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Xenotransplantation - How Bad Science and Big Business Put the World at Risk from Viral Pandemics

ISIS Sustainable Science Audit #2
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Summary

Xenotransplantation -- the transplant of animal organs into human beings -- is a multi-billion dollar business venture built on the anticipated sale of patented techniques and organs, as well as drugs to overcome organ-rejection [1]. It has received strong criticism and opposition from scientists warning of the risks of new viruses crossing from animal organs to human subjects and from there to infect the population at large. But regulators are adopting a permissive attitude for clinical trials to go ahead. Scientific reports of virus crossing from pig to human cells [2] and of viral infections in humans subjects transplanted with baboon livers [3] are being ignored or dismissed, while inconclusive, widely faulted papers are taken as evidence that no viruses are found in xenotransplant patients [4]. This audit exposes the shoddy science that puts the world at risk of viral pandemics for the sake of corporate profit, and concludes that xenotranplantation should not be allowed to continue in any form. Instead, effort should be devoted to developing safer, more sustainable and affordable alternatives that are already showing promise and will be more likely to benefit society as a whole in the industrialized west as well as in the Third World.

Key words: transgenic pigs, hyperacute rejection, xenozoonosis, endogenous retroviruses, xenotropism, viral infections, recombinant viruses, PERV, pig HEV, baboon endogenous virus, simian foamy virus.

A multi-billion dollar business venture

Xenotransplantation -- the transplant of organs or tissues between species -- has become a major issue within the past ten years. Biotech companies are developing genetically engineered ‘humanized’ pigs to meet the demand for spare body parts in the industrialized world. A multi-billion dollar market is anticipated from the sale of patented techniques and organs, as well as existing and new drugs to overcome organ-rejection [1]. Still at the experimental phase, it has received strong criticism and opposition from mainstream scientists warning of the risks of new viruses crossing from animal donor organs to human subjects, and from there to infect the population at large. But these warnings have done little to dampen the enthusiasm for continued research well into clinical trials.

The world-leader in xenotransplant research is the UK biotech company Imutran based in Cambridge, now a subsidiary of Novartis. Novartis already owns the rights to Cyclosporine A, the main anti-rejection drug given to transplant patients to suppress the immune system. Since acquiring Imutran, Novartis have pledged $1 billion for research in xenotransplantation, and thereby to dominate a projected $11 billion a year market for organs and associated immune-suppressive drugs.

An estimated 10 000 pigs and nearly five hundred primates have been in the UK, with very little accomplished. Xenotransplantation is in crisis. At the bottom of the crisis lies some shoddy science that puts the world at risk of viral pandemics for the sake of profit. At least one company, PPL, which produced Dolly the cloned sheep, is reported to be winding up xenotransplantation research, on the possibility that pig virus could infect humans [5].

‘Humanized’ transgenic pigs as organ donors

Transgenic pigs, rather than our close relatives primates, were considered as organ donors because there are greater ethical objections to using primates, many of which are endangered protected species [6]. As pigs are already farmed for food, it was thought that there would be less ethical concern, and that pigs could also be more easily controlled for viral infections and consistent quality. Nevertheless, large numbers of primates are exploited and made to suffer as experimental transplant recipients; and primate to human transplant clinical trials have been authorized in the United States.

The first hurdle in transplanting organs between distant species, as in the case of pig to human, is hyperacute rejection (HAR) of the donor organ by the host. This reaction is swift and severe, and depends on naturally occurring, pre-existing antibodies. Naturally occurring human anti-pig antibodies predominantly recognize the carbohydrate antigen galactose-alpha-(1,3)-galactose attached to cell-surface proteins. Both IgM and IgG (different classes of immunoglobulins or antibodies) in the human blood contain antibodies that bind this antigen; which may comprise up to 1% of the IgG. The enzyme for making the galactose-alpha-(1,3)-galactose exists in all mammals except humans, old world monkeys and the great apes. The binding of these antibodies to the antigens triggers a cascade of reactions -- complement activation -- that results in destruction of the donor organ and cells within minutes. Induced antibodies against the foreign graft, xenograft, are responsible for organ rejection in the longer term.

There are three possible ways to block HAR: by depleting the pre-existing antibodies, by reducing antigen expression in the donor cells, and by inhibiting complement activation. Of these, the last option appeared to be the only clinically viable strategy in combating HAR. One of the five candidate proteins that proved most promising is the decay accelerating factor (DAF), which blocks an early step in complement activation. Transgenic pigs containing hDAF were therefore produced.

