014;14(2):18–20.
30. Chapman A, Hiskes AL. Unscrambling the eggs: cybrid research through an embryonic stem cell research oversight committee (ESCRO) lens. Am J Bioeth. 2008;8(12):44–6.
31. Charland LC. Are there answers? Am J Bioeth. 2003;3(3):1–2.
32. Cheshire WP Jr. The moral musings of a murine chimera. Am J Bioeth. 2007; 7(5):49–50.
33. Cohen CB. Creating human-nonhuman chimeras: of mice and men. Am J Bioeth. 2003;3(3):3–5.
34. Cooley DR. Genetically engineering human-animal chimeras and lives worth living. Between Species. 2008;8:1–19.
35. Coors ME. Considering chimeras: the confluence of genetic engineering and ethics. Natl Cathol Bioeth Q. 2006;6(1):75–87.
36. de Melo-Martin I. Chimeras and human dignity. Kennedy Inst Ethics J. 2008; 18(4):331–46.
37. deGrazia D. Human-animal chimeras: human dignity, moral status, and species prejudice. Metaphilosophy. 2007;38(2–3):310–29.
38. DiSilvestro R. The two-essence problem that wasn't. Am J Bioeth. 2012;12(9):34–5.
39. Eberl JT. Creating non-human persons: might it be worth the risk? Am J Bioeth. 2007;7(5):52–4.
40. Eberl JT. Ontological kinds versus biological species. Am J Bioeth. 2012;12(9): 32–4.
41. Eberl JT, Ballard RA. Exercising restraint in the creation of animal-human chimeras. Am J Bioeth. 2008;8(6):45–6.
42. Eberl JT, Ballard RA. Metaphysical and ethical perspectives on creating animal-human chimeras. J Med Philos. 2009;34(5):470–86.
43. Franklin S. Drawing the line at not-fully-human: what we already know. Am J Bioeth. 2003;3(3):25–7.
44. Gerrek ML. Who really causes the lady to vanish? Am J Bioeth. 2008;8(12): 46–7.
45. Greely HT. Defining chimeras...and chimeric concerns. Am J Bioeth. 2003; 3(3):17–20.
46. Greene M, Schill K, Takahashi S, Bateman-House A, Beauchamp T, Bok H, et al. Ethics: moral issues of human-non-human primate neural grafting. Science. 2005;309(5733):385–6.
47. Haber MH, Benham B. Reframing the ethical issues in part-human animal research: the unbearable ontology of inexorable moral confusion. Am J Bioeth. 2012;12(9):17–25.
48. Heathcotte B, Robert JS. The strange case of the humanzee patent quest. Natl Cathol Bioeth Q. 2006;6(1):51–9.
49. Hermeren G. Ethical considerations in chimera research. Development. 2015; 142(1):3–5.
50. Hyun I. From naive pluripotency to chimeras: a new ethical challenge? Development. 2015;142(1):6–8.
51. Hyun I. What's wrong with human/nonhuman chimera research? PLoS Biol. 2016;14(8):e1002535.
52. Irvine R, Degeling C, Kerridge I. Uncanny animals: thinking differently about ethics and the animal-human relationship. Am J Bioeth. 2012;12(9):30–2.
53. Johnston J, Eliot C. Chimeras and "human dignity". Am J Bioeth. 2003;3(3): W6–w8.
54. Jones DA. The ethics of creating chimeras and other admixed organisms. Ethics Med. 2012;28(3):81–93.
55. Karpowicz P. In defense of stem cell chimeras: a response to "crossing species boundaries". Am J Bioeth. 2003;3(3):17–9.
56. Karpowicz P, Cohen CB, van der Kooy D. Developing human-nonhuman chimeras in human stem cell research: ethical issues and boundaries. Kennedy Inst Ethics J. 2005;15(2):107–34.
57. Knoppers BMJ. Yann Our social genome? Trends Biotechnol. 2007;25(7):284–8.
58. Kobayashi NR. A scientist crossing a boundary: a step into the bioethical issues surrounding stem cell research. Am J Bioeth. 2003;3(3):15–6.
59. Lavieri RR. The ethical mouse: be not like Icarus. Am J Bioeth. 2007;7(5):57–8.
60. Levine S, Grabel L. The contribution of human/non-human animal chimeras to stem cell research. Stem Cell Res. 2017;24:128–34.
61. Masaki H, Nakauchi H. Interspecies chimeras for human stem cell research. Development. 2017;144(14):2544–7.
62. McGee DB. Moral ambiguity? Yes. Moral confusion? No. Am J Bioeth. 2003; 3(3):11–2.
63. Mirkes R. Is it ethical to generate human-animal chimeras? Natl Cathol Bioeth Q. 2006;6(1):109–30.
64. Murphy TF. When is an objection to hybrid stem cell research a moral objection? Am J Bioeth. 2008;8(12):47–9.
65. Nelson JL. Respecting boundaries, disparaging values. Am J Bioeth. 2008; 8(12):33–4.
66. Palacios-Gonzalez C. Ethical aspects of creating human-nonhuman chimeras capable of human gamete production and human pregnancy. Monash Bioeth Rev. 2015;33(2–3):181–202.
67. Palacios-Gonzalez C. Chimeras intended for human gamete production: an ethical alternative? Reprod BioMed Online. 2017;35(4):387–90.
68. Palacios-González C. Human dignity and the creation of human–nonhuman chimeras. Med Health Care Philos. 2015;18(4):487–99.
69. Piotrowska M. Who are my parents? Why assigning moral categories to genealogical relations leads to more confusion. Am J Bioeth. 2012;12(9):28–30.
70. Pusch AF. Splices: when science catches up with science fiction. NanoEthics. 2015;9(1):55–73.

71. Ravelingien A, Braeckman J, Legge M. On the moral status of humanized chimeras and the concept of human dignity. Between Species. 2006;6:1–22.
72. Robert JS. The science and ethics of making part-human animals in stem cell biology. FASEB J. 2006;20(7):838–45.
73. Robert JS, Baylis F. Crossing species boundaries. Am J Bioeth. 2003;3(3):1–13.
74. Robert JS, Baylis F. A response to commentators on "Crossing species boundaries". Am J Bioeth. 2003;3(3):W-c6.
75. Robertson JA. A response to "Crossing species boundaries" by Jason Scott Robert and Francoise Baylis. Am J Bioeth. 2003;3(3):W-c5.
76. Rollin BE. Of mice and men. Am J Bioeth. 2007;7(5):55–7.
77. Rollin BE. On chimeras. Zygon. 2007;42(3):643–8.
78. Sagoff M. Transgenic chimeras. Am J Bioeth. 2003;3(3):30–1.
79. Salter B, Harvey A. Creating problems in the governance of science: bioethics and human/animal chimeras. Sci Public Policy. 2014;41(5):685–96.
80. Saniotis A. Remaking homo: ethical issues on future human enhancement. Ethics Sci Environ Polit. 2013;13(1):15–21.
81. Savulescu J. Human-animal transgenesis and chimeras might be an expression of our humanity. Am J Bioeth. 2003;3(3):22–5.
82. Savulescu J, Skene L. The kingdom of genes: why genes from animals and plants will make better humans. Am J Bioeth. 2008;8(12):35–8.
83. Schaub DJ. Chimeras: from poetry to science. Natl Cathol Bioeth Q. 2006; 6(1):29–35.
84. Seyfer TL. An overview of chimeras and hybrids. Natl Cathol Bioeth Q. 2006; 6(1):37–49.
85. Shaw D, Dondorp W, Geijsen N, de Wert G. Creating human organs in chimaera pigs: an ethical source of immunocompatible organs? J Med Ethics. 2015;41(12):970–4.
86. Siegel AW. The moral insignificance of crossing species boundaries. Am J Bioeth. 2003;3(3):33–4.
87. Streiffer R. In defense of the moral relevance of species boundaries. Am J Bioeth. 2003;3(3):37–8.
88. Streiffer R. At the edge of humanity: human stem cells, chimeras, and moral status. Kennedy Inst Ethics J. 2005;15(4):347–70.
89. Streiffer R. Chimeras, moral status, and public policy: implications of the abortion debate for public policy on human/nonhuman chimera research. J Law Med Ethics. 2010;38(2):238–50.
90. Thompson PB. Crossing species boundaries is even more controversial than you think. Am J Bioeth. 2003;3(3):14–5.
91. Urie KA, Stanley A, Friedman JD. The humane imperative: a moral opportunity. Am J Bioeth. 2003;3(3):20–1.
92. Watt H. Embryos and pseudoembryos: parthenotes, reprogrammed oocytes and headless clones. J Med Ethics. 2007;33(9):554–6.
93. Zwanziger LL. Crossing perspectival chasms about species. Am J Bioeth. 2003;3(3):9–10.
94. Piotrowska M. Transferring morality to human-nonhuman chimeras. Am J Bioeth. 2014;14(2):4–12.
95. van Dijke I, Bosch L, Bredenoord AL, Cornel M, Repping S, Hendriks S. The ethics of clinical applications of germline genome modification: a systematic review of reasons. Hum Reprod. 2018;33(9):1777–96.
96. de Graeff N, Jongsma KR, Johnston J, Hartley S, Bredenoord AL. The ethics of genome editing in non-human animals: a systematic review of reasons reported in the academic literature. Philos Trans R Soc Lond Ser B Biol Sci. 2019;374(1772):20180106.
97. Kars MC, van Thiel GJ, van der Graaf R, Moors M, de Graeff A, van Delden JJ. A systematic review of reasons for gatekeeping in palliative care research. Palliat Med. 2016;30(6):533–48.
98. Abraham NS, Young JM, Solomon MJ. A systematic review of reasons for nonentry of eligible patients into surgical randomized controlled trials. Surgery. 2006;139(4):469–83.
99. Fleming V, Frith L, Luyben A, Ramsayer B. Conscientious objection to participation in abortion by midwives and nurses: a systematic review of reasons. BMC Med Ethics. 2018;19(1):31.
100. Song F, Loke Y, Hooper L. Why are medical and health-related studies not being published? A systematic review of reasons given by investigators. PLoS One. 2014;9(10):e110418.
101. Hubner D. Human-animal chimeras and hybrids: an ethical paradox behind moral confusion? J Med Philos. 2018;43(2):187–210.
102. Farahany NA, Greely HT, Hyman S, Koch C, Grady C, Pasca SP, et al. The ethics of experimenting with human brain tissue. Nature. 2018;556(7702): 429–32.
103. Koplin JJ. Human-animal chimeras: the moral insignificance of uniquely human capacities. Hast Cent Rep. 2019;49(5):23–32.
104. Hyun I. Ethical considerations for human-animal neurological chimera research: mouse models and beyond. EMBO J. 2019;38(21):e103331.
105. Chen HI, Song H, Ming GL. Applications of human brain Organoids to clinical problems. Dev Dyn. 2019;248(1):53–64.
106. De Los AA, Hyun I, Latham SR, Elsworth JD, Redmond DE Jr. Human-monkey chimeras for modeling human disease: opportunities and challenges. Methods Mol Biol. 2019;2005:221–31.
107. Counihan D. Neurological chimeras and the moral staircase. Methods Mol Biol. 2019;2005:195–203.
108. Mann SP, Sun R, Hermeren G. Ethical considerations in crossing the Xenobarrier. Methods Mol Biol. 2019;2005:175–93.
109. Hyun I. Ethical standards for chimera research oversight. Methods Mol Biol. 2019;2005:165–71.
110. Porsdam Mann S, Sun R, Hermeren G. A framework for the ethical assessment of chimeric animal research involving human neural tissue. BMC Med Ethics. 2019;20(1):10.
111. Casal P, Williams A. Human iPSC-Chimera Xenotransplantation and the Non-Identity Problem. J Clin Med. 2019;8(1):95.
112. Koplin J, Wilkinson D. Moral uncertainty and the farming of human-pig chimeras. J Med Ethics. 2019;45(7):440–6.


## Publisher's Note

## IV. Regulatory Hurdles Surrounding Xenotransplantation

### 1. General Aspects of the Topic

Recent advances in clinical development discussed above demonstrate that xenotransplantation of solid organs is close to routine clinical evaluation. This requires particular deliberation on the part of regulators to ensure that clinical trials and application take place in a sound legal environment. However, in general, regulation and guidelines seem to have difficulties in capturing the issues raised by such innovative technologies and are often late to the party. Additionally, the introduction of human iPSCs into organogenesis-disabled pigs as well as gene-editing of the porcine genome both pose distinct regulatory challenges, not least because of jurisdictional differences (globally) in approaching the regulation of life sciences. First, there are issues pertaining to the use of human material, particularly stem cells, which are heterogeneously regulated even within the EU: diverging approaches to the national implementation even of supranational frameworks such as EU instruments reflect the diverse contexts of the various member states [175]. Introducing those cells into animals adds another layer of complexity as the question arises whether these are encompassed by the regulations regarding breeding. Second, genetic editing of organisms has ignited a global debate regarding whether organisms modified by targeted gene disruption fall within the scope of the legal framework for genetically modified organisms traditionally aiming at genetically modified crops [176, 177]. Third, regulation aimed specifically at xenotransplantation initially has thus far mainly focused on dovetailing individual versus collective rights in terms of public health [178].

There are analyses regarding the status of xenotransplantation regulation within particular jurisdictions available [178-182]. Also, supranational bodies like the World Health Organisation and the International Xenotransplantation Association have published their own guidelines [183-185]. The tenor of all of these publications as well as many reviews of the matter, including those from clinicians and scientists, is the emphasis on the need for international harmonisation of rules and regulations [13, 149, 186-188].

The research question for the subsequent publications therefore was whether there were any *specific* hindrances to the harmonisation of international regulations regarding xenotransplantation. We thus set out to identify and detail any and all normative issues tied to all the steps involved in creating chimeric/multitransgenic pigs for xenotransplantation purposes. Further, we sought to analyse regulations in the jurisdictions at the forefront of xenotransplantation research, as well as in two supranational frameworks. Our hypothesis was that contrasting the regulations in place with all normative issues described would probably allow identification of unaddressed regulatory issues specific for xenotransplantation.

