

**U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
SECRETARY'S ADVISORY COMMITTEE ON XENOTRANSPLANTATION**

**REPORT ON THE STATE OF THE SCIENCE
IN XENOTRANSPLANTATION**

**DRAFT
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SACX REPORT ON THE STATE OF THE SCIENCE IN XENOTRANSPLANTATION

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9 Xenotransplantation holds promise for the treatment of many human diseases and disorders and
10 could potentially alleviate the shortage of human organs and tissues available for transplantation.
11 It also raises many interesting and complex scientific, medical, ethical, legal, and social issues.
12 This report focuses on the science of xenotransplantation, an area in which major advances have
13 been made in the last few decades. The report outlines the considerable progress that has been
14 made in understanding and overcoming some of the major hurdles to xenotransplantation and
15 also discusses the public health concerns associated with xenotransplantation.
16

17 **Aim of This Report**
18

19 This report was produced in response to two critical mandates of the U.S. Department of Health
20 and Human Services (DHHS) Secretary’s Advisory Committee on Xenotransplantation (SACX):
21 to advise the DHHS on the current state of scientific knowledge about xenotransplantation and
22 on the potential for transmission of infectious diseases as a consequence of xenotransplantation.
23

24 This report provides an overview of the potential impact of xenotransplantation, the types of
25 xenotransplantation procedures currently being used in research, and the source animals for
26 xenotransplantation products. It then discusses some of the major challenges posed by
27 immunologic and physiologic incompatibilities, as well as strategies to address those challenges.
28 Also discussed are the infectious disease risks associated with animal-to-human transplantation
29 and management strategies to cope with them. Some alternative strategies to xenotransplantation
30 are described to provide a contextual perspective on the field. The report also describes public
31 health concerns about “xenotourism,” a term coined to describe personal travel outside of a
32 country of residence for the purpose of participating in xenotransplantation programs or
33 attending clinics to obtain therapies not presently available or acceptable in the home country.
34 Finally, the SACX presents its recommendations regarding xenotransplantation as an
35 experimental therapeutic strategy.
36

37 **Types of Xenotransplantation Procedures**
38

39 The term *xenotransplantation* refers to the transplantation, implantation, or infusion into a
40 human recipient of live cells, tissues, or organs derived from non-human animals. The procedure
41 includes the use of human body fluids, cells, tissues, or organs that have had *ex vivo* contact with
42 live, non-human animal cells, tissues, or organs. The source animals or their cells may or may
43 not be genetically modified. The different types of xenotransplantation procedures being
44 performed or considered include the following:
45

- 1 • **Solid-organ xenotransplantation** is a procedure in which a source animal organ such as a
2 heart, lung, kidney, or liver is transplanted into a human. In such cases, the vascular supplies
3 of the source animal and the recipient are connected.
4
- 5 • **Cellular and tissue xenotransplantation** is the grafting of tissues and cells from a source
6 animal without surgical connection of any animal blood vessels to the recipient's vessels.
7 These xenotransplantation products may be implanted directly into a recipient's organ.
8
- 9 • **Extracorporeal (natural and artificial organ) perfusion** occurs when human blood is
10 circulated outside of the human body through an animal organ, such as a liver or kidney, or
11 through a bioartificial organ produced by culturing animal cells on an artificial matrix.
12
- 13 • **Exposure to living animal-derived material** occurs when any of a variety of human cell
14 types are grown ex vivo with non-human animal cells. If these human cells are subsequently
15 transplanted or infused into a human patient, the procedure is considered a form of
16 xenotransplantation.
17

18 **Scientific Challenges in Xenotransplantation**

19
20 Technical challenges to xenotransplantation are primarily immunologic and physiologic in
21 nature. Although these challenges are common to both human-to-human transplantation
22 (allotransplantation) and xenotransplantation procedures, the various differences and disparities
23 between species tend to exacerbate these difficulties in xenotransplantation.
24

25 Immunologic rejection processes are faced in any transplantation procedure and are exacerbated
26 by the differences between species. In xenotransplantation, however, a number of factors arising
27 from disparities between species intensify the immunologic processes that mediate rejection:
28