Lack of documentation and molecular characterization of the transgenic pigs

The first and only report in the scientific literature on the experiment creating the transgenic pigs with hDAF was a note [7] less than one and a half pages long, published in *Transplantation Proceedings* in 1994. It contained no molecular genetic documentation of the construct such as genetic map to indicate whether unknown sequences are present, or the promoter-enhancer sequences used. It did not state how the ‘minigene’ construct was introduced, whether by itself, or spliced into a vector. Later papers [6, 8, 9, 10] up to year 2000, all referred back to the same experiment with no further elaboration.

About 2500 fertilized eggs were injected with the minigene. Of 85 surrogate mothers implanted with embryos, 49 delivered litters with 311 piglets, 49 of which were transgenic, ie, contained human DNA, with one to 30 copies of the gene. Only 33 expressed the gene for hDAF, however. The rate of success is thus no better than 1.3%.

There was considerable variability in expression of hDAF in the transgenic animals, not only between animals, but also between organs from the same animal [6]. Liver expression was found in 90% of transgenic pigs, and expression in the heart was the least frequently detected (18/30). But high expression did not guarantee expression in endothelial cells (cells on the surfaces of the organ). No correlation was found between the number of copies of the gene integrated and hDAF expression. Animals with the highest gene copy number (13 copies) expressed very low levels of hDAF in all transplantable tissues. The two most promising lines incorporated between 6-8copies of the gene and expressed hDAF on parenchyma (inside) and endothelium (surfaces) of all the transplantable organs. In 75% of the organs, gene expression levels of hDAF was greater than in equivalent human tissues.

These results underscore the unpredictable, uncontrollable nature of the transgenic process and the low rate of success. There were no attempts to characterise the transgenic inserts, nor to create stable transgenic lines *before* transplant experiments were carried out; thus compromising not only the reproducibility of the experimental findings, but also the safety of the procedure, particularly with regard to the stability of the transgenic inserts (see Box 1) and the potential for creating new viruses (see Box 3). Plans were made [8, 9] to use whole yeast artificial chromosomes (YACs) containing large segments of the human genome to optimize gene expression, but it is not clear if such procedures have been carried out. Introducing YACs may mean that uncharacterized human genome DNA, including endogenous human viruses (see Box 3), will be transferred into the transgenic pigs, which could increase the potential for generating new recombinant viruses (see Box 3).

The 1997 review [6] admitted that production of an ideal hDAF expressing pig was not complete, and that all organs used for xenotransplantation were derived from heterozygous pigs, ie, pigs having hDAF gene(s) on one of a pair of chromosomes. The review did not state whether these were zero-generation transgenic pigs, or transgenic pigs from later generations bred from the original. The ideal pig, according to the authors, would express high levels of hDAF on organs and cells lining the organs, and would be bred to homozygosity, ie, having hDAF genes on both of a pair of chromosomes. That means the transgenic pigs would ‘breed true’. Only one line was reported to fulfill these criteria in 1997. But the authors pointed out that "breeding to homozygosity might cause undesirable effects on the stability and health of the pig". This conceals a major technical problem with creating transgenic lines. Transgenic organisms, plants as well as animals, are genetically unstable and do not breed true (see Box 1).

Intended xenotransplant recipient animals were not screened for viruses and postmortems of transplant recipients did not include examination for viral infections

In the first experiment, eight hearts from transgenic pigs were transplanted into non-immune suppressed cynomolgus monkeys (*Macaca fascicularis*) of unspecified origins, and without pre-screening for viral infections or endogenous viruses. The median survival was 5.1 days (97-126h) with no reported hyperacute rejection. Five of the ten controls that received control hearts survived a surprising mean of 86.4h, while the other five survived for only 2.6h, as typical of HAR.

Box 1

Transgenesis is unpredictable and uncontrollable, and trangenes are unstable

The instability of transgenic plants is well-known and actively researched. Transgenic constructs typically integrate at random into the host genome, and in a scrambled configuration, consisting of repeats, rearrangements and deletions [11]. There is no reason to expect transgenic animals to be different. Indeed, integration of transgenic construct was reported to be random in the transgenic pigs, and the expression of the transgene depended on the site of integration [8].

Transgene integration was examined by fluorescence *in situ* hybridization (FISH) [12], a technique that enables the inserts to be seen on chromosomes. Routine 'slot blot' analysis of total transgenic pig DNA was done first to identify pigs with hDAF DNA. According to the strength of the signal, one line, E14, was estimated to contain 40 copies of the hDAF transgene; while another, A74, contained 6 copies, and a third, C50, two copies. These were referred to as "heterozygous founders lines". Again, it is not stated how many generations were bred after transgenesis. And it is not clear why the "lines" are heterozygous and not homozygous.