**Added Value of These Publications**

To my knowledge, this is the first publication that outlines a) all normative issues arising from the creation of chimeric/multitransgenic pigs and b) identifies three specific obstacles to prospective supranational or international regulatory framework harmonisation, namely:

1. An inconsistent approach across several international jurisdictions with regards to the regulatory framework capturing either a chimeric and/or a genetically engineered/multitransgenic pig.

2. An unanswered question as to what the final product to be regulated is, i.e., the chimeric/multi-transgenic pig or the organ obtained from it.

3. The patchwork of existing laws are not fit for purpose to address (intellectual) property rights tied to the chimeric/multitransgenic pig and/or the respective transplanted organ.

This work therefore aimed at providing the groundwork for steering the discussion of harmonisation of international regulation in concrete directions by identifying specific hot topics, which require a more systematic debate.

Incidentally, the original publication seemed to immediately serve its purpose by drawing two high profile responses, namely by the FDA and the International Association of Xenotransplantation [189, 190]. Our response to these two comments, alongside which it was published, thus further augments the impact of the original publication. It also emphasises that the discussion about harmonisation of regulatory frameworks regarding xenotransplantation has to start with addressing the fragmentation across the various international jurisdictions rather than focusing on any specific jurisdiction.

The original publication together with the reply thus serve as a baseline of rethinking regulatory issues pertaining to xenotransplantation by pointing out specific inadequacies.

# Regulatory and intellectual property conundrums surrounding xenotransplantation

To the Editor — Xenotransplantation seeks to ameliorate the organ transplantation shortage via the use of genetically modified pigs as organ donors for human recipients[1]. Two main paths are being pursued to create these pigs: human–animal chimeras, in which human induced pluripotent stem cells (iPSCs) are introduced into organogenesis-disabled animals; and multi-transgenic pigs, whose genomes are engineered or edited to circumvent host immune reactions following transplantation (reviewed in refs. [2–4]). Many challenges remain in overcoming immune rejection and long-term survival of the transplanted organ; as yet, about 900 days is the longest that pig xenografts have survived in a primate[5]. But even if the scientific and technical challenges to successful long-term xenotransplantation can be addressed, we argue here that a lack of clarity with respect to regulatory oversight and property rights in jurisdictions across the world threatens to thwart clinical translation of these products.

Figure 1 elaborates the necessary steps and associated regulatory, ethical and legal issues to be undertaken for a patient to receive a chimeric or genetically altered pig organ. In Table 1, we summarize relevant regulations in the countries at the forefront of xenotransplantation research, as well as in two supranational frameworks: the European Union and the World Health Organization (WHO). A glance at the regulatory and legal frameworks reveals three key issues related to the generation of human–pig chimeras or multi-transgenic pigs.

The first problem is a lack of clarity as to which legal framework captures a human–animal chimera or a multi-transgenic pig. CRISPR–Cas9 manipulation to generate the latter and introduction of human iPSCs into the pig blastocyst to create the former (steps 1 and 2 in Fig. 1) are not subject to sui generis regulation in most jurisdictions: regarding human–animal chimeras, regulation covering the use of human cells for breeding generally assumes that a human ovum is the basis for this process. Hence, many of the relevant rules regarding breeding are not triggered by pig ova and the process of creating the resulting chimeric pigs is therefore not in toto regulated by these norms. The situation is different in Japan, where the 2013 Act on Pharmaceuticals and Medical Devices creates the therapeutic category 'regenerative medicine products,' which refers to "items [...] obtained after culturing or other processes using human or animal cells [for] reconstruction [or] repairing of [human] bodies"[6]. Thus, it is possible that chimeric pigs would fall within the scope of this regulation.

Regarding multi-transgenic pigs, the question revolves around whether pigs modified by CRISPR–Cas9-mediated targeted gene disruption or the

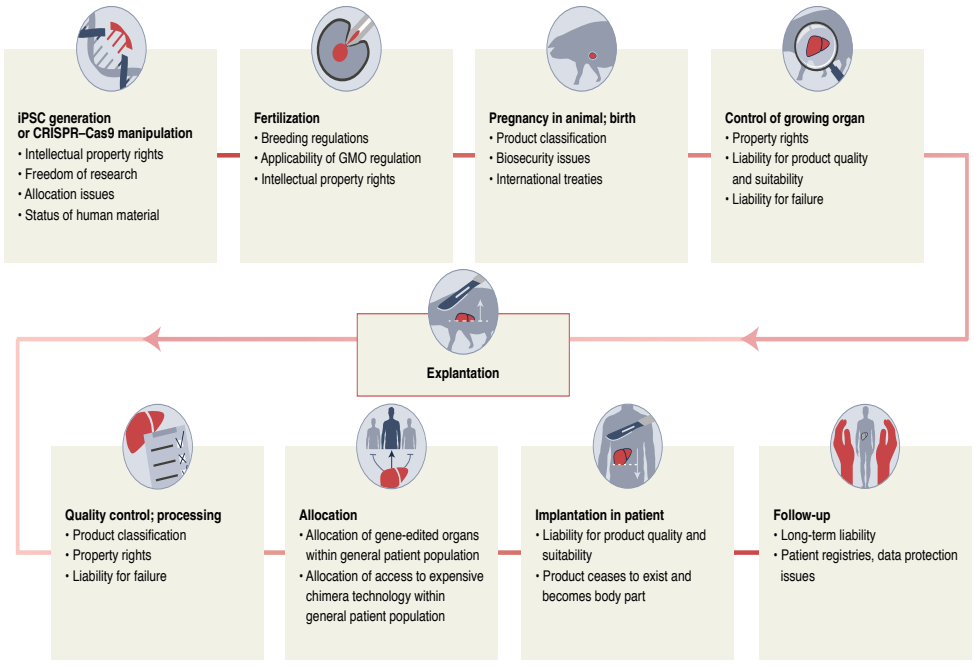



**Fig. 1 | Normative issues in genetically engineered pigs for organ transplantation.** The figure depicts the necessary steps in the process of engineering human–animal chimeras or genetically altered pigs, as well as legal and normative issues associated with each step.

**Table 1 | Overview of regulations in different jurisdictions concerning xenogeneic and cell-based products**

| Jurisdiction | Specific regulation | Domestic or supranational | Public or private regulation | Normative source | Normative weight |
|---|---|---|---|---|---|
| United States | Yes | Domestic | Public and delegated (FDA) | Framework of the Regulation of Biotechnology | Binding |
| Japan | Yes | Domestic | Public | Act on the Regulation of Human Cloning Techniques (Act No. 146, 6 December 2000); Act on Pharmaceuticals and Medical Devices (PMD Act, November 2013) | Binding |
| United Kingdom | Yes | Domestic (partially excluding Scotland) | Public and delegated (HTA) | Human Tissue Act 2004 | Binding |
| China | No | – | China is awaiting international regulatory approaches | – | – |
| European Union | Yes | Supranational | Public | Regulation EC 1394/2007; additional guideline on the quality of biological active substances produced by transgene expression in animals (2013); Directive 2003/94/EC; Directive 2001/83/EC; Directive 2001/20/EC; Directive 2006/86/EC | Regulation includes directly binding directives and domestic implementations in member states |
| WHO | Yes (recommendation) | Supranational | Public (UN) | WHO recommends that member states put in place effective national regulation before allowing xenogeneic transplantation to take place | Non-binding |

FDA, Food and Drug Administration; HTA, Health Technology Assessment; UN, United Nations.

incorporation of human transgenes fall within existing legal frameworks for genetically modified organisms (GMOs). There is a substantial debate on a global level about the regulatory status of these new breeding technologies[7–12]. The United States updated the Coordinated Framework for the Regulation of Biotechnology in 2017, but the US National Academy of Sciences is urging regulatory agencies and the federal government to develop a horizon-scanning strategy for new biotech products[8]. In the European Union, a 2018 judgment from the European Court of Justice suggests that the jurisprudence will classify engineered or edited animals as GMOs[10]. Elsewhere, Japan considers as a GMO any organism containing an insertion of extracellularly processed nucleic acid[13], whereas China has yet to put a regulatory regime in place[12].

This leads to the second regulatory conundrum: what is the product—the chimeric or multi-transgenic pig or the organ to be donated—and under which regulation does it fall? Is the pig as a whole a medical device or a medicinal product? Or is the pig merely an incubator for the organ transplant? The answer to these questions will have consequences for the debate surrounding intangible and tangible property rights (see below). Where an intervention is derived from human material, the provisions of human tissues legislation may be triggered, and in many jurisdictions (for example, the United States and European Union), this may result in a xenotransplant being classified as an Advanced Therapy Medicinal Product, where the processing of cells implies a manipulation that alters their biological characteristics[14].

Japan is one territory where this issue has been addressed by creating specific legislation that could cover chimeric or multi-transgenic pigs and their organs. In that country, the regenerative medicine products category encompasses the processing of human cells such as iPSCs for the reconstruction, repair or formation of the structure or function of the human (or animal) body (that is, tissue-engineered products)[15].

Apart from the above regulatory questions, there is also a third problem relating to property rights: to whom does the xenotransplant product belong? Given the enormous investment required to create a scaled-up 'manufacturing' process for xenotransplant organs, there will likely be intense interest around the protection of property rights. This relates to not only tangible property rights (that is, in the tangible, material cells of the transplant[16]), but also the ethically and legally much more complex issue of intangible property rights (that is, the special knowledge that produces the xenotransplant cells).

Regarding intellectual property rights and their protection, the European Union, according to Directive 98/44, excludes "processes to produce chimeras from germ cells or totipotent cells of humans and animals [...] from patentability"[17]. Similar provisions are in the Japanese Patent Act, Section 32, which states that biotechnological inventions that "contravene public order, morality or public health" are excluded from patentability[18]. In the United States, at least some forms of human–non-human chimeras have already been declared unpatentable following the Newman case[19]. At the same time, there is an ongoing debate on whether the notoriously wide scope of patentability in the United States may have also left the door open for human–non-human chimeras[20]. However, guidelines aimed at illustrating the scope of patentability of stem cell related therapies suggest that patents may be obtainable where material is removed from a human body to produce medical reagents that are returned to the same body[21]. It will be interesting to

see whether this material making a detour through a chimeric or engineered animal before being reintroduced to the material donor also qualifies for patenting.

Moreover, in cases where an organ is classified as separate product, European Union and Japanese regulations limit patent rights for products for medical treatment or cure of a patient; the United States theoretically allows them, but in reality limits the enforcement of such patents. The upshot of this is that medical innovation surrounding xenotransplants is difficult to patent in all three jurisdictions[22]. In addition, human donors of iPSCs might try to enforce property rights (though this will likely meet with limited success, as the cells are no longer the original material). Competing claims could potentially clash with intellectual property rights regarding the genetically altered animal, with more than one individual asserting a good claim to the animal. What the scientific community should strive to avoid is a case similar to that of HeLa cells, where entitlements to informational self-determination and physical property were in conflict[23].

Finally, with regards to steps 5 (quality control) and 7 (implantation) in Fig. 1, there is the issue of long-term liability: product liability laws are ubiquitous, and there is no reasonable argument why xenobiotic pig-derived products should be excluded from this regime. Moreover, regardless of whether xenobiotic pigs will ultimately contain human material or not, organs will not likely be produced on an industrial scale any time soon; therefore, the problem of allocating a scarce resource (step 6 in Fig. 1) remains a live one, with the novel twist being the conundrum of how surplus organs are allocated.

In summary, a patchwork of different regulatory and legal approaches means that it is unlikely that an international consensus can be found to oversee xenotransplants. The moral charge of the subject matter and the related intercultural divergence will likely steer different jurisdictions in different directions. This will inevitably exacerbate cross-border issues in international research cooperation and therefore unnecessarily hamper patients' access to a much-needed resource. A continued, systematic debate on common standards should therefore be a priority across jurisdictions. ❐

Koko Kwisda[1,2,4]✉, Tobias Cantz[2,3,4] and Nils Hoppe[1,2,4]

[1]*CELLS – Centre for Ethics and Law in the Life Sciences, Leibniz University Hannover, Hannover, Germany.* [2]*REBIRTH-AG Translational Hepatology and Stem Cell Biology, Hannover, Germany.* [3]*Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany.* [4]*These authors contributed equally: Koko Kwisda, Tobias Cantz, Nils Hoppe.*
✉*e-mail: koko.kwisda@philos.uni-hannover.de*

Published online: 7 July 2021
https://doi.org/10.1038/s41587-021-00976-7

## References

1. Rashid, T., Kobayashi, T. & Nakauchi, H. *Cell Stem Cell* **15**, 406–409 (2014).
2. Niemann, H. & Petersen, B. *Transgenic Res* **25**, 361–374 (2016).
3. Hryhorowicz, M., Zeyland, J., Słomski, R. & Lipiński, D. *Mol. Biotechnol.* **59**, 435–444 (2017).
4. Ekser, B., Li, P. & Cooper, D. K. C. *Curr. Opin. Organ Transplant.* **22**, 513–521 (2017).
5. Mohiuddin, M. M. et al. *Nat. Commun.* **7**, 11138 (2016).
6. Government of Japan. *Act on Securing Quality, Efficacy and Safety of Products Including Pharmaceuticals and Medical Devices* (1960) Act No. 145. English translation: http://www.japaneselawtranslation.go.jp/law/detail/?id=3213&vm=04&re=02 (accessed 5 May 2021).
7. Taupitz, J. & Weschka, M. (eds.). Chimbrids — Chimeras and Hybrids in Comparative European and International Research: Scientific, Ethical, Philosophical and Legal Aspects (Springer, 2009).
8. US National Academies of Sciences Engineering and Medicine. *Preparing for Future Products of Biotechnology* (National Academies Press, 2017).
9. Jones, H. D. *Nat. Plants* **1**, 14011 (2015).
10. Callaway, E. *Nature* **560**, 16 (2018).
11. Inoue, Y., Shineha, R. & Yashiro, Y. *Cell Stem Cell* **19**, 152–153 (2016).
12. Groth, C. G. *Xenotransplantation* **14**, 358–359 (2007).
13. Tsuda, M., Watanabe, K. N. & Ohsawa, R. *Front. Bioeng. Biotechnol.* **7**, 387 (2019).
14. Iglesias-López, C., Agusti, A., Obach, M. & Vallano, A. *Front. Pharmacol.* **10**, 921 (2019).
15. Azuma, K. *Curr. Stem Cell Rep* **1**, 118–128 (2015).
16. Hoppe, N. *Bioequity – Property and the Human Body* (Ashgate, 2009).
17. The European Parliament and the Council of the European Union. *Directive 98/44/EC of the European Parliament and of the Council on the Legal Protection of Biotechnological Inventions* (1998); https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A31998L0044
18. Government of Japan. *The Patent Act* (1959) Act No. 121. English translation: http://www.japaneselawtranslation.go.jp/law/detail/?%20printID=&ft=1&co=01&x=32&y=19&k%20y=%20%20%20%20%20&page=10&id=42&lvm=&re=02&vm=02 (accessed 5 May 2021).
19. Kopinksi, N. E. *Boston Coll. Law Rev* **45**, 619–666 (2004).
20. Hagglund, R. *Santa Clara High Technol. Law J* **25**, 51–104 (2008).
21. Eyre, D. E. & Schlich, G. W. *Pharm. Pat. Anal.* **4**, 431–441 (2015).
22. Schütt, C. *Patents for Biotechnological Inventions: Current Legal Situation and Case Law in Europe, the US and Japan* (Swiss Federal Institute of Technology Zurich, 2004).
23. Beskow, L. M. *Annu. Rev. Genomics Hum. Genet.* **17**, 395–417 (2016).