- 29 • **Hyperacute rejection** is the nearly immediate and catastrophic destruction of a graft, a
30 process that is initiated by natural antibodies directed to a specific sugar molecule called
31 galactose-(α 1-3)-galactose (Gala 1-3Gal) (abbreviated aGal). Because human cells do not
32 express this sugar molecule, the human immune system recognizes it as “non-self,” or
33 foreign, and produces antibodies against it in response to its presence on common
34 microorganisms. These antibodies and the process of complement-mediated destruction are
35 elements of natural human immunity against invading microorganisms.
36
- 37 • **Delayed vascular rejection** occurs over days to a few months and involves the vascular
38 system of the xenotransplantation product. Pig kidneys and hearts transplanted into non-
39 human primates stimulate progressive destruction of the pig blood vessels. Antibodies and
40 inflammatory immune cells are found in these vascular lesions. The exact immune
41 mechanisms responsible for delayed vascular rejection are not yet fully known.
42
- 43 • **Acute cellular rejection** occurs over weeks or months after transplantation. This
44 phenomenon is mediated predominantly by T-cells, which constitute the cellular arm of the
45 immune system. Acute cellular rejection can target blood vessels but usually involves
46 infiltration of attacking host T-cells (and other immune cells) into the transplant tissues and

1 destruction of the epithelial cells that are responsible for the function of the xenotransplanted
2 organ.

- 3
- 4 • **Chronic rejection** is the progressive destruction of a transplant over months to many years.
5 Antibodies induced by the organ graft may play a role in the process. For some transplanted
6 organs (e.g., hearts), a poorly understood, distinct pathological process results in such severe
7 narrowing of blood vessels that the underlying tissue is starved of essential nutrients. This
8 process, as well as direct attacks on the cells that form the structure and maintain the function
9 of the transplant, may eventually produce extensive tissue destruction and replacement by
10 fibrotic scars.

11

12 Additional species differences that may affect the function of a xenograft include complement
13 and coagulation systems; adhesion molecules, cytokines, and growth factors; organ-specific
14 physiologic considerations; and size considerations. Current approaches to the challenges facing
15 xenotransplantation include genetic modification of source animals, encapsulation and other
16 bioartificial isolation devices, methods to induce tolerance in xenotransplantation recipients,
17 gene therapy to modify either source animals or recipient cells, targeted molecular therapies to
18 inhibit the activation of the complement and blood clotting systems that follow antibody binding
19 and complement activation, and a number of other host treatments, such as removal of anti-aGal
20 antibodies before xenotransplantation.

21

22 **Risks of Infectious Diseases Associated with Xenotransplantation**

23

24 Although all allotransplant procedures are associated with a risk of infectious diseases caused by
25 known and emerging human pathogens, the risks associated with xenotransplantation have
26 unique features that could pose a threat to public health. Xenotransplantation may allow
27 infectious agents from source animals to circumvent natural barriers, such as mucosal surfaces
28 and skin, through the surgical placement of a xenograft product into a human. The magnitude of
29 the risk associated with xenotransplantation would be influenced by the requirement for
30 immunosuppressive agents, the species of the source animal and the applicable husbandry
31 practices, the type of tissue or organ used, and the duration and type of recipient exposure. If an
32 infectious agent from a source animal is transmitted to a human recipient, the agent could result
33 in the undetected spread and establishment of novel infections in humans. Risks from
34 xenotransplantation include both acute and chronic persistent viral infections. Importantly, some
35 infectious agents may cause no disease in the source animal and may remain unrecognized.
36 Person-to-person spread of infection without detection for years represents a significant problem
37 due to potential long clinical latency periods that can extend to decades, thus allowing for the
38 spread of an animal virus to the general population. As an example, HIV-1 is asymptomatic in
39 chimpanzees, but its transmission to humans resulted in a new clinical disease (AIDS) after a
40 long clinical latency. Therefore, the risks from xenogeneic infections pose unique concerns that
41 need to be addressed during research and clinical trials involving xenotransplantation.

42

43

44 ***Risks from Porcine Xenotransplant Products***

45

1 At present, pigs are the source animal of choice for whole-organ xenotransplants. It is
2 recommended that nonhuman primates not be used as source animals for xenotransplants
3 because of problems associated with infectious diseases in monkeys and their risk to humans.
4 Porcine infectious diseases are currently being studied and assays are being developed to detect
5 infection in pigs and humans. Many porcine agents are non-infectious for humans, and others
6 can be eliminated through screening and husbandry practices, including closed breeding
7 colonies. Unrecognized infections are likely to circulate in porcine populations and could be a
8 potential risk to humans in xenotransplantation. Efforts should be directed toward preventive
9 measures by 1) developing new assays and methods to detect novel porcine agents and 2)
10 developing methods to reduce infections in swine colonies.