In the cross E14 x A74, one from a litter of 13, and another from a litter of four were the only piglets that showed a signal similar to that of one parent. In the cross C50 x A74, three out of 9, and 4 out of 11, respectively showed signals that were similar to those of the parents. But when analyzed by FISH, only 4 piglets were actually found to have inherited any transgenes from their parents. Thus, the actual transmission of transgenes is 4/37 or 10.8%. This is much lower than the 75% predicted (assuming both parents were heterozygous), and is typical of the instability of transgenic inserts, which can become lost in subsequent generations. This raises the question as to whether the lost insert can be tranferred again, unintentionally, to unrelated species, a process referred to as horizontal gene transfer, with its own attendant hazards [13].

In a second experiment, ten cynomolgus monkeys receiving heart xenotransplants from transgenic pigs were dosed with a regimen of immune suppressing drugs: 80-180mg/kg/day of cyclosporine and 10-20mg/kg of cyclophosphamide on alternate days. Methylprednisolone was also administered at 1mg/kg. This regimen produced median survival of 40 days (2 to 62 days). The five non-transgenic hearts were rejected hyperacutely (median 55 mins). Five animals from the transgenic heart group had to be euthanased (killed out of compassion, to relieve suffering) due to gastrointestinal toxicity, resulting in severe diarrhoea. All hearts were reported to be normal with no evidence of complement or immunoglobulin deposition.

In immune-suppressed animals, rejection was considered not the primary cause of graft failure. Only two out of ten were due to rejection, while drug toxicity resulted in 50% having to be euthanized. That accounted for seven of the ten xenotransplant recipients. So, what did

the remaining three die of? The report did not specify. There was no indication that post-mortem examination for viral infections had been carried out.

An experiment involving a single transgenic pig heart transplanted to a baboon was described in a subsequent paper [10]. The animal survived 39 days with an immunosupressive regimen of cyclophosphamide, cyclosporine A, mycophenolate mofetila and cortecosteroids. It was reported to be active and energetic until day 39, when it underwent sudden and rapid decline, leading to collapse almost immediately following the routine administration of drugs. The cause of death was recorded as "unclear". Postmortem examination was limited to ascertaining that organ rejection was not to blame. Again, no investigations for viral infections were reported.

Hazards of cross-species viruses arising from xenotransplantation

The problem of infectious viruses arising from xenotransplantation was first raised by Robin Weiss and his coworkers at the Institute of Cancer Research [2]. They showed that a pig endogenous retrovirus (see Box 2) can infect cultured human cells. And once the virus has gone through a complete life-cycle in human cells, it is then able to infect a wide range of other human cells. Many copies of pig endogenous retroviruses (PERV) exist in the pig genome and it will be extremely difficult, if not impossible, to breed pigs free of PERV. Robin Weiss argued that accidents have already occurred (reported in ref. 1). Millions have become infected with the monkey SV40 virus through polio and adenovirus vaccines made in monkey kidney cells. Many viruses lying dormant in animals, in particular herpes viruses and retroviruses, can become activated and deadly in humans. Activation of animal viruses might be favoured under transplant conditions, which compromise many barriers to natural infection. Robin Weiss stressed that virus adaptation or recombination with other retroviruses in the new host cannot be dismissed.

Another important safety consideration is that the creation of transgenic pigs with human genes, such as hDAF, to suppress hyperacute rejection, actually increases the potential for creating infectious cross-species viruses. It suppresses the body’s defense against bacteria and viral infections, and also provides more opportunities for the viruses to gain access to the host cells (see Box 3).

Risks considered disproportionate to benefits by many scientists

Jonathan Allen, a virologist on FDA’s advisory subcommittee, accused the FDA of the failure to adhere to the precautionary principle (see ref. 1). It may take decades for a xenozoonosis -- infectious diseases arising from cross-species viruses -- like the AIDs virus or Human T-cell Leukemia Virus to spread and become detected. The FDA’s requirement that all future xenotransplant recipients be monitored for infectious diseases over their life time, and prohibiting them and their close contacts from donating blood, amount to shutting the barn door after the horse has bolted.

Box 2

What are endogenous retroviruses and why are they dangerous?