## Acknowledgements

This study was supported by the German Research Foundation through the excellence cluster 'From Regenerative Biology to Reconstructive Therapies' (REBIRTH; EXC 62/3) and through the CRC/TRR 127 'Biology of Xenogeneic Cell and Organ Transplantation: From Bench to Bedside'.

## Competing interests

The authors declare no competing interests.

# Reply to 'Clarifying US regulations on xenotransplantation' and 'International standards and guidelines for xenotransplantation'

Kwisda et al. reply — The purpose of our Correspondence[1] was to elicit a frank discussion on better ways to reach common regulatory frameworks for xenotransplantation, and the comments from Marks and Solomon[2] and from Hawthorne et al.[3] show that our piece was timely and the ensuing debate necessary. A common theme from the two responses is a criticism generally not of what we have written, but what we have not written about. The two responses thus augment our Correspondence and, taken together, provide an overview broader than the explicit remit of our original paper. This is one of the main mechanisms of academic debate, and we are delighted that our publication has spurred this discussion.

The response provided by International Xenotransplantation Association representatives Hawthorne et al. criticizes the lack of mention of their scientific organization; however, an appraisal of professional self-regulation at supranational level was outside the scope of our short analysis. Although these authors indicate that our modest suggestion for more systematic debate was "misleading," their overview provides just that: more systematic debate. We are pleased that this aspect of our work has already come to fruition and would like to thank Hawthorne et al. for their contribution.

We share Marks and Solomon's enthusiasm in relation to the potential that lies in the area of xenotransplantation. We appreciate that their outline of the regulatory process in the United States provides additional, and enriching, detail to our comparison paper — which, by necessity and design, does not provide such detail for the different jurisdictions and areas of law to


which we alluded. We respectfully disagree with their suggestion that the information provided in our Correspondence is "inaccurate and incomplete" in relation to regulatory oversight.

Marks and Solomon object to the overall aim of our paper: to outline hindrances to prospective supranational or international regulatory frameworks. They do so because they erroneously read it so that it applies specifically to the US regulatory position. The sentence they quote that provides the hook for their criticism ("… a lack of clarity with respect to regulatory oversight …") goes on to make this clear ("… across the world …"). The focus of the argument therefore lies in the fragmentation across jurisdictions, rather than fragmentation within any one jurisdiction like the United States.

In addition, it is clear that the brevity of the Correspondence format means that it cannot (and should not) provide a detailed (or even complete) overview of the regulatory landscape in the United States or other jurisdictions, which would quite clearly be sufficiently sizeable subject matter for a book. We nevertheless welcomed Marks and Solomon's comments as they provide additional detail. We are also certain that the outline they provide will assist colleagues seeking to register xenotransplantation products in the United States, giving a better understanding of the regulatory frameworks within which the US Food and Drug Administration undertakes its important work. ❐

Koko Kwisda[1,2]✉, Tobias Cantz[2,3] and Nils Hoppe[2,3]

[1]*CELLS – Centre for Ethics and Law in the Life Sciences, Leibniz University Hannover, Hannover, Germany.* [2]*REBIRTH-AG Translational Hepatology and Stem Cell Biology, Hannover, Germany.* [3]*Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany.*
✉*e-mail: koko.kwisda@philos.uni-hannover.de*

Published online: 2 December 2021
https://doi.org/10.1038/s41587-021-01151-8

References
1. Kwisda, K., Cantz, T. & Hoppe, N. *Nat. Biotechnol.* **39**, 796–798 (2021).
2. Marks, P. & Solomon, S. *Nat. Biotechnol.* https://doi.org/10.1038/s41587-021-01144-7 (2021).
3. Hawthorne, W.J., Cowan, P.J., Buhler, L. & Wolf, E. *Nat. Biotechnol.* https://doi.org/10.1038/s41587-021-01148-3 (2021).

Competing interests
The authors declare no competing interests.

## 2. Practical Application in a Concrete Jurisdiction

Moving away from theoretical considerations we wanted to evaluate how the three identified open questions would play out in a concrete jurisdiction. Germany was chosen for reasons of practicality and because it has a traditionally restrictive approach to regulating novel technologies.

In Germany, over 139.000 organs have been transplanted since the 1970s. Still, approximately 1000 people die annually waiting for an organ with 9000 people on the waiting list [191]. The first law regulating transplantation specifically, the German Transplant Act (Transplantationsgesetz, TPG), was passed in 1997, with amendments in 2007 and 2012 [192]. However, the TPG is not applicable to xenotransplantation as it only covers the removal and donation of human organ and tissues as per § 1 TPG. Specific legislation regarding xenotransplantation has not been passed as yet.

We therefore decided to analyse the normative aspects of xenotransplantation on the basis of a fictitious but realistic case study and showcase how it could unfold in the current regulatory context of Germany. Our hypothesis was that the current German regulatory framework would not adequately capture and address the three previously identified regulatory issues.

### Added Value of This Publication

This is the first publication to discuss what the shortcomings of the current xenotransplantation regulatory framework are by using a concrete example in a specific jurisdiction. It shows that the current German legislation only inadequately addresses the three issues pertaining to xenotransplantation:

1. Even though there appears to be a consensus regarding the regulatory framework capturing a multitransgenic pig for xenotransplantation purposes, it is unclear whether the same rules would apply if human tissue in the form of iPSCs is added to the mixture.

2. The question as to what the final product to be regulated is, i.e., the chimeric/multi-transgenic pig or the organ obtained from it and whether separate approvals need to be obtained for their respective creation remains unanswered.

3. To my knowledge, neither property nor intellectual property rights tied to a multitransgenic/chimeric pig and/or the transplanted organ have been discussed in the context of xenotransplantation in Germany so far.

This publication thus provides a basis for discussing regulatory issues pertaining to xenotransplantation with respect to the current German legislation; but also serves as a proof of concept. Even though many national and international bodies have started to provide frameworks for xenotransplantation, most of them pertain to multitransgenic animals only; and the two downstream issues have not been specifically addressed in Germany or elsewhere.

frontiers | Frontiers in Transplantation

TYPE Policy Brief
PUBLISHED 20 September 2023
DOI 10.3389/frtra.2023.1222031



OPEN ACCESS

EDITED BY
Friedrich Thaiss,
University of Hamburg, Germany

REVIEWED BY
George Bayliss,
Brown University, United States
Kathleen Fenton,
National Heart, Lung, and Blood Institute (NIH), United States

*CORRESPONDENCE
Koko Kwisda
koko.kwisda@philos.uni-hannover.de

RECEIVED 13 May 2023
ACCEPTED 22 August 2023
PUBLISHED 20 September 2023

CITATION
Kwisda K (2023) Unaddressed regulatory issues in xenotransplantation: a hypothetical example. Front. Transplant. 2:1222031. doi: 10.3389/frtra.2023.1222031



# Unaddressed regulatory issues in xenotransplantation: a hypothetical example

Koko Kwisda*

Centre for Ethics and Law in the Life Sciences, Leibniz University Hannover, Hanover, Germany

The last few years have seen a significant increase in the use of technology to manipulate genetic sequences and generate animals as a source of xeno-organs. This has made the generation of genetically bespoke organisms a reality. This paper will analyze the regulatory and practical aspects of such an innovative approach to xenotransplantation on the basis of a hypothetical case study applied to Germany and highlight the gaps in the current regulation. This paper thus provides the basis for legal debate within a specific country. In addition, the identified gaps also pose a barrier toward the harmonization of international regulation. This publication therefore lays the groundwork for guiding the international debate regarding the regulatory framework for solid organ xenotransplantation toward specific issues.

KEYWORDS

transplantation, organ shortage, regulation, xenotransplantation, interspecies blastocyst complementation, genome editing technologies, transgenic pigs, biotechnology

## Introduction

The shortage of human donor organs is a global problem. Emerging xenotransplantation approaches suggest two possible solutions: genetic modification of the porcine genome to yield organs from these multitransgenic pigs, which are immune-compatible with humans (reviewed in (1–3)); or the introduction of human induced pluripotent stem cells (iPSCs) into pigs to rescue organogenesis of a previously knocked-out target organ via blastocyst complementation: the resulting chimeric animal would ideally have an organ made up of human cells, which would enable proper physiological functions in the recipient's organism (reviewed in (4, 5)).

There are still some obstacles to overcome regarding immune-compatibility and the long-term survival of porcine organs. However, a pig xenograft has been able to survive over 900 days in primates already (6). Notably, the most recent successes were a pig kidney being transplanted into a human, brain-dead recipient, which remained functional for roughly 3 days before the experiment was terminated (7), as well as the first pig to human cardiac xenotransplantation, with the patient surviving for 2 months (8).

In general, multitransgenic pigs could become a seemingly unlimited source of organs. This could have several beneficial effects (reviewed in (9)), namely, avoiding deaths of patients waiting on the transplant list; avoiding considerable costs for managing end-stage care, e.g., for end-stage kidney disease (10); and expanding indications for organ transplantation by including patients ineligible for transplantation per the current standards. The latter would include patients not sick enough or too sick to be eligible according to the current criteria because an unlimited pool of donors would allow individual assessment of every patient because he or she would not be competing for an organ.

Xenotransplantation via blastocyst complementation would most likely be a complementary approach to multitransgenic pigs, as the latter would be the ideal host animals into which to insert

the human iPSCs. However, xenotransplantation of a chimeric organ offers several additional possibilities over xeno-organs from "just" multitransgenic pigs (reviewed in (9)):

- *Avoiding immunosuppression*: as the organ would consist of the patient's own cells, immunosuppression could potentially be avoided, or at least limited.
- *Correcting genetic defects*: the iPSCs from the patient could be edited before inserting them into the pig blastocyst and thus correct defective genes. This could be beneficial for patients with genetically determined organ diseases, such as polycystic kidney disease, hemochromatosis, arrhythmogenic cardiomyopathy, cardiac channelopathies, or X-linked chronic granulomatous disease.
- *Compensating for human-specific organ needs*: specific organs or cells are not easily replaceable by their porcine counterparts. This is particularly true for pancreatic islet cells as their insulin secretion capacity apparently does not mirror the human demand for insulin (11); and the liver, as it plays a central role in the production of roughly 2,000 proteins and it seems unlikely that all of those produced by a transplanted porcine liver would function properly in humans (12).

It is clear that numerous regulatory concerns need to be addressed before such an approach can be translated into routine practice. In our previous work, we described three key issues pertaining to the creation of human–pig chimeras/multitransgenic pigs, namely: (1) the potential uncertainty as to which framework captures human–animal chimeras or multitransgenic pigs; (2) what the end product is and by which regulation it is captured; and (3) who the owner of the xenoproduct is (13, 14). While we previously discussed these in terms of hindrances to prospective supranational or international regulatory frameworks, we will now play out the scenario in a concrete jurisdiction by describing the necessary steps to be undertaken in order for a patient to receive a chimeric organ for two reasons. First, the science pertaining to multitransgenic pigs is much advanced if compared to blastocyst complementation and all recent successes described above were achieved with transplanting xeno-organs from multitransgenic pigs. Acknowledging that, two important supranational bodies, i.e., the World Health Organization and the International Xenotransplantation Association, have published their own guidelines in an effort to harmonize the approach to xenotransplantation (15–17). Second, human stem cell research is very differently regulated even within EU member states, and mixing them with animal material adds another intriguing level of complexity. Therefore, based on a hypothetical but realistic case study, we will outline the major associated normative and practical issues as described in **Figure 1** and address the abovementioned issues in the context of the German jurisdiction.

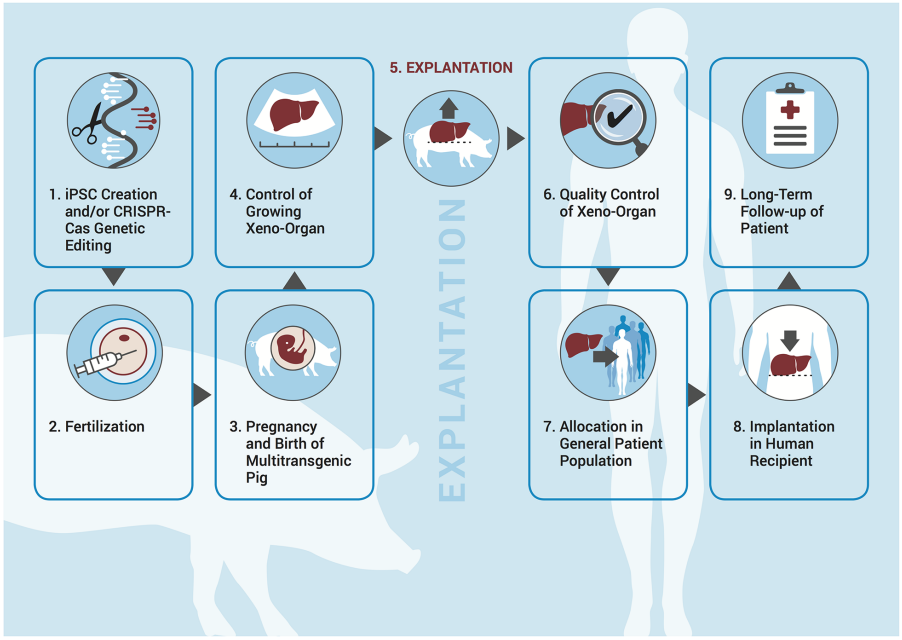



FIGURE 1
Necessary steps in the process of creating human–animal chimeras/genetically altered pigs for the purposes of xenotransplantation.