11
12 Of particular concern as a potential emerging infection from xenotransplantation has been
13 porcine endogenous retrovirus (PERV). PERV is essentially embedded in all pig genomes and
14 could potentially be transmitted to humans with the xenotransplant product. Previous
15 retrospective studies to detect transmission to humans exposed to porcine products have failed to
16 detect transmission of PERV; however, as clinical trials become successful, longer exposure to
17 xenotransplant products may enhance the risk from PERV. Recent studies suggest that the risk
18 from PERV might be reduced through selective breeding practices using pigs that do not transmit
19 the virus to human cells (e.g. some MGH mini-pigs have been shown to be non-transmitters).

20
21 The overall risk from porcine xenografts can be minimized through good animal husbandry
22 practices, construction of barrier-contained breeding facilities, and screening of source animals
23 for known infections. Because of the potential for an infected blood donation or other recipient
24 contact with an infected individual to spread new, unidentified pathogens, infectious risks from
25 animal sources will continue to warrant more intensive investigation as the field of
26 xenotransplantation matures.

27 28 29 **“Xenotourism”: An Emerging Global Public Health Concern**

30
31 The SACX defines “xenotourism” as the travel of U.S. residents to foreign nations to participate
32 in xenotransplantation programs or clinics for the purpose of obtaining therapies not presently
33 available or acceptable in the United States. The SACX believes that xenotourism constitutes a
34 public health risk, because the xenotransplantation procedures may be performed under
35 circumstances that would not be allowed in the U.S. After the xenotransplantation procedure,
36 American xenotourists return to the U.S., where they receive the majority of their health care and
37 participate in the daily activities of life. Xenotourists unaware of the potential for transmitting
38 infections are not likely to take appropriate precautions to reduce the risk of transmission of a
39 potential xenogeneic infection. For example, they might donate blood or organs or engage in
40 other behaviors that might be associated with risk of transmission of viral infection. Furthermore,
41 unless they inform their health care providers of their participation in a xenotransplantation
42 procedure, a potential xenogeneic infection may not be recognized and properly monitored.

43
44 The SACX believes that a systematic effort should be undertaken to identify xenotransplantation
45 programs in other countries and to assess their use by U.S. citizens. This will inform U.S. efforts

1 to educate the public about the potential dangers of xenotourism and to develop strategies for
2 further discussions with the international community on the regulation of xenotransplantation.

4 **Knowledge Gaps and Resource Limitations**

6 Although increased understanding of molecular immunobiology and cell and organ physiology
7 has permitted significant progress in xenotransplantation in recent years, many challenges must
8 be addressed before organ and cellular xenotransplantation can be clinically valuable.

9 Following are several areas in which major gaps in knowledge or limitations in resources may
10 hinder the progress of xenotransplantation:

- 12 • **Molecular incompatibilities between species** will require more research to generate new
13 molecular and genetic strategies for avoiding delayed vascular rejection and other deleterious
14 outcomes.
- 16 • **Animal models**, in particular non-human primate models, are particularly important in the
17 setting of xenotransplantation, in which the unknown risk of infection from the source animal
18 imposes an unusual ethical requirement to justify clinical trials. Efforts are needed to
19 overcome the limitations of non-human primate models that diminish the utility and
20 predictive value of these models.
- 22 • **Sharing of resources** through new partnership arrangements between industry and
23 government, as well as public support of research leading to future modifications, might
24 better ensure the sharing of genetically modified pigs and other reagents that are in limited
25 supply and are costly and time-consuming to generate. The science of xenotransplantation is
26 unlikely to proceed expeditiously without sharing of such reagents.
- 28 • **Support for xenotransplantation** from the biotechnology industry is currently low. In
29 addition, there are significant challenges regarding sharing of data, animals, and reagents.
30 Extensive additional basic research in xenotransplantation and a long-term investment of
31 resources and effort will be required to determine if xenotransplantation is a viable clinical
32 approach.

34 **Parallel or Alternative Strategies to Xenotransplantation**

36 In addition to xenotransplantation, a number of other approaches are under development for the
37 treatment of conditions involving cellular, tissue, and organ destruction:

- 39 • **Prevention** of the acute and chronic conditions that lead to the need for replacement organs,
40 cells, and tissue is the ideal approach, and prevention activities need to be promoted by all
41 available means.
- 43 • **Gene therapy**, a relatively new and highly experimental technology for treating human
44 disease, has recently enjoyed some limited clinical success. In organ transplantation, gene
45 therapy approaches could one day be useful in preventing transplant rejection, inducing

1 tolerance, prolonging graft survival, and ameliorating some of the problems associated with
2 systemic immunosuppression.