A retrovirus is a RNA virus that is reverse-transcribed into complementary DNA (cDNA) and integrated into the host cell genome to replicate and complete its life-cycle. Endogenous retroviruses (ERVs) are elements in the genomes of all higher organisms including human beings, which are very similar to the genomes of retroviruses. They are flanked by long terminal repeats (LTRs) and carry genes coding for structural and coat proteins of the virus as well as the reverse transcriptase and integrase enzymes (required for reverse-transcription and integration of the viral genome into the host genome) [14].

There are two theories on how ERVs may have evolved. Howard Temin, Nobel laureate who co-discovered the enzyme reverse transcriptase, suggested that they have evolved from retro-transposons -- mobile genetic elements with reverse transcriptase -- which are part of the genomes of all higher organisms. Alternatively, ERVs may have evolved from exogenous viruses, foreign viruses that have become integrated into the genome. There is no reason to believe that these alternatives are mutually exclusive. Exogenous viruses, which may have arisen from retrotransposons, can indeed re-invade the genome of higher organisms to become endogenous retroviruses. In general, most endogenous retroviruses appear to have been acquired millions of years ago, but there is evidence that new retroviruses can be acquired. Under certain circumstances, endogenous retroviruses can also give rise to infectious retroviruses, although most ERVs are in a dormant, non-infectious state.

Some ERVs have retained their ability to code for virus that can infect the cells of other species, a phenomenon known as xenotropism, and this is of particular safety concern with regard to xenotransplantation. For example, xenotropic retroviruses in mice have been described that cannot replicate in mouse cells, but can propagate profusely in human cells in culture. Also, chick and pig ERVs rarely replicate in their own species but readily infect cultured cells of other species, including those of humans. Likewise, a cat ERV replicates in human cells, as does one from baboon, although neither replicates in its own host species.

Box 3

Transgenic pigs increase the likelihood of generating cross-species viruses

Robin Weiss [15] points out that many animal viruses with lipid envelopes are sensitive to inactivation by the human complement cascade. The virus undergoes lysis (breaking open), triggered by the binding of anti-alpha-Gal antibodies to

alpha-Gal on the viral envelope. Viruses grown in non-primate cells are sensitive to inactivation by fresh human serum, whereas the same viruses propagated in human cells are not because they have lost the alpha-Gal. Other enveloped viruses grown in animal cells are also sensitive to lysis by human complement, including arenavirus, paramyxovirus, alphavirus and the rhabdoviral pig pathogen, vesicular stomatitis virus. If alpha-Gal is on the host cell, then the viral envelope becomes sensitive to rapid lysis by human serum. In other words, virus inactivation occurs by precisely the same mechanism as hyperacute rejection of xenograft. So, modifications to make pig xenografts resistant to hyperacute rejection may also make any enveloped viruses of pigs similarly resistant to breakdown in the human host.

The key proteins are CD46 (membrane cofactor protein, MCP-1), CD55 (decay accelerating factor, DAF) and CD59 (prolectin). They all inhibit downstream steps in the complement cascade, and several transgenic pig herds have been developed expressing one or more of these human genes. All of these are present in the envelope of HIV, the AIDS virus, and protect the virus from lysis.

CD46 is the cell-surface receptor for measles virus and CD55 can serve as a binding receptor for Echo and Coxsackie B picornaviruses. Coxsackie B virus causes myocarditis and might endanger the pig heart in an immune suppressed recipient of a xenograft. Transgenic pigs may therefore also provide an opportunity for animal viruses to adapt to a human host range. Coxsackie B virus, for example, can be adapted to grow in mice, and in some human cell cultures, it increases its infectivity a million-fold by adopting the CD55 receptor. If pigs were to harbour picornaviruses that use the pig equivalent of CD55, such viruses may readily adapt to recognize human CD55 in transgenic pigs that express both pig and human equivalents. These viruses would then be pre-adapted to transmit to the xenograft recipient and to be transmitted from human-to-human. There is already concern that mice transgenic for human poliovirus receptor should not escape and become a non-human reservoir for a human pathogen.

Animal morbilliviruses (measles-related viruses such as canine distemper virus and rinderpest virus) might become pre-adapted for human transmission in CD46 transgenic pigs. Morbilliviruses are known to jump host species as in the recent epidemic in seals and dolphins. In Australia, a vet and a stable-hand died after an autopsy of a horse with a new type of morbillivirus which in turn was probably acquired from fruit bats.

Human tumour tissue transplanted into immunodeficient mice frequently becomes infected by endogenous xenotropic mouse retrovirus. Two or three distinct pig retroviruses can infect some human cells in culture.

Researchers are identifying many new pig viruses. One pig virus, closely related to human hepatitis virus E [16], was found in the majority of pigs, three months or older, in herds from mid-western United States. This raised concerns over the creation of cross-species pathogens in xenotransplantation.