## The hypothetical case: chimeric humanized organs as an alternative to liver transplant

A 35-year-old patient is diagnosed with intermediate stage hepatocellular carcinoma (HCC) consisting of two separate nodules with sizes of 4 and 1.5 cm, respectively. Subsequent tumor staging analyses confirm the absence of extrahepatic tumor manifestations and no affections of larger branches of the liver vasculature are detectable. The patient's estimated survival rate is 21–30 months (18–20). In principle, HCC is an indication for liver transplantation, which is able to provide a definitive cure to a subset of patients. However, eligibility for receiving organs from the UNOS or Eurotransplant registry is linked to the "Milan criteria" for HCC staging (one lesion ≤5 cm; alternatively, up to three lesions, each <3 cm; no extrahepatic manifestations; no evidence of gross vascular invasion) (21). The patient's HCC stage does not meet the Milan criteria because one nodule is larger than 3 cm; however, since his tumor has not progressed to gross vascular invasion, he would still have a fairly good oncologic survival chance if transplanted. His physicians see two potential options: related living donor liver transplantation or transplantation of a liver made up of the patient's cells from a chimeric pig. As a potential living donor, the brother of the patient is willing to donate a liver lobe. He is a healthy 42-year-old craftsman, has a family with three children, and runs a small workshop with seven employees.

An institutional ethics committee reviews the case and comes to the following conclusion: as the patient is not eligible for receiving an organ from a deceased donor but may have a considerable chance of a complete cure, the experimental humanized pig liver transplantation approach is endorsed. On the one hand, the double equipoise principle is challenged by the fact that the patient's HCC stage impairs his chance of overall survival, and the donor risks, such as prolonged recovery and long-term health issues with incapacity to work, are not easily justified in this scenario. On the other hand, the patient would benefit from a functional human liver without the need of immunosuppression. The committee therefore suggests the experimental approach as a first-line therapy and the living donor transplantation as a back-up strategy in the case of graft failure (initial non-function) of the humanized pig liver approach (the current status regarding chimeric liver transplantation using blastocyst complementation is reviewed in (22)).

## Applying our scenario in Germany as example

Returning to our fictitious patient with liver carcinoma, we will run the scenario as it could play out in the German jurisdiction with respect to the steps outlined in **Figure 1**.

The German research group will pursue the two-pronged approach, deciding to combine human iPSCs with a liver defective porcine embryo via blastocyst complementation. For an overview of potential applicable laws, see **Table 1**.

## Which framework captures the chimeric pig?

There are several laws in Germany that could potentially be applied but a general problem is that the introduction of iPSCs into a pig blastocyst to create a chimeric animal is under-regulated: norms aiming at the regulation of the use of human cells for breeding assume that a human ovum is the basis for the process and thus do not trigger breeding laws, as per the national animal welfare law (TierSchG). The process of creating the resulting chimeric entity, which is part animal and part human, is therefore not regulated by these norms. At the same time, even statutory documents such as the German Embryo Protection Act (ESchG), which for the reasons outlined does not, prima facie, seem to apply, sometimes contain provisions dealing

**TABLE 1** Illustration of German laws as to the question of which framework could capture the chimeric pig, the human iPSCs, and the xeno-organ.

| Entity | Legal framework | Reason it might apply | Obstacle/problem |
|---|---|---|---|
| Chimeric animal | National animal welfare law (TierSchG) | TierSchG ensures the protection of the lives and wellbeing of animals to ensure no pain, suffering, or harm is done without good reason. Chimeric animals should logically enjoy the same protection. | The breeding law as per TierSchG is not triggered. Norms aiming at the regulation of the use of human cells for breeding assume that a human ovum is the basis for the process. |
| | Embryo Protection Act (ESchG) | Contains provisions regarding changing human germline cells. Inserting human iPSCs into the pig blastocyst is creating a part-human embryo. | An embryo is defined as fertilized human egg or a totipotent cell removed from an embryo. The status of human material when inserted into an animal is unclear. |
| | German Technology Act (GenTG) | Regulates genetically modified organisms and includes genetically altered animals. | Unclear whether mixing cells from humans and animals is in scope. Unclear whether further use of the chimeric animal for xenotransplantation purposes would be permitted. |
| | Advanced Therapeutic Medicinal Product (ATMP) acc. to § 4b AMG and ATMP directive | … defines a medicinal product as a substance, which includes living animals, to be used in or on the human body. | Unclear whether the pig itself will be viewed as an MP for application on humans or just as a vessel for the actual ATMP. |
| Induced pluripotent stem cells (iPSCs) | ATMP acc. to § 4b AMG and ATMP directive | Applies | Opens the question of whether iPSCs will be viewed as a tissue-engineered product or a gene therapy medicinal product. |
| Xeno-organ | ATMP acc. to § 4b AMG and ATMP directive | Applies | Opens the question of whether xeno-organs will be viewed as a tissue-engineered product or a gene therapy medicinal product. |

with germline modification (§ 5 ESchG). It has been argued, though, that the ESchG, due to prohibition of analogy, cannot be applied to artificially created germ cells (23).

In theory, genetically modified organisms are captured by the European GMO directive and thus, in our case, the German genetic engineering law (GenTG), which describes them as "*an organism […] whose genetic material has been changed in a way […] which would not occur naturally*" (24). Indeed, the clarifying opinion by the Court of Justice of the European Union from 2018 seems to suggest classification of edited animals as genetically modified organisms (GMOs) and it thus seems to suggest classification of edited animals as GMOs (25) and it thus seems clear that multitransgenic pigs are captured by this law. However, it is unclear whether the law would apply to human–animal mixtures as well even though it allows mixing of cells from different organisms and adding foreign DNA.

An alternative route would be classification of the pig as an Advanced Therapy Medicinal Product (ATMP). The German Medicinal Product Act (AMG) defines medicinal products as substances, which, in turn, can be "*bodies of animals, including those of living animals*" (26). The question is, then, whether the chimeric pig itself can and should be classified as a medicinal product (MP).

Still, in light of the above, the health care professional (HCP) decides to file the request for approval for the creation of the chimeric pig as a GMO with the competent state authority since GMO approval lies with the state in Germany.

## What is the end product and by which regulation is it captured?

Unarguably, there are at least two components that need separate regulatory approval: on the one hand, the human iPSCs derived from the patient, which constitute an ATMP, more precisely, either a tissue-engineered product (TEP) or, if they are indeed genetically modified upfront, e.g., to knock out central nervous system contribution, they might be classified as a gene therapy medicinal product.

On the other hand, the creation of the chimeric pig needs to be approved as well, as described above. The key question here is whether the pig as a whole can and should be viewed as an entity that will be applied to humans. This is interdependent with the question of what it will be classified as in the first place. For example, the GenTG explicitly excludes the application of GMOs on humans. As the xenoliver will be transplanted into the patient, though, it seems intuitive that it will be viewed as a MP, regardless of what the pig is classified as, which means that it will very likely need a separate approval process as an ATMP, i.e., a tissue-engineered product or gene therapy medicinal product before transplantation.

Indeed, the Paul Ehrlich Institute had a prospective meeting regarding the regulatory classification of xenotransplantation products and decided that the AMG will be applied together with the ATMP directive (Directive (EG) Nr. 1394/2007); however, this discussion happened in the context of genetically edited, i.e., multitransgenic, pigs (27) and in practice has not been applied to a chimeric animal or its organ, respectively.

The treating physicians, therefore, file for approval for the generation of two more ATMPs with the Paul Ehrlich Institute: (1) the iPSCs from the patient and (2) the xenoliver.

## Who is the owner of the xenoproduct?

After these respective approvals have been granted, iPSCs are created from the patient's skin sample; any contribution to the central nervous system and the germ line has been knocked out. The patient signs a waiver of property rights to ensure that he has no claim to either the iPSC lines produced or the later resulting organs (step 1 in **Figure 1**). The HCPs decide that in order to maximize the chances of success, more than one pig should be generated. They therefore insert the iPSCs into five pig blastocysts, which are then transferred into a sow (step 2). After 114 days, four healthy piglets are born (step 3). Over the next 12–24 weeks, their livers are monitored through functional imaging as well as invasive diagnostics (step 4). After 24 weeks, the pigs and their livers, respectively, have grown enough to allow for transplantation, which succeeds at the first attempt (step 5). The livers from the four remaining pigs are not used at this point, as it is still an experimental approach. In future cases, though, it is conceivable that the unused organs from the pigs generated in excess would be distributed via the Eurotransplant system. The patient has to sign a waiver for liability claims (steps 6–8). Follow-up of the patient is analogous to human transplantation with extra "xenovigilance" in relation to the implanted organ (step 9).

# Results and discussion

With the high unmet medical need for organs, pig xenotransplantation could potentially cure millions of patients with life-threatening diseases. Recent advances in primate models as well as the first transplantations of xenogeneic organs into human recipients make clinical trials in the near future more likely. It is therefore necessary that regulatory authorities start to think about how such approaches would pan out in their respective jurisdictions. Our hypothetical case study elaborated how xenotransplantation could potentially play out in the current German regulatory situation. However, the three questions highlighted above have not been conclusively answered.

Regarding question 1 and which framework would apply, it seems to have been answered for multitransgenic pigs, but it is not at all clear whether this holds true for chimeric pigs.

In addition, question 2 regarding what the end product is might not end up being answered by simply splitting the "product" in several parts and treating them as separate entities from a regulatory standpoint. On top of that, since classification as an ATMP happens at the national level, ATMPs are not regulated concisely within Europe, with some states classifying biotechnologically altered tissue products as ATMPs and some as medicinal products.

Question 3 regarding who the owner of the xenoproduct is seems to be the most complex. Will there be a difference regarding patentability between human–animal chimeric and multitransgenic pigs? How will (non-) patentability influence downstream property rights? How will those in turn affect to whom the excess organs belong and how they will be distributed—if at all?

In summary, the example of Germany shows the fragmented nature of regulations governing human–animal chimeras for the purposes of xenotransplantation, which creates a technological context devoid of legal certainty. The moral charge of the subject matter, and the related intercultural divergence, might steer jurisdictions in different directions even within Europe. Where research takes place in countries with significantly different ethico-legal approaches, a common set of norms will be difficult to agree upon. A continued, systematic debate on common standards should therefore be a priority.

In addition, there are numerous ethical issues pertaining to xenotransplantation, which have been discussed for years (28–33) and fall into three broad categories: the first argues that certain scientific experiments should simply not be undertaken; the second warns of unforeseen consequences of genetically altering organisms; and the third pertains to the suffering of involved animals. In addition, there is a continuous and heated debate about the permissibility of mixing animal and human material in the academic (34–36) as well as the public sector (37–39).

These ethical and legal questions need to be addressed before such an approach ever becomes routine.

## Author contributions

The author confirms sole responsibility for the conception of the work as well as manuscript preparation.

## Funding

This study was supported by the German Research Foundation through the excellence cluster "From Regenerative Biology to Reconstructive Therapies" (REBIRTH; EXC 62/3).

## Acknowledgments

The author sincerely thanks Jürgen Scherer from the Paul Ehrlich Institute for providing clarifying explanations regarding the regulatory environment in Germany. The author also thanks Tobias Cantz and Nils Hoppe for their comments on earlier versions of this article.

## Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## References

1. Lu T, Yang B, Wang R, Qin C. Xenotransplantation: current status in preclinical research. *Front Immunol.* (2019) 10:3060. doi: 10.3389/fimmu.2019.03060

2. Zhang X, Wang Q, Zhao J, Li X, Peng W, Yang Z, et al. The resurgent landscape of xenotransplantation of pig organs in nonhuman primates. *Sci China Life Sci.* (2021) 64(5):697–708. doi: 10.1007/s11427-019-1806-2

3. Cooper DKC, Gaston R, Eckhoff D, Ladowski J, Yamamoto T, Wang L, et al. Xenotransplantation—the current status and prospects. *Br Med Bull.* (2018) 125 (1):5–14. doi: 10.1093/bmb/ldx043

4. Lu Y, Zhou Y, Ju R, Chen J. Human-animal chimeras for autologous organ transplantation: technological advances and future perspectives. *Ann Transl Med.* (2019) 7(20):576. doi: 10.21037/atm.2019.10.13

5. Kobayashi E, Tohyama S, Fukuda K. Organ fabrication using pigs as an in vivo bioreactor. *Keio J Med.* (2020) 69(2):30–6. doi: 10.2302/kjm.2019-0006-OA

6. Mohiuddin MM, Singh AK, Corcoran PC, Thomas ML. Chimeric 2C10R4 anti-CD40 antibody therapy is critical for long-term survival of GTKO.hCD46.hTBM pig-to-primate cardiac xenograft. *Nat Commun.* (2016) 7:11138. doi: 10.1038/ncomms11138

7. Weintraub K. Pig kidneys to the rescue? Groundbreaking transplant a step toward solving organ shortage. USA Today News (2021). Available at: https://eu.usatoday.com/in-depth/news/health/2021/10/19/pig-kidney-organ-transplant-into-human-milestone-science/5948819001/ (Accessed December 22, 2022).