- 3
- 4 • **Stem cell therapy** offers the possibility of treatment for a variety of diseases and disorders
5 involving tissue destruction or cellular injury and dysfunction. It offers hope for treating a
6 multitude of clinical diseases and has both advantages and disadvantages when compared
7 with xenotransplantation. Stem cells may have considerable potential for cellular
8 replacement and repair, but their potential for whole-organ replacement is currently
9 unknown.
- 10
- 11 • **Artificial organs** include left ventricular assist devices, which can improve cardiac function
12 in patients with isolated left-sided heart failure, and an artificial heart (Abiocror) has been
13 developed and evaluated in a small group of patients. Such devices, however, currently face
14 several obstacles, and the potential for the success of these technologies in terms of
15 improving quality of life and longevity are currently unknown.
- 16

17 **Findings and Recommendations**

18

19 The SACX makes the following recommendations for pursuing xenotransplantation as a strategy
20 for treating a variety of medical disorders:

- 21
- 22 1. Continue to evaluate pigs as a suitable source animal for xenotransplantation. Due to
23 heightened risks and ethical concerns apparent with nonhuman primates, these animals
24 should not be considered as source animals for xenotransplantation. The establishment of
25 specific pathogen-free closed colonies of pigs will ultimately be needed to raise animals for
26 clinical trials.
- 27
- 28 2. Support existing federal guidelines on source animals for xenotransplantation.
- 29
- 30 3. Further development of diagnostic tools, including antibody and nucleic acid-based assays, to
31 detect known and unrecognized porcine pathogens that pose a risk to humans should be
32 supported. Continue research on the risks of zoonotic infection in xenotransplantation
33 recipients and gauging the potential for new emerging diseases is needed.
- 34
- 35 4. Initiate research studies that will use the new tools of molecular biology and genetics to
36 reveal physiologic and immunologic incompatibilities between source animals and humans.
- 37
- 38 5. Develop facilities where pig-to-non-human primate models could be used to gauge the
39 efficacy of xenotransplantation of pig organs, tissues, and cells to humans.
- 40
- 41 6. Encourage scientists from diverse disciplines to apply their expertise in the discovery of
42 solutions for successful xenotransplantation.
- 43
- 44 7. Establish repositories in which reagents, genetically modified pigs, and other valuable
45 materials can be maintained and distributed to researchers and laboratories engaged in
46 xenotransplantation research.

- 1
- 2 **8.** Build government-industrial-academic partnerships that ensure the sharing of reagents and
- 3 research animals.
- 4
- 5 **9.** Provide counseling to industry early in their development of xenotransplantation products on
- 6 issues related to compliance with federal regulatory and safety issues.
- 7
- 8 **10.** The problem of broad liability for the consequences of possible zoonotic infections is
- 9 perceived by some as a deterrent to participation by industry in xenotransplantation research.
- 10 Investigate this issue and identify solutions.
- 11
- 12 **11.** Periodically re-evaluate federal guidelines on xenotransplantation and institute a system of
- 13 review and oversight of regulations.
- 14
- 15 **12.** Investigate the scope of xenotransplantation in countries lacking stringent oversight and the
- 16 extent of risks posed by entry into the United States of persons receiving xenotransplants in
- 17 such countries.
- 18
- 19 **13.** Educate U.S. residents about the risks of unregulated xenotransplantation procedures and
- 20 discourage their participation in those lacking regulatory oversight as stringent as that in the
- 21 United States.
- 22
- 23 **14.** Work closely with international health agencies to promote regulations and guidelines for
- 24 xenotransplantation that are as rigorous as those developed by the PHS and assist other
- 25 countries in implementing them.

REPORT ON THE STATE OF THE SCIENCE IN XENOTRANSPLANTATION

INTRODUCTION

Xenotransplantation holds promise for the treatment of many human diseases and disorders and could potentially alleviate the shortage of human organs and tissues available for transplantation. Xenotransplantation also raises many interesting and complex scientific, medical, ethical, legal, and social issues. This report focuses on the science of xenotransplantation, an area in which major advances have been made in the last few decades. The report outlines the considerable progress that has been made in understanding and overcoming some of the major hurdles to xenotransplantation, including hyperacute rejection, delayed xenograft rejection, the role of antibodies against the aGal sugar in these processes, and the potential infectious complications of xenotransplantation.