The American Society of Transplant Physicians also want tougher guidelines, and accuse the capital-hungry biotech companies of excessive hype, and creating unrealistic expectations among patients, fuelling pressure to proceed to clinical trials.

Fritz Bach, xenotransplant scientist from Harvard among others, called for a moratorium in 1998, as potential risk of xenotransplants would affect the general public who are being exposed without informed consent. He argued for a wide "informed" public debate on whether such trials should be allowed to proceed at all, as it is an ethical question.

According to the United Network for Organ Sharing, the number of transplants increased from 12 000 to 20 000 between 1988 and 1996; while the number on the waiting list soared from 16 000 to 50 000 and the number of deaths rose from about 1 000 to 3 000 [17]. David Sachs of Harvard Medical School estimated that more than 400 000 could benefit from heart transplants when the official waiting list in 1996 was 3 698. Many on the waiting list are for repeat procedures to replace failed transplants. Was Sachs' estimate overblown? Did it reflect the over-enthusiasm on the part of the medical establishment for spare-organ trafficking rather than real demand or benefit? Chronic rejection is the major cause of the loss of allotransplants from unrelated human donors. So it can be predicted that xenotransplants will be much worse.

A new study published in the British Medical Journal suggests that even transplants from unrelated humans save lives only in patients on the verge of death [18]. The study was carried out in Germany. Researchers looked back at 889 patients listed for a first heart transplant in 1997. The patients were categorised into groups with low, medium or high risk of dying and compared the mortality of those on the waiting list with those who had a transplant. It turns out that there were no differences in mortality for the low and medium risk groups. Only in the sickest patients was there an improvement in survival due to the transplant.

There is evidence for cross-species viruses in xenotransplant recipients

Evidence that baboon viruses have arisen in two human subjects transplanted with baboon livers emerged two years ago [3]. DNA of two retroviruses, the simian foamy virus (SFV) and baboon endogenous virus (BaEV), were found in many tissues of the patients. The presence of baboon mitochondrial DNA (evidence of baboon cells) were also founded in the same tissues, suggesting that baboon leukocytes harboring latent or active viral infections had migrated from the xenografts to distant sites in the human transplant recipients. The authors stressed, "The persistence of SFV and BaEV in human recipients throughout the posttransplant period underscores the potential infectious risks associated with xenotransplantation."

These were the first baboon-to-human liver transplants. One was performed in June 1992 in an HIV-infected 35-yr old man, who survived 70 days, and the second, in Jan. 1993, in a 62-yr man who also received donor bone marrow intravenously and survived for 27 days. Both patients had hepatitis B virus-associated liver cirrhosis. Neither transplanted baboon liver functioned normally. In addition, both patients developed kidney failures and multiple post-transplant infectious complications. Both received an immunosuppressive regimen of FD-506 prednisone and cyclophosphamide. The two adult male baboon donors were

screened against a panel of simian and human viruses and were negative for Simian T-cell Leukemia Virus, Simian Immunodeficiency Virus and simian retrovirus.

Antibodies against SFV were detected in samples from both donor baboon samples prior to transplantation, whereas the human patients were non-reactive during several time points after receiving the transplant. However, a faint positive result was recorded at day 22 in patient 2. The absence of antiviral antibody in the patients may be due to insufficient time in case of patient 2, and AIDs in patient 1. Furthermore, immunosuppressants may have suppressed the antiviral response.

Nevertheless, SFV DNA was detected by PCR (Polymerase Chain Reaction) probes in tissues from both patients. In patient 2, SFV DNA was detected in the liver graft on day 24 but not day 12. The liver sample from patient 1 on day 16 was negative for the viral DNA, but positive results in both lymph node and kidney were obtained on the day 70. DNA sequence analyses confirmed that the SFV in the transplant recipients were closely related to the baboon virus rather than those of other primates. The life cycle of SFV includes integration of viral DNA into the host genome.

Baboon mitochondrial DNA and BaEV were simultaneously detected in every sample in which SFV was present.

The authors stated, "These findings demonstrate the potential for both exogenous and endogenous viruses to reside in human recipients of animal organs for a significant period after transplantation. It is possible that these circulating xenogeneic cells could also act as conduits for new human infections....Since retroviruses commonly exist as persistent latent infections, with an incidence of disease that varies because of both host and viral factors, the possibility that baboon foamy viruses might cause disease in humans remains a consideration in discussing future animal sources for xenotransplantation. Theoretically, other yet to be characterized viruses carried by baboons might also be transmitted to human recipients." (p.824).