8. Mohiuddin MM, Singh AK, Scobie L, Goerlich CE, Grazioli A, Saharia K, et al. Graft dysfunction in compassionate use of genetically engineered pig-to-human cardiac xenotransplantation: a case report. *Lancet.* (2023) 402(10399):397–410. doi: 10.1016/s0140-6736(23)00775-4

9. Oldani G, Peloso A, Lacotte S, Meier R, Toso C. Xenogeneic chimera-generated by blastocyst complementation—as a potential unlimited source of recipient-tailored organs. *Xenotransplantation.* (2017) 24(4). doi: 10.1111/xen.12327

10. Collins AJ, Foley RN, Chavers B, Gilbertson D, Herzog C, Ishani A, et al. US renal data system 2013 annual data report. *Am J Kidney Dis.* (2014) 63(1 Suppl): A7. doi: 10.1053/j.ajkd.2013.11.001

11. Graham ML, Bellin MD, Papas KK, Hering BJ, Schuurman HJ. Species incompatibilities in the pig-to-macaque islet xenotransplant model affect transplant outcome: a comparison with allotransplantation. *Xenotransplantation.* (2011) 18 (6):328–42. doi: 10.1111/j.1399-3089.2011.00676.x

12. Ibrahim Z, Busch J, Awwad M, Wagner R, Wells K, Cooper DK. Selected physiologic compatibilities and incompatibilities between human and porcine organ systems. *Xenotransplantation.* (2006) 13(6):488–99. doi: 10.1111/j.1399-3089.2006.00346.x

13. Kwisda K, Cantz T, Hoppe N. Regulatory and intellectual property conundrums surrounding xenotransplantation. *Nat Biotechnol.* (2021) 39(7):796–8. doi: 10.1038/s41587-021-00976-7

14. Kwisda K, Cantz T, Hoppe N. Reply to 'Clarifying US regulations on xenotransplantation' and 'International standards and guidelines for xenotransplantation'. *Nat Biotechnol.* (2021) 39(12):1503. doi: 10.1038/s41587-021-01151-8

15. Kim MK, Choi HJ, Kwon I, Pierson RN 3rd, Cooper DK, Soulillou JP, et al. The international xenotransplantation association consensus statement on conditions for undertaking clinical trials of xenocorneal transplantation. *Xenotransplantation.* (2014) 21(5):420–30. doi: 10.1111/xen.12129

16. World Health Organization. First WHO global consultation on regulatory requirements for xenotransplantation clinical trials, Changsha, China, 19–21 November 2008. World Health Organization (2008). Available at: https://apps.who.int/iris/handle/10665/341812 (Accessed October 25, 2022).

17. World Health Organization. Second WHO global consultation on regulatory requirements for xenotransplantation clinical trials, Geneva, Switzerland, 17–19 October 2011. World Health Organization (2011). Available at: https://apps.who.int/iris/handle/10665/341817 (Accessed October 25, 2022).

18. Wang CY, Li S. Clinical characteristics and prognosis of 2887 patients with hepatocellular carcinoma: a single center 14 years experience from China. *Medicine*. (2019) 98(4):e14070. doi: 10.1097/md.0000000000014070

19. Burrel M, Reig M, Forner A, Barrufet M, de Lope CR, Tremosini S, et al. Survival of patients with hepatocellular carcinoma treated by transarterial chemoembolisation (TACE) using drug eluting beads. Implications for clinical practice and trial design. *J Hepatol*. (2012) 56(6):1330–5. doi: 10.1016/j.jhep.2012.01.008

20. Elshaarawy O, Gomaa A, Omar H, Rewisha E, Waked I. Intermediate stage hepatocellular carcinoma: a summary review. *J Hepatocell Carcinoma*. (2019) 6:105–17. doi: 10.2147/jhc.s168682

21. Mazzaferro V, Llovet JM, Miceli R, Bhoori S, Schiavo M, Mariani L, et al. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet Oncol*. (2009) 10(1):35–43. doi: 10.1016/S1470-2045(08)70284-5

22. Aravalli RN. Generating liver using blastocyst complementation: opportunities and challenges. *Xenotransplantation*. (2021) 28(2):e12668. doi: 10.1111/xen.12668

23. Faltus T. Stammzellenreprogrammierung der rechtliche status und die rechtliche handhabung sowie die rechtssystematische bedeutung reprogrammierter stammzellen. *Ordnung der Wissenschaft*. (2016) 4:239–46.

24. Gesetz zur Regelung der Gentechnik (Gentechnikgesetz - GenTG). Bundesministerium der Justiz (1990). Available at: https://www.gesetze-im-internet.de/gentg/BJNR110800990.html (Accessed November 30, 2022).

25. Judgment in Case C-528/16. Organisms obtained by mutagenesis are GMOs and are, in principle, subject to the obligations laid down by the GMO directive. Court of Justice of the European Union (2018). Available at: https://curia.europa.eu/jcms/upload/docs/application/pdf/2018-07/cp180111en.pdf (Accessed November 11, 2022).

26. Gesetz über den Verkehr mit Arzneimitteln (Arzneimittelgesetz - AMG). Bundesministerium der Justiz Gesetz über den Verkehr mit Arzneimitteln (Arzneimittelgesetz—AMG) (2005). Available at: https://www.gesetze-im-internet.de/amg_1976/BJNR024480976.html (Accessed August 17, 2023).

27. Reichart B, Längin M, Denner J, Schwinzer R, Cowan PJ, Wolf E. Pathways to clinical cardiac xenotransplantation. *Transplantation*. (2021) 105(9):1930–43. doi: 10.1097/tp.0000000000003588

28. Cooper DK. Ethical aspects of xenotransplantation of current importance. *Xenotransplantation*. (1996) 3:264–74. doi: 10.1111/j.1399-3089.1996.tb00147.x

29. Smetanka C, Cooper DK. The ethics debate in relation to xenotransplantation. *Rev Sci Tech*. (2005) 24(1):335–42.

30. Rollin BE. Ethical and societal issues occasioned by xenotransplantation. *Animals*. (2020) 10(9):1695. doi: 10.3390/ani10091695

31. Morris PJ, Monaco AP. Ethical issues and xenotransplantation. *Transplantation*. (2004) 78(8):1099–100. doi: 10.1097/01.tp.0000146577.84256.07

32. Cortesini R. Ethical aspects in xenotransplantation. *Transplant Proc*. (1998) 30 (5):2463–4. doi: 10.1016/s0041-1345(98)00687-3

33. Entwistle JW, Sade RM, Drake DH. Clinical xenotransplantation seems close: ethical issues persist. *Artif Organs*. (2022) 46(6):987–94. doi: 10.1111/aor.14255

34. Deitch R. Government looks for guidance through Warnock minefield. *Lancet*. (1984) 2(8411):1107–8. doi: 10.1016/S0140-6736(84)91550-2

35. Birke L, Michael M. The heart of the matter: animal bodies, ethics, and species boundaries. *Soc Anim*. (1998) 6(3):245–61. doi: 10.1163/156853098x00186

36. Robert JS, Baylis F. Crossing species boundaries. *Am J Bioeth*. (2003) 3(3):1–13. doi: 10.1162/15265160360706417

37. Bilger M. Scientists create human-pig hybrids for organ transplants that could develop into "monsters". Lifenews.com (2017). Available at: https://www.lifenews.com/2017/01/27/scientists-create-human-pig-hybrids-for-organ-transplants-that-could-develop-into-monsters/ (Accessed May 30).

38. McDermott N. FRANKENSWINE scientists make PIG HUMAN babies by splicing stem cells in amazing new trial. The Sun (2017). Available at: https://www.thesun.co.uk/news/2713619/scientists-make-pig-human-babies-by-splicing-stem-cells-in-amazing-new-trial/ (Accessed May 30).

39. Brock B. The uneasy truth about human-animal hybrids. BBC (2017). Available at: https://www.bbc.com/future/article/20170222-the-uneasy-truth-about-human-animal-hybrids (Accessed May 30).

## V. Summary and Outlook

Animal-to-human xenotransplantation unarguably offers an intriguing and much needed solution for the global problem of organ shortage. The rapid advances in preclinical animal studies have made in-human clinical trials a realistic possibility in the near future. To ensure their smooth start, several issues will need to be mitigated: First, the genetic engineering of animals and/or the mixing of animal and human material will very likely cause public unease - either in the form of a human receiving an animal organ, or, by adding a twist, a human receiving an organ consisting largely of her own cells but derived from a pig. Therefore, public opinion needs to be shaped and trust built among the general public through early dialogue [187]. In addition to the public discourse, the academic debate surrounding xenotransplantation, and, particularly, the creation of human-animal chimeras in this context, asks for an integrated assessment of the associated ethical issues. Finally, the law typically trails behind science given the impossibility of predicting every circumstance that could arise within the scope of a particular regulation. However, if certain novel technologies are on the horizon they should be discussed prior to possible translation. This is particularly relevant regarding xenotransplantation as any unnecessary delay in the process results in avoidable deaths.

The results of this thesis have set the basis for the ethical as well as the regulatory discussion regarding xenotransplantation:

The first-of-its-kind systematic review regarding the ethical issues associated with human-animal chimeras provides a ground zero for the continued debate. The identification of categories as well as trends and patterns will hopefully have

a two-fold effect: One, to provide an overview of the breadth and depth of the ethical debate; and two, prevent the continued fragmentation of the discussion.

The three papers regarding the regulatory issues have also achieved some notable *firsts:* one, they comprehensively detailed all normative issues pertaining to every step regarding the generation of chimeric and/or multitransgenic pigs; two, they identified specific unanswered regulatory question; and three, they showed how existing regulation is unfit to answer all associated issues and questions in a concrete jurisdiction. Together these papers lay the groundwork for steering the discussion regarding the harmonisation of international regulation in concrete directions, which has been identified as a key issue by researchers in the field.

Taken together, all publications will hopefully provide a basis for the development of relevant policy and legislation by objectivising the moral charge through standardising the ethical debate; and by directing the international discussion towards specific issues and thus enable a coherent framework for the regulation of xenotransplantation.

## VI. Bibliography


1. Health Resources and Services Administration. Organ Donation Statistics. 2020 [cited 8 November 2021]. Available from: https://www.organdonor.gov/learn/organ-donation-statistics.
2. T.E. Starzl, T.L. Marchioro, G.N. Peters*, et al.* Renal Heterotransplantation from Baboon to Man: Experience with 6 Cases. *Transplantation*, 1964. 2: p. 752-76.
3. C.N. Barnard, A. Wolpowitz, and J.G. Losman. Heterotopic cardiac transplantation with a xenograft for assistance of the left heart in cardiogenic shock after cardiopulmonary bypass. *South African Medical Journal*, 1977. 52(26): p. 1035-8.
4. L.L. Bailey, S.L. Nehlsen-Cannarella, W. Concepcion, and W.B. Jolley. Baboon-to-human cardiac xenotransplantation in a neonate. *JAMA*, 1985. 254(23): p. 3321-9.
5. D.K.C. Cooper, R. Gaston, D. Eckhoff*, et al.* Xenotransplantation-the current status and prospects. *British Medical Bulletin*, 2018. 125(1): p. 5-14.
6. E. Kobayashi, S. Tohyama, and K. Fukuda. Organ Fabrication Using Pigs as An in Vivo Bioreactor. *Keio J Med*, 2020. 69(2): p. 30-36.
7. M. Hryhorowicz, J. Zeyland, R. Slomski, and D. Lipinski. Genetically Modified Pigs as Organ Donors for Xenotransplantation. *Mol Biotechnol*, 2017. 59(9-10): p. 435-444.
8. B. Ekser, P. Li, and D.K.C. Cooper. Xenotransplantation: past, present, and future. *Curr Opin Organ Transplant*, 2017. 22(6): p. 513-521.
9. H. Niemann and B. Petersen. The production of multi-transgenic pigs: update and perspectives for xenotransplantation. *Transgenic Res*, 2016. 25(3): p. 361-74.
10. K. Weintraub. Pig kidneys to the rescue? Groundbreaking transplant a step toward solving organ shortage. USA Today News. 2021 [cited 22 December 2022]. Available from: https://eu.usatoday.com/in-depth/news/health/2021/10/19/pig-kidney-organ-transplant-into-human-milestone-science/5948819001/.

11. Eurotransplant. Waiting list mortality in 2021, by country, by organ. 2021 [cited 29 May 2022]. Available from: https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&ved=2ahUKEwiKraSr8IT4AhX5_rsIHXUVBSAQFnoECAIQAQ&url=https%3A%2F%2Fstatistics.eurotransplant.org%2Freportloader.php%3Freport%3D10716-32926%26format%3Dpdf%26download%3D1&usg=AOvVaw22DNV8PB9mtIQvK_DD2b7K.
12. K. Fischer, A. Kind, and A. Schnieke. Assembling multiple xenoprotective transgenes in pigs. *Xenotransplantation*, 2018: p. e12431.
13. T. Lu, B. Yang, R. Wang, and C. Qin. Xenotransplantation: Current Status in Preclinical Research. *Frontiers in Immunology*, 2019. 10: p. 3060.
14. N. Ryczek, M. Hryhorowicz, J. Zeyland*, et al.* CRISPR/Cas Technology in Pig-to-Human Xenotransplantation Research. *Int J Mol Sci*, 2021. 22(6).
15. R. Oriol, Y. Ye, E. Koren, and D.K. Cooper. Carbohydrate antigens of pig tissues reacting with human natural antibodies as potential targets for hyperacute vascular rejection in pig-to-man organ xenotransplantation. *Transplantation*, 1993. 56(6): p. 1433-42.
16. A.H. Good, D.K. Cooper, A.J. Malcolm*, et al.* Identification of carbohydrate structures that bind human antiporcine antibodies: implications for discordant xenografting in humans. *Transplant Proc*, 1992. 24(2): p. 559-62.
17. T. Kobayashi and D.K. Cooper. Anti-Gal, alpha-Gal epitopes, and xenotransplantation. *Subcell Biochem*, 1999. 32: p. 229-57.
18. U. Galili. Interaction of the natural anti-Gal antibody with alpha-galactosyl epitopes: a major obstacle for xenotransplantation in humans. *Immunol Today*, 1993. 14(10): p. 480-2.
19. U. Galili, S.B. Shohet, E. Kobrin*, et al.* Man, apes, and Old World monkeys differ from other mammals in the expression of alpha-galactosyl epitopes on nucleated cells. *Journal of Biological Chemistry*, 1988. 263(33): p. 17755-62.