Xenotransplantation refers to procedures in which live cells, tissues, or organs derived from a non-human animal are transplanted, implanted, or infused into a human patient. It also includes procedures in which human body fluids, cells, tissues, or organs are removed from the body, come into contact with live animal cells, tissues, or organs, and are then placed back into a human patient.^a Xenotransplantation is raised to a level of special public interest not only because of its potential to meet a critical health care need but also for the potential risk it poses: that new disease-causing agents could be transmitted from a xenotransplantation product to patients and their close contacts and, ultimately, to the public at large. Major headway has been made toward minimizing these risks through both the development of regulatory guidelines by the U.S. Public Health Service and scientific research that has improved our understanding of and ability to diminish the risk of transmitting endogenous infections from source animals to humans. Despite all of the encouraging progress, many major hurdles remain before the potential of clinical organ and tissue xenotransplantation can be fully realized. In this report, we summarize the limitations of the progress that has been made and identify areas in which considerable effort may still be needed in order to optimize progress in xenotransplantation.

Aim of This Report

The U.S. Department of Health and Human Services (DHHS), which includes the U.S. Public Health Service (PHS), has been developing tools for the oversight and coordination of xenotransplantation activities. These public health tools include a DHHS interagency working group; a regulatory framework; the PHS *Guideline on Infectious Disease Issues in Xenotransplantation* (<http://www.fda.gov/cber/gdlns/xenophs1000.htm>);¹ a national database of xenotransplantation clinical trials and biomedical animal facilities supplying animals and tissues for these trials; a national centralized archive of biological specimens for public health

^a This is a paraphrase of the definition of xenotransplantation used by the DHHS and set forth in *PHS Guideline on Infectious Disease Issues in Xenotransplantation*¹: “Any procedure that involves the transplantation, implantation, or infusion into a human recipient of either (a) live cells, tissues, or organs from a non-human animal source or (b) human body fluids, cells, tissues, or organs that have had ex vivo contact with live nonhuman animal cells, tissues, or organs” (p. 4).

1 investigations; and a national advisory panel on xenotransplantation: the Secretary’s Advisory
2 Committee on Xenotransplantation (SACX), which has produced this report.

3
4 In the mid- to late 1990s, there were a number of calls for a national advisory body on
5 xenotransplantation to serve as a public forum for input, public education, and deliberation on
6 this topic by researchers, health care providers, policy makers, patients and their families, public
7 health officials, ethicists, animal welfare representatives, and other members of the public. The
8 SACX was established by the DHHS in 1999 and first convened in 2001. The Committee is
9 charged with advising the Department on the scientific, medical, social, ethical, and public health
10 issues and concerns raised by xenotransplantation, as well as with recommending policies and
11 procedures related to the scientific development and clinical application of xenotransplantation
12 procedures.

13
14 This report was produced in direct response to two critical mandates of the SACX: to advise the
15 Department on (1) the current state of scientific knowledge about xenotransplantation and (2) the
16 potential for transmission of infectious diseases as a consequence of xenotransplantation. In
17 keeping with these and related mandates, this report also addresses certain ongoing practices that
18 the SACX considers to be global public health risks and provides recommendations for
19 appropriate responses.

20
21 This report provides an overview of the potential impact of xenotransplantation, the types of
22 xenotransplantation procedures currently being used in research, and the source animals for
23 xenotransplantation products. It then discusses some of the major challenges posed by
24 immunologic and physiologic incompatibilities, as well as strategies to address those challenges.
25 Also discussed are the infectious disease risks associated with animal-to-human transplantation
26 and management strategies to cope with them. Some alternative strategies to xenotransplantation
27 are described to provide a contextual perspective on the field. The report also describes public
28 health concerns about “xenotourism,” a term coined to describe personal travel outside of a
29 country of residence for the purpose of participating in xenotransplantation programs or
30 attending clinics to obtain therapies not presently available or acceptable in the home country.
31 Finally, SACX presents its recommendations for pursuing xenotransplantation as a therapeutic
32 strategy.

33 34 **Background**

35
36 Although sporadic (and unsuccessful) attempts at xenotransplantation can be documented as far
37 back as the 1600s,² the field is still very much in its infancy. In the United States,
38 xenotransplantation products are regulated using applicable guidelines by the U.S. Food and
39 Drug Administration (FDA). The FDA has received approximately 40 investigational new drug
40 (IND) applications for xenotransplantation in the last decade. Approximately 500 patients in
41 clinical studies in the United States have received experimental xenotransplantation products^b for
42 liver failure, Parkinson’s disease, Huntington’s disease, diabetes, intractable pain of cancer,
43 melanoma, or burns. At least 1,000 burn patients have been treated with autologous skin cells
44 that have been grown on mouse feeder layer cells.

^b Xenotransplantation products are the live cells, tissues or organs used in xenotransplantation (as defined in *PHS Guideline on Infectious Disease Issues in Xenotransplantation*²).