As mentioned earlier, none of the published papers up to year 2000 from the Imutran group gave any indication that post-mortem pathological investigations included tests for viral infections.

A brief note (less than one page) from Imutran-Novartis, published later in the same year, reported an experiment in which pig alveolar macrophages (PAM) from pig blood, infected with pig cytomegalovirus, PCMV, were cultivated for up to 15 passages together with human cell lines, and monitored for the presence of PCMV [19] at three time points: passages 5, 10 and 15. It reported "no evidence of PCMV infection of the human cells at passage 15, the farthest time point in this study, despite evidence that PAM and PCMV were present in the co-culture up to at least passage 10. On the basis of this evidence, PCMV is unlikely to be a significant zoonotic agent in clinical xenotransplantation of pig organs to human."

The experimental results were equivocal, to say the least. It is bad science to draw any conclusions on the basis of such limited, inconclusive data. Cell culture conditions are obviously different from the conditions in which a xenograft is transplanted into a living body. Furthermore, positive indications for PCMV were obtained at both passages 5 and 10.

Clinical trials to go ahead based on faulted study

White and Nicholson [20] reviewed xenotransplantation research at the end of 1999, and concluded that xenograft rejection cannot be prevented without significant immune suppression and toxic side-effects. They highlighted the risk of pig endogenous retrovirus transmission, but state that some of the important issues will never be solved "until carefully regulated clinical trials are allowed to begin." They take at face-value a report [4] published by Imutran/Novartis and other biotech companies claiming no retroviral cross-infection in patients exposed to pig tissues or receiving pig xenografts; and which has been criticized by many scientists.

The study tracked 160 patients in 9 countries exposed to living pig tissue over a 12-year period. One hundred and thirty one patients had their blood "filtered" and re-circulated through pig spleens, kidneys, livers, or devices made with pig liver cells; 15 received pig skin grafts for burns, and 14 received injections of pig pancreas cells for diabetes.

As pointed out by Peter Collignon of the Infectious Diseases Unit, Canberra Hospital, Australia [21], pig endogenous viral (PERV) genes were detected in 30 of the patients, and pig cells persisted in 23 xenotransplant recipients for up to 8.5 years. Although the authors found no active infection, the possibility of infection remains in the four patients with positive antibodies to PERV, and in another four patients with unexplained symptoms (skin rashes). In addition, lack of antibodies to PERV may not exclude the existence of infection, as for example, prion diseases (which include mad cow disease) cannot be detected by antibody or cellular immune responses. Immune suppressive drugs could also prevent the development of anti-viral antibodies [3]. Collignon asked, "Who would have predicted that so many patients only transiently exposed to pig tissue would have persistent pig cells (and PERV) in their blood?" Even though the authors claim that there is no conclusive evidence of human infection by PERV [4], they admit that "PERV infection [cannot] be excluded."

Emanuel Goldman, Professor of Microbiology and Molecular Genetics at New Jersey Medical School in Newark noted that a majority of the samples tested were from patients whose blood had been flushed through pig organs/tissues, and recirculated into their bodies for very short periods -- of the order of minutes to hours. Such data are hardly relevant to the kinds of conditions that would apply in whole organ xenotransplants. Data from the 14 subjects who received pig pancreatic islet cells could be taken more seriously. But, as with the burn victims, important information about these patients' exposure times to the xenografts and health and immunological status was missing [22].

Moreover, Goldman pointed out that the patients in the study were treated, and serum samples handled and stored in 9 separate countries, making quality control almost impossible. Looking for PERV RNA is always suspect with serum stored for several years. Plasma samples are frozen at -70C and thawed at very high temperatures. Many viruses are very unstable; it is unknown whether such extreme temperature changes might alter PERV and affect test results.

Another problem with the study is that the PCR probes are only good for two genes of one PERV, and will not detect other viruses, such as Hepatitis E virus or Cytomegalovirus, nor recombinant viruses, which are hybrids of pig and human viruses. Finally, none of the

patients have been exposed to transgenic pig tissues. And it has already been pointed out that transgenic pig tissue may be more likely to give rise to new viruses (see Box 3).

To address the risks of infection, the US Food and Drug Administration (FDA) established an Advisory Panel on Xenotransplantation, and the British government set up the UK Xenotransplantation Interim Regulatory Authority (UKXIRA) in 1997.