20. U. Galili. The alpha-gal epitope and the anti-Gal antibody in xenotransplantation and in cancer immunotherapy. *Immunology & Cell Biology*, 2005. 83(6): p. 674-86.
21. J.L. Platt, R.J. Fischel, A.J. Matas*, et al.* Immunopathology of hyperacute xenograft rejection in a swine-to-primate model. *Transplantation*, 1991. 52(2): p. 214-20.
22. H.J. Schuurman, J. Cheng, and T. Lam. Pathology of xenograft rejection: a commentary. *Xenotransplantation*, 2003. 10(4): p. 293-9.
23. L. Lai, D. Kolber-Simonds, K.W. Park*, et al.* Production of alpha-1,3-galactosyltransferase knockout pigs by nuclear transfer cloning. *Science*, 2002. 295(5557): p. 1089-92.
24. V.T. Dai Y, Boone J, Chen SH, Phelps CJ, Ball S. Targeted disruption of the alpha1,3-galactosyl- transferase gene in cloned pigs. *Nat Biotechnol*, 2002. 21: p. 111–115.
25. Q.H. Chen G, Starzl T, Sun H, Garcia B, Wang X. Acute rejection is associated with antibodies to non-Gal antigens in baboons using Gal-knockout pig kidneys. *Nat Med*, 2005. 11: p. 1295–8.
26. G.W. Byrne, P.G. Stalboerger, E. Davila*, et al.* Proteomic identification of non-Gal antibody targets after pig-to-primate cardiac xenotransplantation. *Xenotransplantation*, 2008. 15(4): p. 268-76.
27. B. Gollackner, S.K. Goh, I. Qawi*, et al.* Acute vascular rejection of xenografts: roles of natural and elicited xenoreactive antibodies in activation of vascular endothelial cells and induction of procoagulant activity. *Transplantation*, 2004. 77(11): p. 1735-41.
28. G. Chen, H. Qian, T. Starzl*, et al.* Acute rejection is associated with antibodies to non-Gal antigens in baboons using Gal-knockout pig kidneys. *Nat Med*, 2005. 11(12): p. 1295-8.
29. D.K. Cooper, B. Ekser, and A.J. Tector. Immunobiological barriers to xenotransplantation. *Int J Surg*, 2015. 23(Pt B): p. 211-6.
30. K.H. Song, Y.J. Kang, U.H. Jin*, et al.* Cloning and functional characterization of pig CMP-N-acetylneuraminic acid hydroxylase for the synthesis of N-glycolylneuraminic acid as the xenoantigenic determinant in pig-human xenotransplantation. *Biochemical Journal*, 2010. 427(1): p. 179-88.

31. G. Byrne, S. Ahmad-Villiers, Z. Du, and C. McGregor. B4GALNT2 and xenotransplantation: A newly appreciated xenogeneic antigen. *Xenotransplantation*, 2018. 25(5): p. e12394.
32. A. Varki. Loss of N-glycolylneuraminic acid in humans: Mechanisms, consequences, and implications for hominid evolution. *Am J Phys Anthropol*, 2001. Suppl 33(Suppl): p. 54-69.
33. D. Bouhours, C. Pourcel, and J.E. Bouhours. Simultaneous expression by porcine aorta endothelial cells of glycosphingolipids bearing the major epitope for human xenoreactive antibodies (Gal alpha 1-3Gal), blood group H determinant and N-glycolylneuraminic acid. *Glycoconj J*, 1996. 13(6): p. 947-53.
34. N.B. Basnet, K. Ide, H. Tahara*, et al.* Deficiency of N-glycolylneuraminic acid and Galalpha1-3Galbeta1-4GlcNAc epitopes in xenogeneic cells attenuates cytotoxicity of human natural antibodies. *Xenotransplantation*, 2010. 17(6): p. 440-8.
35. A. Cadili and N. Kneteman. The role of macrophages in xenograft rejection. *Transplantation Proceedings*, 2008. 40(10): p. 3289-93.
36. M.R. Ganji and B. Broumand. Acute cellular rejection. *Iran J Kidney Dis*, 2007. 1(2): p. 54-6.
37. T. Lu, B. Yang, R. Wang, and C. Qin. Xenotransplantation: Current Status in Preclinical Research. *Front Immunol*, 2019. 10: p. 3060.
38. X. Zhang, Q. Wang, J. Zhao*, et al.* The resurgent landscape of xenotransplantation of pig organs in nonhuman primates. *Sci China Life Sci*, 2021. 64(5): p. 697-708.
39. M.B. Ezzelarab, B. Ekser, A. Azimzadeh*, et al.* Systemic inflammation in xenograft recipients precedes activation of coagulation. *Xenotransplantation*, 2015. 22(1): p. 32-47.
40. C.C. Lin, D. Chen, J.H. McVey*, et al.* Expression of tissue factor and initiation of clotting by human platelets and monocytes after incubation with porcine endothelial cells. *Transplantation*, 2008. 86(5): p. 702-9.
41. M. Gawaz. Role of platelets in coronary thrombosis and reperfusion of ischemic myocardium. *Cardiovasc Res*, 2004. 61(3): p. 498-511.

42. H. Iwase, E.C. Klein, and D.K. Cooper. Physiologic Aspects of Pig Kidney Transplantation in Nonhuman Primates. *Comp Med*, 2018. 68(5): p. 332-340.
43. Z. Ibrahim, J. Busch, M. Awwad*, et al.* Selected physiologic compatibilities and incompatibilities between human and porcine organ systems. *Xenotransplantation*, 2006. 13(6): p. 488-99.
44. J. Denner and N.J. Mueller. Preventing transfer of infectious agents. *Int J Surg*, 2015. 23(Pt B): p. 306-311.
45. M. Hryhorowicz, J. Zeyland, R. Słomski, and D. Lipiński. Genetically Modified Pigs as Organ Donors for Xenotransplantation. *Mol Biotechnol*, 2017. 59(9-10): p. 435-444.
46. N. Kim, J. Choi, S. Kim*, et al.* Transmission of Porcine Endogenous Retrovirus Produced from Different Recipient Cells In Vivo. *PLoS One*, 2016. 11(11): p. e0165156.
47. J. Drost and H. Clevers. Translational applications of adult stem cell-derived organoids. *Development*, 2017. 144(6): p. 968-975.
48. H. Matsunari, H. Nagashima, M. Watanabe*, et al.* Blastocyst complementation generates exogenic pancreas in vivo in apancreatic cloned pigs. *Proc Natl Acad Sci U S A*, 2013. 110(12): p. 4557-62.
49. A. Isotani, H. Hatayama, K. Kaseda*, et al.* Formation of a thymus from rat ES cells in xenogeneic nude mouse<-->rat ES chimeras. *Genes Cells*, 2011. 16(4): p. 397-405.
50. T. Yamaguchi, H. Sato, M. Kato-Itoh*, et al.* Interspecies organogenesis generates autologous functional islets. *Nature*, 2017. 542(7640): p. 191-196.
51. J. Wu, A. Platero-Luengo, M. Sakurai*, et al.* Interspecies Chimerism with Mammalian Pluripotent Stem Cells. *Cell*, 2017. 168(3): p. 473-486 e15.
52. Y. Huang, R. Osorno, A. Tsakiridis, and V. Wilson. In Vivo differentiation potential of epiblast stem cells revealed by chimeric embryo formation. *Cell Rep*, 2012. 2(6): p. 1571-8.
53. V.L. Mascetti and R.A. Pedersen. Human-Mouse Chimerism Validates Human Stem Cell Pluripotency. *Cell Stem Cell*, 2016. 18(1): p. 67-72.

54. M. Tachibana, M. Sparman, C. Ramsey*, et al.* Generation of chimeric rhesus monkeys. *Cell*, 2012. 148(1-2): p. 285-95.
55. J. Nichols and A. Smith. Naive and primed pluripotent states. *Cell Stem Cell*, 2009. 4(6): p. 487-92.
56. H. Kim, J. Wu, S. Ye*, et al.* Modulation of β-catenin function maintains mouse epiblast stem cell and human embryonic stem cell self-renewal. *Nat Commun*, 2013. 4: p. 2403.
57. D. James, S.A. Noggle, T. Swigut, and A.H. Brivanlou. Contribution of human embryonic stem cells to mouse blastocysts. *Dev Biol*, 2006. 295(1): p. 90-102.
58. Y. Takashima, G. Guo, R. Loos*, et al.* Resetting transcription factor control circuitry toward ground-state pluripotency in human. *Cell*, 2014. 158(6): p. 1254-1269.
59. T.W. Theunissen, B.E. Powell, H. Wang*, et al.* Systematic identification of culture conditions for induction and maintenance of naive human pluripotency. *Cell Stem Cell*, 2014. 15(4): p. 471-487.
60. C. Zhong, J. Wu, and J.C. Izpisua Belmonte. Pig Chimeric Model with Human Pluripotent Stem Cells. *Methods Mol Biol*, 2019. 2005: p. 101-124.
61. H. Masaki, M. Kato-Itoh, A. Umino*, et al.* Interspecific in vitro assay for the chimera-forming ability of human pluripotent stem cells. *Development*, 2015. 142(18): p. 3222-30.
62. H. Masaki, M. Kato-Itoh, Y. Takahashi*, et al.* Inhibition of Apoptosis Overcomes Stage-Related Compatibility Barriers to Chimera Formation in Mouse Embryos. *Cell Stem Cell*, 2016. 19(5): p. 587-592.
63. K. Huang, Y. Zhu, Y. Ma*, et al.* BMI1 enables interspecies chimerism with human pluripotent stem cells. *Nat Commun*, 2018. 9(1): p. 4649.
64. M.A. Brehm, W.J. Racki, J. Leif*, et al.* Engraftment of human HSCs in nonirradiated newborn NOD-scid IL2rγ null mice is enhanced by transgenic expression of membrane-bound human SCF. *Blood*, 2012. 119(12): p. 2778-88.
65. S. Takagi, Y. Saito, A. Hijikata*, et al.* Membrane-bound human SCF/KL promotes in vivo human hematopoietic engraftment and myeloid differentiation. *Blood*, 2012. 119(12): p. 2768-77.

66. A. Rongvaux, T. Willinger, H. Takizawa*, et al.* Human thrombopoietin knockin mice efficiently support human hematopoiesis in vivo. *Proc Natl Acad Sci U S A*, 2011. 108(6): p. 2378-83.
67. X. Han, M. Chen, F. Wang*, et al.* Forebrain engraftment by human glial progenitor cells enhances synaptic plasticity and learning in adult mice. *Cell Stem Cell*, 2013. 12(3): p. 342-53.
68. T. Yamaguchi. Hurdles to Generating Human Islets in Animals via Blastocyst Complementation. *Curr Diab Rep*, 2019. 19(8): p. 45.
69. F. Suchy and H. Nakauchi. Interspecies chimeras. *Curr Opin Genet Dev*, 2018. 52: p. 36-41.
70. F. Zeng, M.J. Chen, D.A. Baldwin*, et al.* Multiorgan engraftment and differentiation of human cord blood CD34+ Lin- cells in goats assessed by gene expression profiling. *Proc Natl Acad Sci U S A*, 2006. 103(20): p. 7801-6.
71. F. Zeng, S.Z. Huang, Z.J. Gong*, et al.* Long-term deregulated human hematopoiesis in goats transplanted in utero with BCR-ABL-transduced lin(-)CD34(+) cord blood cells. *Cell Res*, 2013. 23(6): p. 859-62.
72. T. Ito, Y. Sendai, S. Yamazaki*, et al.* Generation of recombination activating gene-1-deficient neonatal piglets: a model of T and B cell deficient severe combined immune deficiency. *PLoS One*, 2014. 9(12): p. e113833.
73. J. Huang, X. Guo, N. Fan*, et al.* RAG1/2 knockout pigs with severe combined immunodeficiency. *J Immunol*, 2014. 193(3): p. 1496-503.
74. R.M. Binns, D.E. McFarlin, and J.R. Sugar. Lymphoid depletion and immunosuppression after thymectomy in the young pig. *Nat New Biol*, 1972. 238(84): p. 181-3.
75. H.C. Hsu, S. Enosawa, T. Yamazaki*, et al.* Enhancing Survival of Human Hepatocytes by Neonatal Thymectomy and Partial Hepatectomy in Micro-miniature Pigs. *Transplant Proc*, 2017. 49(1): p. 153-158.
76. M. Itoh, Y. Mukae, T. Kitsuka*, et al.* Development of an immunodeficient pig model allowing long-term accommodation of artificial human vascular tubes. *Nat Commun*, 2019. 10(1): p. 2244.

77. J.E. Fisher, J.B. Lillegard, T.J. McKenzie*, et al.* In utero transplanted human hepatocytes allow postnatal engraftment of human hepatocytes in pigs. *Liver Transpl*, 2013. 19(3): p. 328-35.
78. S. Lei, J. Ryu, K. Wen*, et al.* Increased and prolonged human norovirus infection in RAG2/IL2RG deficient gnotobiotic pigs with severe combined immunodeficiency. *Sci Rep*, 2016. 6: p. 25222.
79. A.N. Boettcher, Y. Li, A.P. Ahrens*, et al.* Novel Engraftment and T Cell Differentiation of Human Hematopoietic Cells in ART (-/-) IL2RG (-/Y) SCID Pigs. *Front Immunol*, 2020. 11: p. 100.
80. H. Hara, H. Shibata, K. Nakano*, et al.* Production and rearing of germ-free X-SCID pigs. *Exp Anim*, 2018. 67(2): p. 139-146.
81. J.T. Kang, B. Cho, J. Ryu*, et al.* Biallelic modification of IL2RG leads to severe combined immunodeficiency in pigs. *Reprod Biol Endocrinol*, 2016. 14(1): p. 74.
82. C.J. Phelps, C. Koike, T.D. Vaught*, et al.* Production of alpha 1,3-galactosyltransferase-deficient pigs. *Science*, 2003. 299(5605): p. 411-4.
83. K. Kuwaki, Y.L. Tseng, F.J. Dor*, et al.* Heart transplantation in baboons using alpha1,3-galactosyltransferase gene-knockout pigs as donors: initial experience. *Nat Med*, 2005. 11(1): p. 29-31.
84. K. Yamada, K. Yazawa, A. Shimizu*, et al.* Marked prolongation of porcine renal xenograft survival in baboons through the use of alpha1,3-galactosyltransferase gene-knockout donors and the cotransplantation of vascularized thymic tissue. *Nat Med*, 2005. 11(1): p. 32-4.
85. P. Li, J.L. Estrada, C. Burlak*, et al.* Efficient generation of genetically distinct pigs in a single pregnancy using multiplexed single-guide RNA and carbohydrate selection. *Xenotransplantation*, 2015. 22(1): p. 20-31.
86. A.J. Lutz, P. Li, J.L. Estrada*, et al.* Double knockout pigs deficient in N-glycolylneuraminic acid and galactose α-1,3-galactose reduce the humoral barrier to xenotransplantation. *Xenotransplantation*, 2013. 20(1): p. 27-35.
87. A.B. Adams, S.C. Kim, G.R. Martens*, et al.* Xenoantigen Deletion and Chemical Immunosuppression Can Prolong Renal Xenograft Survival. *Ann Surg*, 2018. 268(4): p. 564-573.