1
2 **Potential Impact of Xenotransplantation on U.S. Chronic Disease Burden**
3

4 Statistics from the United Network for Organ Sharing (UNOS) provide some indication of the
5 number of people who suffer from organ failure.³ Nearly 83,000 patients were on the UNOS
6 waiting lists at the end of 2002, and more than 6,000 died before an organ from a human donor
7 became available (see Table 1). Approximately 2 million people die in the United States each
8 year. However, a recent study found that only 10,500–13,800 deceased individuals are eligible
9 to donate organs each year, and only about 42% of eligible donors during the period studied
10 became actual donors.⁴ Even if all eligible donors became actual donors, it would not meet the
11 demand for transplantable human organs.

12
13 The waiting list reflects only those individuals who are listed as candidates for transplantation
14 and so is not a reflection of the prevalence of organ failure. For example, the vast majority of
15 people with type 1 diabetes, which is endocrine pancreas failure, are not listed as candidates for
16 transplant because there is no widely available, reliable source of acceptable donor material.
17

Table 1. Patients on UNOS waiting list, transplants, and death while waiting in 2002

Organ	No. of patients on list*	No. of patients transplanted	No. of deaths
Total	111,716	24,544	6,385
Kidney	68,468	14,523	3,396
Liver	26,326	5,060	1,818
Heart	6,990	2,111	558
Pancreas [†]	1,884	517	29

* Includes patients on waiting list at start of 2002 and patients added to the list at any time during 2002.

[†] Includes pancreas transplant alone and pancreas after kidney transplant candidates.

Source: U.S. Department of Health and Human Services/United Network for Organ Sharing/Universal Renal Research and Education Association. 2003 Organ Procurement and Transportation Network/Scientific Registry of Transplant Recipients Annual Report: Transplant Data 1993–2002. Available at: <http://www.ustransplant.org>. Retrieved June 16, 2004.

1 Type 1 diabetes provides an example of a medical
2 condition that could be significantly affected, both
3 on an individual and a public health level, if
4 xenotransplantation were to become a standard
5 medical treatment. This disease results from the
6 failure of pancreatic islet cells to produce insulin,
7 which regulates blood glucose. It affects
8 approximately 1 million Americans, who must
9 take daily injections of insulin to survive, and
10 results in lost work, high medical costs, and
11 diminished quality of life.⁵

12
13 Type 1 diabetes could potentially be treated by
14 transplantation of either pancreatic islet cells or
15 the entire pancreas. Because this form of diabetes
16 can also cause kidney damage or failure,
17 xenotransplantation of an animal kidney could
18 also be considered. Preliminary research⁷
19 suggests that it may be possible to use non-human
20 islet cells to produce levels of insulin that would
21 be sufficient to reduce or eliminate the need for
22 insulin injections. In solid-organ
23 xenotransplantation, the transplanted organ might
24 completely replace the malfunctioning pancreas.

25 Human adult pancreatic tissue cannot meet this need because of the inadequate availability of
26 human organs or tissues for transplantation.

27
28 In 2002, almost 100,000 people in the United States progressed to end-stage kidney failure, with
29 the result that 300,000 patients were sustained on dialysis and 80,000 had functioning
30 transplanted kidneys. These numbers had doubled since 1990 and are expected to double again
31 by 2010.⁶ More than 40% of cases of end-stage kidney failure are caused by diabetes.⁷ Current
32 standard treatment for kidney failure is hemodialysis or kidney transplantation of a kidney from a
33 living or deceased human donor (kidney allotransplant). Survival is greatly increased after a
34 kidney allotransplant: up to 83% of these patients are alive after 5 years. In contrast, only about
35 33% of patients receiving dialysis treatment survive for 5 years.⁸ The annual cost of
36 hemodialysis for one person is approximately \$73,500 per year, and the cost after kidney
37 transplantation is approximately \$8,000–10,000 per year.⁹ Islet-kidney xenografts, in which
38 porcine islets are engrafted under the pig's kidney capsule before transplantation,¹⁰ has the
39 potential to cure both end-stage kidney disease and diabetes simultaneously. The potential
40 effectiveness, safety, and cost of kidney xenotransplantation require further investigation and
41 evaluation.