The report [4] on the lack of evidence for PERV infection in xenotransplant recipients allows the Novartis/Centers for Disease Control teams to conclude that only cautious progress in closely monitored, prospective clinical trials will help to assess the safety and efficacy of xenotransplantation. Both the FDA and UKXIRA are taking this same attitude, and are ready to approve small-scale human trials of pig cell therapy. To proceed on this basis not only exhibits flagrant violation of the precautionary principle, it is to adopt the *anti*-precautionary approach [23], where failure to rule out viral infection (due to faults in data collection or handling) is taken as evidence that there is no risk of viral infection.

Robin Weiss [24] compares the present situation in xenotransplantation to the short-lived Asilomar moratorium on genetic engineering declared in mid 1970s. The parallel is closer than perhaps he thinks, as some of us have indeed questioned whether the exponential growth in genetic engineering biotechnology since the 1970s may have contributed to the recent resurgence of drug and antibiotic resistance diseases [25]. In genetic engineering as in xenotransplantation, species barriers are undermined, and conditions are created which favour the generation of new viruses through horizontal gene transfer and recombination.

It turns out that PERVs not only infect human cells but produce products of the infection that inhibit human immune cell functions. Thus, PERV infection in transplant recipients could lead to an immunodeficiency disease [26]. The suitability of baboons as models for human transplantation was previously questioned on grounds that pig cells do not release PERVS when they contact baboon cells or following pig to baboon cell transplants [27]. However, a subsequent study showed that human, gorilla, and *Papio hamadryas* primary skin fibroblasts, as well as baboon B-cell lines, are permissive for PERV infection [28]. There are probably no barriers to the transfer of viruses across species under conditions of co-culture of cells, or xenotransplantation of tissues and organs.

British scientists have now found that cancer-causing retroviruses can also spread relatively easily across species in the wild [29]. Mouse leukaemia viruses, close relatives of the cancer retroviruses known to infect pigs, were found in a range of mammalian species, suggesting that pig retroviruses may also be capable of infecting other animals -- including humans -- with relative ease. This has prompted the Western health authorities to impose a moratorium on all xenotransplant surgery.

Professor George Griffen, a member of the UK Xenotransplantation Interim Regulatory Authority, admitted that viruses jumping species from xenotransplant organs is possible, but draws attention to the ‘fact’ that "hundreds of pre-moratorium xenotransplant recipients have yet to show reactions to retroviruses."

Governments disregard scientific evidence to put their citizens at risk from cross-species viral pandemics

In January 2000, the US FDA's Xenotransplant Subcommittee met in Gaithersburg, Maryland to review its proposed guidelines to "indefinitely defer" blood and plasma donations from xenotransplant recipients and their "close contacts" [29].

Phil Noguchi, Director of FDA's Division of Cellular and Gene Therapies, acknowledged that xenotransplantation is "fraught with danger." Yet he revealed that there are currently 12 FDA-approved xenotransplant clinical trials going on in the U.S. Most, if not all, are industry-sponsored, and involve the use of pig cells to treat diabetes and neurological diseases, and whole pig livers and cells to perfuse the blood of patients with acute liver failure.

In order to perform such trials, companies must submit an Investigational New Drug (IND) application. But Jay Siegel, Director of FDA's Office of Therapeutics Research and Review indicated that he would be shocked if there weren't activities being done that are not under IND that should be.

Genzyme, a Cambridge, Massachusetts-based biotech company, had been treating about 100 burn patients per year since 1987 with a xenotransplant product called Epicel, regulated as a 'device'. The company use 3T3 mouse cells to grow layers of human skin,which are then applied to the patient. The mouse cells are allegedly irradiated to prevent them, and any viruses, from proliferating; though when pressed, Genzyme's President admitted that the company was still assessing the efficacy of its irradiation method. And, it had not performed FDA-required tests to determine whether its mouse cells could infect human cells. Most shocking was the company's admission that it had not kept a registry of the patients it treated, nor followed up to see whether any of them might have developed signs of illness or infection. Genzyme said it would be "impractical" to try to find these patients. The FDA seemed to have no knowledge of this situation.

Andrew Dayton of the FDA's Division of Transfusion Transmitted Diseases, and architect of the guidelines, acknowledged that if a xenotransplant-related virus entered the blood supply by mistake, the results would be "disastrous" and the necessary withdrawal of contaminated blood products would cause serious blood shortages.

While some Subcommittee members seemed to downplay the threat of infection by pig viruses, virologist Jonathan Allan commented that, for FDA to recognize infectious disease risks in non-human primates, but not in pigs, is arbitrary. Prem Paul, a veterinary researcher at Iowa State University, warned that new pig viruses were continually being discovered; they had not been extensively studied; and the potential existed for them to mutate and infect humans.