88. G.R. Martens, L.M. Reyes, P. Li*, et al.* Humoral Reactivity of Renal Transplant-Waitlisted Patients to Cells From GGTA1/CMAH/B4GalNT2, and SLA Class I Knockout Pigs. *Transplantation*, 2017. 101(4): p. e86-e92.
89. Z.Y. Wang, G.R. Martens, R.L. Blankenship*, et al.* Eliminating Xenoantigen Expression on Swine RBC. *Transplantation*, 2017. 101(3): p. 517-523.
90. J.R. Butler, L.L. Paris, R.L. Blankenship*, et al.* Silencing Porcine CMAH and GGTA1 Genes Significantly Reduces Xenogeneic Consumption of Human Platelets by Porcine Livers. *Transplantation*, 2016. 100(3): p. 571-6.
91. D.K.C. Cooper, R. Gaston, D. Eckhoff*, et al.* Xenotransplantation-the current status and prospects. *Br Med Bull*, 2018. 125(1): p. 5-14.
92. J.L. Estrada, G. Martens, P. Li*, et al.* Evaluation of human and non-human primate antibody binding to pig cells lacking GGTA1/CMAH/β4GalNT2 genes. *Xenotransplantation*, 2015. 22(3): p. 194-202.
93. Q. Li, S. Shaikh, H. Iwase*, et al.* Carbohydrate antigen expression and anti-pig antibodies in New World capuchin monkeys: Relevance to studies of xenotransplantation. *Xenotransplantation*, 2019. 26(3): p. e12498.
94. D.J. White, T. Oglesby, M.K. Liszewski*, et al.* Expression of human decay accelerating factor or membrane cofactor protein genes on mouse cells inhibits lysis by human complement. *Transplant Proc*, 1992. 24(2): p. 474-6.
95. A.P. Dalmasso, G.M. Vercellotti, J.L. Platt, and F.H. Bach. Inhibition of complement-mediated endothelial cell cytotoxicity by decay-accelerating factor. Potential for prevention of xenograft hyperacute rejection. *Transplantation*, 1991. 52(3): p. 530-3.
96. H. Zhou, H. Hara, and D.K.C. Cooper. The complex functioning of the complement system in xenotransplantation. *Xenotransplantation*, 2019. 26(4): p. e12517.
97. L. Burdorf, T. Stoddard, T. Zhang*, et al.* Expression of human CD46 modulates inflammation associated with GalTKO lung xenograft injury. *Am J Transplant*, 2014. 14(5): p. 1084-95.
98. C.Y. Zhou, E. McInnes, L. Copeman*, et al.* Transgenic pigs expressing human CD59, in combination with human membrane cofactor protein and

human decay-accelerating factor. *Xenotransplantation*, 2005. 12(2): p. 142-8.

99. J. Huang, D. Gou, C. Zhen*, et al.* Protection of xenogeneic cells from human complement-mediated lysis by the expression of human DAF, CD59 and MCP. *FEMS Immunol Med Microbiol*, 2001. 31(3): p. 203-9.
100. C.G. McGregor, D. Ricci, N. Miyagi*, et al.* Human CD55 expression blocks hyperacute rejection and restricts complement activation in Gal knockout cardiac xenografts. *Transplantation*, 2012. 93(7): p. 686-92.
101. A.M. Azimzadeh, S.S. Kelishadi, M.B. Ezzelarab*, et al.* Early graft failure of GalTKO pig organs in baboons is reduced by expression of a human complement pathway-regulatory protein. *Xenotransplantation*, 2015. 22(4): p. 310-6.
102. A. Shimizu, K. Yamada, S. Yamamoto*, et al.* Thrombotic microangiopathic glomerulopathy in human decay accelerating factor-transgenic swine-to-baboon kidney xenografts. *J Am Soc Nephrol*, 2005. 16(9): p. 2732-45.
103. H. Iwase, H. Hara, M. Ezzelarab*, et al.* Immunological and physiological observations in baboons with life-supporting genetically engineered pig kidney grafts. *Xenotransplantation*, 2017. 24(2): p. 10.1111/xen.12293.
104. M.M. Mohiuddin, A.K. Singh, P.C. Corcoran*, et al.* Genetically engineered pigs and target-specific immunomodulation provide significant graft survival and hope for clinical cardiac xenotransplantation. *Journal of Thoracic & Cardiovascular Surgery*, 2014. 148(3): p. 1106-13; discussion 1113-4.
105. K. Ide, H. Wang, H. Tahara*, et al.* Role for CD47-SIRPalpha signaling in xenograft rejection by macrophages. *Proc Natl Acad Sci U S A*, 2007. 104(12): p. 5062-6.
106. A.A. Tena, D.H. Sachs, C. Mallard*, et al.* Prolonged Survival of Pig Skin on Baboons After Administration of Pig Cells Expressing Human CD47. *Transplantation*, 2017. 101(2): p. 316-321.
107. Y. Wang, H.Q. Yang, W. Jiang*, et al.* Transgenic expression of human cytoxic T-lymphocyte associated antigen4-immunoglobulin (hCTLA4Ig) by porcine skin for xenogeneic skin grafting. *Transgenic Res*, 2015. 24(2): p. 199-211.

108. M.G. Levisetti, P.A. Padrid, G.L. Szot*, et al.* Immunosuppressive effects of human CTLA4Ig in a non-human primate model of allogeneic pancreatic islet transplantation. *J Immunol*, 1997. 159(11): p. 5187-91.
109. H. Hara, W. Witt, T. Crossley*, et al.* Human dominant-negative class II transactivator transgenic pigs - effect on the human anti-pig T-cell immune response and immune status. *Immunology*, 2013. 140(1): p. 39-46.
110. L.M. Reyes, J.L. Estrada, Z.Y. Wang*, et al.* Creating class I MHC-null pigs using guide RNA and the Cas9 endonuclease. *J Immunol*, 2014. 193(11): p. 5751-7.
111. B.C. Baumann, P. Forte, R.J. Hawley*, et al.* Lack of galactose-alpha-1,3-galactose expression on porcine endothelial cells prevents complement-induced lysis but not direct xenogeneic NK cytotoxicity. *J Immunol*, 2004. 172(10): p. 6460-7.
112. Y. Miwa, K. Yamamoto, A. Onishi*, et al.* Potential value of human thrombomodulin and DAF expression for coagulation control in pig-to-human xenotransplantation. *Xenotransplantation*, 2010. 17(1): p. 26-37.
113. H. Iwase, B. Ekser, H. Hara*, et al.* Regulation of human platelet aggregation by genetically modified pig endothelial cells and thrombin inhibition. *Xenotransplantation*, 2014. 21(1): p. 72-83.
114. D.G. Wheeler, M.E. Joseph, S.D. Mahamud*, et al.* Transgenic swine: expression of human CD39 protects against myocardial injury. *J Mol Cell Cardiol*, 2012. 52(5): p. 958-61.
115. M.M. Mohiuddin, A.K. Singh, P.C. Corcoran, and M.L. Thomas. Chimeric 2C10R4 anti-CD40 antibody therapy is critical for long-term survival of GTKO.hCD46.hTBM pig-to-primate cardiac xenograft. *Nat Commun*, 2016. 7: p. 11138.
116. A.K. Singh, J.L. Chan, L. DiChiacchio*, et al.* Cardiac xenografts show reduced survival in the absence of transgenic human thrombomodulin expression in donor pigs. *Xenotransplantation*, 2019. 26(2): p. e12465.
117. E. Cantu, K.R. Balsara, B. Li*, et al.* Prolonged function of macrophage, von Willebrand factor-deficient porcine pulmonary xenografts. *Am J Transplant*, 2007. 7(1): p. 66-75.

118. L.L. Paris, R.K. Chihara, L.M. Reyes, *et al.* ASGR1 expressed by porcine enriched liver sinusoidal endothelial cells mediates human platelet phagocytosis in vitro. *Xenotransplantation*, 2011. 18(4): p. 245-51.
119. C.L. Lau, E. Cantu, 3rd, G.V. Gonzalez-Stawinski, *et al.* The role of antibodies and von Willebrand factor in discordant pulmonary xenotransplantation. *Am J Transplant*, 2003. 3(9): p. 1065-75.
120. L.L. Paris, J.L. Estrada, P. Li, *et al.* Reduced human platelet uptake by pig livers deficient in the asialoglycoprotein receptor 1 protein. *Xenotransplantation*, 2015. 22(3): p. 203-10.
121. K. Fischer, S. Kraner-Scheiber, B. Petersen, *et al.* Efficient production of multi-modified pigs for xenotransplantation by 'combineering', gene stacking and gene editing. *Sci Rep*, 2016. 6: p. 29081.
122. A. Hinrichs, E.O. Riedel, N. Klymiuk, *et al.* Growth hormone receptor knockout to reduce the size of donor pigs for preclinical xenotransplantation studies. *Xenotransplantation*, 2021. 28(2): p. e12664.
123. Food and Drug Administration. PHS Guideline on Infectious Disease Issues in Xenotransplantation. 2019 [cited 28 May 2022]. Available from: https://www.fda.gov/media/73803/download.
124. A. Regalado. The gene-edited pig heart given to a dying patient was infected with a pig virus. MIT Technology Review. 2022 [cited 28 May 2022 2022]. Available from: https://www.technologyreview.com/2022/05/04/1051725/xenotransplant-patient-died-received-heart-infected-with-pig-virus/.
125. D. Niu, H.J. Wei, L. Lin, *et al.* Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9. *Science*, 2017. 357(6357): p. 1303-1307.
126. J. Denner, V. Specke, U. Thiesen, *et al.* Genetic alterations of the long terminal repeat of an ecotropic porcine endogenous retrovirus during passage in human cells. *Virology*, 2003. 314(1): p. 125-33.
127. V.A. Morozov, S. Wynyard, S. Matsumoto, *et al.* No PERV transmission during a clinical trial of pig islet cell transplantation. *Virus Res*, 2017. 227: p. 34-40.

128. C.G.A. McGregor, Y. Takeuchi, L. Scobie, and G. Byrne. PERVading strategies and infectious risk for clinical xenotransplantation. *Xenotransplantation*, 2018. 25(4): p. e12402.
129. T. Argaw, W. Colon-Moran, and C. Wilson. Susceptibility of porcine endogenous retrovirus to anti-retroviral inhibitors. *Xenotransplantation*, 2016. 23(2): p. 151-8.
130. Y. Yue, W. Xu, Y. Kan*, et al.* Extensive germline genome engineering in pigs. *Nat Biomed Eng*, 2021. 5(2): p. 134-143.
131. D.K. Cooper, V. Satyananda, B. Ekser*, et al.* Progress in pig-to-non-human primate transplantation models (1998-2013): a comprehensive review of the literature. *Xenotransplantation*, 2014. 21(5): p. 397-419.
132. L. Higginbotham, D. Mathews, C.A. Breeden*, et al.* Pre-transplant antibody screening and anti-CD154 costimulation blockade promote long-term xenograft survival in a pig-to-primate kidney transplant model. *Xenotransplantation*, 2015. 22(3): p. 221-30.
133. S.C. Kim, D.V. Mathews, C.P. Breeden*, et al.* Long-term survival of pig-to-rhesus macaque renal xenografts is dependent on CD4 T cell depletion. *Am J Transplant*, 2019. 19(8): p. 2174-2185.
134. D.K.C. Cooper. Genetically engineered pig kidney transplantation in a brain-dead human subject. *Xenotransplantation*, 2021. 28(6): p. e12718.
135. P.M. Porrett, B.J. Orandi, V. Kumar*, et al.* First clinical-grade porcine kidney xenotransplant using a human decedent model. *Am J Transplant*, 2022. 22(4): p. 1037-1053.
136. G.W. Byrne, Z. Du, Z. Sun*, et al.* Changes in cardiac gene expression after pig-to-primate orthotopic xenotransplantation. *Xenotransplantation*, 2011. 18(1): p. 14-27.
137. M. Längin, T. Mayr, B. Reichart*, et al.* Consistent success in life-supporting porcine cardiac xenotransplantation. *Nature*, 2018. 564(7736): p. 430-433.
138. First pig-to-human heart. *Nat Biotechnol*, 2022. 40(2): p. 145.
139. B. Ekser, C.C. Lin, C. Long*, et al.* Potential factors influencing the development of thrombocytopenia and consumptive coagulopathy after

genetically modified pig liver xenotransplantation. *Transpl Int*, 2012. 25(8): p. 882-96.