42
43 Diabetes is just one of many disorders that could be treated with xenotransplantation were this
44 procedure to become proven and accepted. Among the others are congestive heart failure,
45 Parkinson's disease, cystic fibrosis, Alzheimer's disease, and Huntington's chorea (see
46 box).^{5,11,12,13,14,15,16} However, significant technical challenges and public health risks remain and

Some chronic and degenerative diseases that might be treated with xenotransplantation:

- ⚡ **Congestive heart failure** affects 4.8 million Americans, half of whom die within 5 years of diagnosis.⁸
- ⚡ More than 1.5 million people in the United States have **Parkinson's disease**, a progressive disorder of the central nervous system characterized by the loss of dopamine-producing brain cells.^{9,10}
- ⚡ About 30,000 Americans have **Huntington's disease**, and at least 150,000 others have a 50% chance of developing the disease because they have an affected parent. There is no effective treatment for this devastating disorder, which results from genetically programmed degeneration of neurons in certain areas of the brain.¹¹
- ⚡ About 2,500 babies are born with **cystic fibrosis** each year in the United States. Approximately 30,000 Americans of all ages have this chronic, progressive, and frequently fatal genetic disease of the body's mucous glands.¹²
- ⚡ An estimated 250,000–400,000 Americans have **spinal cord injuries**, about 60% of which occur before age 30. These injuries account for millions of dollars in medical costs and unemployment.¹³
- ⚡ In 2002, 6,385 patients **died while on the waiting list** for human organ transplantation. At the end of 2002, more than 82,000 patients were **still on the waiting list** for human organs.³

1 must be addressed before xenotransplantation can become a clinical reality. These technical
2 issues and public health considerations are discussed in detail in the following section.

3 4 ***Types of Xenotransplantation Procedures***

5
6 The term *xenotransplantation* refers to a complex array of procedures involving the
7 transplantation, implantation, or infusion into a human recipient of live cells, tissues, or organs
8 derived from a non-human animal source. The procedure includes the use of human body fluids,
9 cells, tissues, or organs that have had *ex vivo* contact with live, non-human animal cells, tissues,
10 or organs. The source animals or their cells may or may not be genetically modified. A number
11 of different types of xenotransplantation procedures are being performed or considered and are
12 associated with various challenges. The categories of xenotransplantation procedures, as well as
13 some of the challenges involved in their use, include the following:

- 14
15 • **Solid-organ xenotransplantation** is a procedure in which a source animal organ such as a
16 heart, lung, kidney, or liver is transplanted into a human. In such cases, the vascular supplies
17 of the source animal and the recipient are connected. As a result, the recipient's blood
18 circulates through the animal organ, where it encounters endothelial cells that line the animal
19 organ's blood vessels. This contact between components of the recipient's circulating blood
20 and the source animal's endothelial cells may initiate certain types of rejection processes,
21 which are described below.
22
- 23 • **Cellular and tissue xenotransplantation** is the grafting of tissues and cells from a source
24 animal without surgical connection of any animal blood vessels to the recipient's vessels.
25 These xenotransplantation products may be implanted directly into a recipient's organ.
26 Examples of this type of procedure are the implantation of porcine neural tissue into a
27 recipient's brain or of animal-derived cartilage into a recipient's joint. Alternatively, animal
28 cells may be infused into the recipient's bloodstream, such as when islet cells are infused into
29 the portal vein (and are intended ultimately to take up residence in the liver) or hematopoietic
30 cells, such as bone marrow cells, are infused into the recipient's peripheral veins (and
31 ultimately take up residence in the bone marrow or other sites of hematopoietic tissue). All
32 cellular and tissue xenotransplants are subject to immune-mediated rejection, but the
33 mechanisms of rejection differ from those that are activated specifically by endothelial cells
34 (see "Immunologic Rejection Processes").
35
- 36 • **Extracorporeal (natural and artificial organ) perfusion** occurs when human blood is
37 circulated outside of the human body through an animal organ, such as a liver or kidney, or
38 through a bioartificial organ produced by culturing animal cells on an artificial matrix. To
39 date, extracorporeal perfusion has been used in patients with hepatic failure to keep them
40 alive until an allogenic liver transplant becomes available or until the patient's liver
41 regenerates sufficient function to support life.
42
- 43 • **Exposure to living animal-derived material** occurs when any of a variety of human cell
44 types are grown *ex vivo* with non-human animal cells. If these human cells are subsequently
45 transplanted or infused into a human patient, the procedure is considered a form of
46 xenotransplantation. An example of this technique is the growth of human skin cells

1 (keratinocytes) on a mouse cell line in order to generate a layer of autologous tissue to
2 provide a temporary wound covering in patients with severe burns. Another example is the
3 exposure of human immune cells to animal cells for sensitization. Finally, some existing
4 human embryonic stem cell lines, if used to generate cells or tissues for treatment of patients,
5 would be defined as xenografts if they have been previously grown on mouse feeder cell
6 layers. The considerations regarding these types of transplants mainly relate to the risks of
7 infectious disease (see “Infectious Disease Risks Associated with Xenotransplantation”).
8