British veterinary pathologist David Onions concurred. He warned that pig parvovirus can change hosts and escape inactivation treatments; and has already been found in Porcine Factor 8 used to treat hemophiliacs.

In May, 2000, a new US Public Health Service (PHS) Guideline on Infectious Disease Issues

in Xenotransplantation was published [30]. It involves a complex series of measures to store tissue samples for future study and to establish a national xenotransplant database -- something that should have been done before clinical trials were approved. As it is, they will only serve to detect disease and virus after it is too late.

The PHS acknowledge that viruses from animals used in xenotranplantation could infect patients, their offspring, health workers and the general public. And even admits that, "all xenotransplantation products pose a risk of infection and disease to humans", "baboon endogenous retrovirus in human recipients of baboon [livers] has been documented", "new viruses capable of infecting humans have been identified in pigs", "all species pose infectious disease risks", and "[xenotransplant recipients] may represent a biohazard to healthy livestock".

According to the PHS guidelines, the sponsors are entrusted to design and monitor xenotransplant trials, tailor complex informed consent documents, educate workers, to effectively screen source animals for viruses, maintain proper documentation, and reliably report crucial information about patient and animal health to federal agencies. There is no mention of who will be held responsible if a novel virus is unleashed, and no emergency procedures to deal with an outbreak have been proposed, even though the PHS acknowledges that "airborne transmission of infectious agents" is possible.

PHS further suggests that some animals from xenotransplant facilities may be considered "safe for human food use or as feed ingredients", in flagrant disregard of the fact that the safety of transgenic food is yet to be established, and the international community has found it necessary to negotiate and agree a Biosafety Protocol regulating the safe use and transfer of genetic engineered products under the UN Convention on Biological Diversity.

If xenotransplantation is to go ahead, it will involve levels of animal suffering unacceptable to the majority of people. As it is extremely inefficient, it will also generate many abnormal failures and surplus animals which have to be disposed of safely. There is as yet no documented, true-breeding transgenic line established to-date.

PHS, in their current guidelines, state that Americans have neither endorsed nor rejected xenotransplantation. But documents obtained through the Freedom of Information Act reveal otherwise. In response to its 1996 draft guideline, PHS received over 160 comments: 115 against xenotransplantation, 29 in favor, and 19 neither for nor against, with 8 of these strongly opposing the use of nonhuman primates. Furthermore, the Food and Drug Administration received almost 6,000 postcards, and over 350 letters protesting its April 1999 guidelines on the use of non-human primates in xenotransplant trials.

The Campaign for Responsible Transplantation (CRT), an international coalition of physicians, scientists, and 90 public interest groups, have denounced the PHS Guidelines as irrational and in violation of the precautionary principle.

Conclusion:
stop xenotransplantation for safer, more humane and effective alternatives

Our investigations have revealed how bad science has been involved in the xenotransplant project from the start:

- lack of proper documentation of the transgenic process and characterization of the transgenic pigs
- lack of quality control
- failure to obtain well-characterized stable transgenic lines before transplantation experiments were attempted
- failure to screen for viral infections in experimental xenograft recipients
- use of inconclusive studies to push for clinical trials in humans
- systematic disregard of existing scientific evidence of cross-species viruses arising from xenografts

It is nothing short of a scandal to allow xenotranplantation to go ahead in the light of existing scientific evidence, especially when there are safer, more humane and effective alternatives [17].

Much can be done to increase human organ donation in the short term, especially if an assurance can be made to the donor that the organ will be offered free of commercial interest to the recipient. The use of artificial organs and human cells and tissues will both avoid the risk of cross-species viral epidemics.

One of the most exciting recent development is the possibility of regenerating organs and tissues from the patients' own stem cells [31], cells which retain the ability to multiply and differentiate into a number of different cell types even in the adult. This would avoid immune rejection as well as viral epidemics. We reject the claim that human *embryonic* stem cells have to be used, which are obtained from human embryos created solely for the purpose. It has now been demonstrated that adult human liver cells can be derived from stem cells originating in the bone marrow (which normally produce blood cells) or circulating outside the liver. This raises the possibility that bone-marrow stem cells, either from a donor or from the patient could be used to generate liver cells for replacing damaged tissue, thus obviating the need for organ transplant altogether [32]. Better yet, why not find out how to encourage adult stem cells to regenerate *in situ*? These alternatives are infinitely preferable to xenotransplantation in being safe, humane, sustainable and affordable; and hence more likely to benefit society as a whole in the industrialized west as well as in the Third World.

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