140. K. Kim, C. Schuetz, N. Elias*, et al.* Up to 9-day survival and control of thrombocytopenia following alpha1,3-galactosyl transferase knockout swine liver xenotransplantation in baboons. *Xenotransplantation*, 2012. 19(4): p. 256-64.
141. J.A. Shah, M.S. Patel, N. Elias*, et al.* Prolonged Survival Following Pig-to-Primate Liver Xenotransplantation Utilizing Exogenous Coagulation Factors and Costimulation Blockade. *Am J Transplant*, 2017. 17(8): p. 2178-2185.
142. L. Burdorf, A.M. Azimzadeh, and R.N. Pierson, 3rd. Progress and challenges in lung xenotransplantation: an update. *Curr Opin Organ Transplant*, 2018. 23(6): p. 621-627.
143. H. Watanabe, Y. Ariyoshi, T. Pomposelli*, et al.* Intra-bone bone marrow transplantation from hCD47 transgenic pigs to baboons prolongs chimerism to >60 days and promotes increased porcine lung transplant survival. *Xenotransplantation*, 2020. 27(1): p. e12552.
144. D.K. Cooper. Ethical aspects of xenotransplantation of current importance. *Xenotransplantation*, 1996. 3: p. 264-274.
145. C. Smetanka and D.K. Cooper. The ethics debate in relation to xenotransplantation. *Rev Sci Tech*, 2005. 24(1): p. 335-42.
146. B.E. Rollin. Ethical and Societal Issues Occasioned by Xenotransplantation. *Animals (Basel)*, 2020. 10(9): p. 1695.
147. P.J. Morris and A.P. Monaco. Ethical issues and xenotransplantation. *Transplantation*, 2004. 78(8): p. 1099-100.
148. R. Cortesini. Ethical aspects in xenotransplantation. *Transplant Proc*, 1998. 30(5): p. 2463-4.
149. J.W. Entwistle, R.M. Sade, and D.H. Drake. Clinical xenotransplantation seems close: Ethical issues persist. *Artif Organs*, 2022. 46(6): p. 987-994.
150. A.J. Klotzko. Dolly, cloning, and the public misunderstanding of science: a challenge for us all. *Camb Q Healthc Ethics*, 1998. 7(2): p. 115-6.
151. B.R. Sharma. Cloning controversies: an overview of the science, ethics and politics. *Med Sci Law*, 2005. 45(1): p. 17-26.

152. J. Harris. "Goodbye Dolly?" The ethics of human cloning. *J Med Ethics*, 1997. 23(6): p. 353-60.
153. A. Rizzieri. Stem cell research on embryonic persons is just. *J Bioeth Inq*, 2012. 9(2): p. 195-203.
154. N. Manzar, B. Manzar, N. Hussain*, et al.* The ethical dilemma of embryonic stem cell research. *Sci Eng Ethics*, 2013. 19(1): p. 97-106.
155. K. Hug. Therapeutic perspectives of human embryonic stem cell research versus the moral status of a human embryo--does one have to be compromised for the other? *Medicina (Kaunas)*, 2006. 42(2): p. 107-14.
156. A.S. Bawa and K.R. Anilakumar. Genetically modified foods: safety, risks and public concerns-a review. *J Food Sci Technol*, 2013. 50(6): p. 1035-46.
157. A. Greenfield. Editing mammalian genomes: ethical considerations. *Mamm Genome*, 2017. 28(7-8): p. 388-393.
158. P.M. Sharp and B.H. Hahn. Origins of HIV and the AIDS pandemic. *Cold Spring Harb Perspect Med*, 2011. 1(1): p. a006841.
159. C. Santana. COVID-19, other zoonotic diseases and wildlife conservation. *Hist Philos Life Sci*, 2020. 42(4): p. 45.
160. L.R. Dragstedt. Ethical considerations in the use and care of laboratory animals. *J Med Educ*, 1960. 35: p. 1-3.
161. C.W. Hume. The ethics of experiments on animals. *Nature*, 1951. 167(4241): p. 213-5.
162. W. Lane-Petter. Animal welfare--principles and problems. *Med Sci Law*, 1967. 7(4): p. 167-76.
163. C.G. Mole and M. Heyns. Animal Models in Forensic Science Research: Justified Use or Ethical Exploitation? *Sci Eng Ethics*, 2019. 25(4): p. 1095-1110.
164. R.L. Walker. Virtue Ethics and Laboratory Animal Research. *Ilar j*, 2021. 60(3): p. 415-423.
165. M.L. Andersen and L.M.F. Winter. Animal models in biological and biomedical research - experimental and ethical concerns. *An Acad Bras Cienc*, 2019. 91(suppl 1): p. e20170238.
166. R. Deitch. Government looks for guidance through Warnock minefield. *Lancet*, 1984. 2(8411): p. 1107-8.

167. S. Gorovitz. Will we still be 'human' if we have engineered genes and animal organs? Washington Post. 1984 [cited 12 October 2022]. Available from: https://www.washingtonpost.com/archive/opinions/1984/12/09/will-we-still-be-human-if-we-have-engineered-genes-and-animal-organs/06084cf5-32ed-4674-8c10-c072d351f14e/.
168. L. Birke and M. Michael. The heart of the matter: animal bodies, ethics, and species boundaries. *Soc Anim*, 1998. 6(3): p. 245-61.
169. D. Hubner. Human-Animal Chimeras and Hybrids: An Ethical Paradox behind Moral Confusion? *J Med Philos*, 2018. 43(2): p. 187-210.
170. J.S. Robert and F. Baylis. Crossing species boundaries. *Am J Bioeth*, 2003. 3(3): p. 1-13.
171. J. Rossi. Toward a zoocentric animal ethics. *Am J Bioeth*, 2008. 8(6): p. 50-2.
172. M. Bilger. Scientists Create Human-Pig Hybrids for Organ Transplants That Could Develop Into "Monsters". Lifenews.com. 2017 [cited 30 May 2022]. Available from: https://www.lifenews.com/2017/01/27/scientists-create-human-pig-hybrids-for-organ-transplants-that-could-develop-into-monsters/.
173. N. McDermott. FRANKENSWINE Scientists make PIG HUMAN babies by splicing stem cells in amazing new trial. The Sun. 2017 [cited 30 May 2022]. Available from: https://www.thesun.co.uk/news/2713619/scientists-make-pig-human-babies-by-splicing-stem-cells-in-amazing-new-trial/.
174. B. Brock. The uneasy truth about human-animal hybrids. BBC. 2017 [cited 30 May 2022]. Available from: https://www.bbc.com/future/article/20170222-the-uneasy-truth-about-human-animal-hybrids.
175. R. Isasi, M. Ginoza, K. Jongsma*, et al.* Mending the gaps: ethically sensitive cells and the evolution of European stem cell policy. *Regenerative Medicine*, 2022. 17(8): p. 581-595.
176. E. Callaway. CRISPR plants now subject to tough GM laws in European Union. *Nature*, 2018. 560(7716): p. 16.

177. H.D. Jones. Regulatory uncertainty over genome editing. *Nat Plants*, 2015. 1: p. 14011.
178. E.T. Bloom. National policies for xenotransplantation in the USA. *Xenotransplantation*, 2007. 14(4): p. 345-6.
179. W.J. Hawthorne and P.J. Cowan. Xenotransplantation in Australia: Development of the regulatory process. *Xenotransplantation*, 2020. 27(3): p. e12603.
180. I. Kwon, C.G. Park, and S. Lee. Regulatory aspects of xenotransplantation in Korea. *Xenotransplantation*, 2020. 27(3): p. e12602.
181. Y. Liu, L. Qin, R. Tong*, et al.* Regulatory changes in China on xenotransplantation and related products. *Xenotransplantation*, 2020. 27(3): p. e12601.
182. M. Tsuda, K.N. Watanabe, and R. Ohsawa. Regulatory Status of Genome-Edited Organisms Under the Japanese Cartagena Act. *Front Bioeng Biotechnol*, 2019. 7: p. 387.
183. M.K. Kim, H.J. Choi, I. Kwon*, et al.* The International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of xenocorneal transplantation. *Xenotransplantation*, 2014. 21(5): p. 420-30.
184. World Health Organization. First WHO global consultation on regulatory requirements for xenotransplantation clinical trials, Changsha, China, 19-21 November 2008. World Health Organization. 2008 [cited 25 October 2022]. Available from: https://apps.who.int/iris/handle/10665/341812.
185. World Health Organization. Second WHO global consultation on regulatory requirements for xenotransplantation clinical trials, Geneva, Switzerland, 17-19 October 2011. World Health Organization. 2011 [cited 25 October 2022]. Available from: https://apps.who.int/iris/handle/10665/341817.
186. D.K.C. Cooper, R.N. Pierson, B.J. Hering*, et al.* Regulation of Clinical Xenotransplantation-Time for a Reappraisal. *Transplantation*, 2017. 101(8): p. 1766-1769.

187. E. Cozzi, S. Schneeberger, M.I. Bellini*, et al.* Organ transplants of the future: planning for innovations including xenotransplantation. *Transpl Int*, 2021. 34(11): p. 2006-2018.
188. B. Reichart, M. Längin, J. Denner*, et al.* Pathways to Clinical Cardiac Xenotransplantation. *Transplantation*, 2021. 105(9): p. 1930-1943.
189. P. Marks and S. Solomon. Clarifying US regulations on xenotransplantation. *Nat Biotechnol*, 2021. 39(12): p. 1500-1501.
190. W.J. Hawthorne, P.J. Cowan, L. Buhler, and E. Wolf. International standards and guidelines for xenotransplantation. *Nat Biotechnol*, 2021. 39(12): p. 1501-1502.
191. F. Braun and A. Rahmel. Amendments to the Transplantation Act and impact on the donor situation in Germany. *Chirurg*, 2020. 91(11): p. 905-912.
192. Gesetz über die Spende, Entnahme und Übertragung von Organen und Geweben (Transplantationsgesetz - TPG). 1997 [cited 18 September 2022 2022]. Available from: https://www.gesetze-im-internet.de/tpg/.

## Acknowledgements

I would to take the opportunity to thank everyone who has made this thesis possible.

First and foremost, my sincere gratitude goes to my supervisors, Thomas Scheper and Thomas Reydon, who have patiently stuck with me throughout the years. Thank you for your support, your flexibility and most of all your trust in me, in particular regarding the evolution of my ideas.

Second, a huge thank you to my fantastic co-authors of the systematic review, Dietmar Hübner and Lucie White. Your willingness to explore something new with me has meant so much! I will never forget our hour-long discussions and the fun we had in developing and applying our framework.

Third, thank you to my co-authors regarding the regulatory challenges, Nils Hoppe and Tobias Cantz. We achieved something great.

Last, I would like to express my deepest gratitude to my husband, Sebastian Kwisda. This thesis would not have been completed without your patient support through all those years.

**Thank you!**

# Curriculum Vitae

**Name:** Koko Reyna Kwisda (née Wurmboeck)
**Nationality:** Austrian

## EDUCATION

| | |
|---|---|
| 04.2014 – 09.2023 | **Ph.D. in Technical Chemistry (Dr. rer. nat),** Leibniz University Hannover<br>**Topic:** Ethical and Regulatory Aspects Regarding Xenotransplantation<br>Graduated **summa cum laude** |
| 09.2003 – 04.2012 | **Diplom Studies in Philosophy (Mag. phil.),** University of Vienna<br>Graduated **summa cum laude**<br>**Thesis (Grade A):** "The Embryo as Scientific Object and as Baby: Ethical Problems Caused by Pre-Implantation Diagnosis and Human Embryonic Stem Cell Research" |
| 09.2001 – 06.2012 | **Diplom Studies Molecular Biology (Mag. rer. nat.),** University of Vienna<br>Graduated cum laude<br>**Thesis (Grade A):** "Method Development for the Molecular Biological Detection of Food Pathogens" |
| 09.1993 – 06.2001 | **Classical Gymnasium Gymnasiumstraße,** Vienna<br>Specialisation in Biology, Latin, Ancient Greek, Mathematics, German & English |

## EMPLOYMENT

| | |
|---|---|
| 04.2022 – current | **Takeda Switzerland,** Opfikon<br>New Product Planning Lead |
| 09.2020 – 03.2022 | **Takeda Switzerland**<br>Medical Advisor Rare Hematology & Oncology |
| 08.2017 – 08.2020 | **Shire Switzerland,** Zug<br>Senior Account Lead Hematology |
| 10.2012 – 12.2016 | **Centre for Ethics and Law in the Life Sciences (CELLS),** Hannover<br>Research Fellow in Bioethics |
| 05.2004 – 12.2011 | **"Charming Penguins" Event Service Ges.b.R.,** Vienna<br>Co-Founder and Co-Director |
| 12.2009 – 01.2011 | **Christian-Doppler Laboratory for Molecular Biological Food Analysis**<br>Vienna, Research Assistant |
| 05.2007 – 08.2007 | **Yale University School of Medicine,** New Haven<br>Lab Rotation |

## Complete List of Publications

- Hoppe N, Kwisda K. (2014): "Gewebeforschung" in Lenk, C., Duttge, G., Fangerau, H. (eds.) *Handbuch Ethik und Recht der Forschung am Menschen*. Berlin/Heidelberg: Springer, 43-46.
- Hoppe N, Kwisda K. (2014): "Bioethikkonvention des Europarates " in Lenk, C., Duttge, G., Fangerau, H. (eds.) *Handbuch Ethik und Recht der Forschung am Menschen*. Berlin/Heidelberg: Springer, 501-506.
- Hoppe N, Kwisda K. (2014): "Geistiges Eigentum" in Lenk, C., Duttge, G., Fangerau, H. (eds.) *Handbuch Ethik und Recht der Forschung am Menschen*. Berlin/Heidelberg: Springer, 159-162.
- Kwisda K, White L, Hübner D. (2020) Ethical arguments concerning human-animal chimera research: a systematic review. *BMC Medical Ethics* 2020; 21(1):24.
- Kwisda K, Cantz T, Hoppe N. Regulatory and intellectual property conundrums surrounding xenotransplantation. *Nature Biotechnology* 2021; 39(7):796-798.
- Kwisda K, Cantz T, Hoppe N. Reply to 'International standards and guidelines for xenotransplantation' and 'Clarifying US regulations on xenotransplantation'. *Nature Biotechnology* 2021; 39:1503.
- Kwisda K. Unaddressed Regulatory Issues in Xenotransplantation: a Fictional Example. *Frontiers in Transplantation* 2023; 2:1222031.