9 ***Potential Source Animals for Xenotransplantation***

10
11 Recent research in xenotransplantation has focused largely on the pig as a potential source
12 animal for a number of reasons. In particular, the widespread availability and excellent breeding
13 characteristics of pigs make it possible to generate large numbers of animals in closed colonies
14 and to develop transgenic and cloned animals. In addition, the similar size of pig and human
15 organs (particularly those of certain breeds of pig known as miniature swine) and the similar
16 physiology of the two species make the pig particularly suitable as a potential xenograft source.¹⁷
17 Considerable human experience with the husbandry and veterinary care of pigs also favors their
18 use as source animals. Sheep and cows have equally long histories of commercial breeding,
19 genetic manipulations, and animal health experience, and they might be considered as source
20 animals in the future.

21
22 A number of other diverse source species also appear to be suitable. Certain species of fish, such
23 as tilapia (*Oreochromis* spp.), are a potential source of pancreatic islets.¹⁸ Mouse and insect cells
24 have been used for growing human cells in culture. Animal cells that produce viral gene
25 delivery vectors have been injected into humans in a type of cancer therapy. Genetically altered
26 mammal, fish, or insect cells that produce therapeutic biological agents might be placed within
27 special isolation capsules to be injected or introduced into humans for the treatment of genetic or
28 chronic inflammatory diseases.

29
30 Non-human primates may seem to be logical source animals because their genetic proximity to
31 humans might present less formidable immunological barriers to successful xenotransplantation.
32 Major barriers to the use of non-human primates, however, have effectively removed them from
33 consideration. The major reasons are the risks of transmitting viruses to humans¹⁹ and the
34 realization that most monkeys and baboons are considerably smaller than adult humans.
35 Moreover, it would be difficult, expensive, and time-consuming to breed large numbers of
36 captive non-human primates in germ-free isolation facilities. In addition, the breeding of non-
37 human primates as a source of xenografts is considered unethical and unacceptable by many
38 members of the lay and scientific community.

39
40 The importance of the ethical and humane treatment of animals in xenotransplantation, both
41 those used in research and those eventually serving as source animals for human recipients, is a
42 topic that deserves consideration in itself and is beyond the scope of this report. Animals used in
43 research, and eventually in therapeutic applications of xenotransplantation, should be treated
44 humanely and with respect.
45
46

1 **SCIENTIFIC CHALLENGES IN XENOTRANSPLANTATION**

2
3 Technical challenges to xenotransplantation are primarily immunologic and physiologic in
4 nature. Although these challenges are common to both human-to-human transplantation
5 (allotransplantation) and xenotransplantation procedures, the various differences and disparities
6 between species tend to exacerbate these issues in xenotransplantation.

7 8 **Immunologic Rejection Processes**

9
10 Immunologic problems such as acute cellular rejection and chronic rejection are faced in any
11 transplantation procedure. In xenotransplantation, however, a number of factors arising from
12 disparities between species intensify the immunologic processes that mediate rejection.

13 14 *Hyperacute Rejection*

15
16 Hyperacute rejection²⁰ is the nearly immediate and catastrophic destruction of a graft. This
17 process is initiated by antibodies, often called natural antibodies, that are present and circulating
18 in all humans. For xenotransplantation, the most important natural antibodies are directed to a
19 specific sugar molecule expressed by most species, including pigs, called galactose-(a1-3)-
20 galactose (Gala 1-3Gal) (abbreviated aGal). Because human cells do not express this sugar
21 molecule, the human immune system recognizes it as “non-self,” or foreign, and produces
22 antibodies against it in response to its presence on common microorganisms. Most other
23 mammalian species (except Old World monkeys and Chinese hamsters) express aGal on the
24 proteins and lipids that are present on their cell surfaces. When a vascularized
25 xenotransplantation product that expresses these aGal sugars is placed into a human or Old-
26 World, non-human primate recipient, the circulating natural antibodies quickly bind to the sugars
27 and activate a destructive cascade of protein interactions along what is known as the complement
28 pathway. These antibodies and the process of complement-mediated destruction are elements of
29 natural human immunity against invading microorganisms.

30
31 Most antibody binding occurs first in the bloodstream, on the surface of endothelial cells that line
32 the blood vessels. With complement activation, these endothelial cells are activated, injured, and
33 often killed. Simultaneous activation of the blood coagulation systems leads to the generation of
34 fibrin clots, which can obstruct the blood vessels feeding the xenotransplant and cause tissue
35 destruction due to lack of blood flow.

36
37 Both the complement and coagulation cascades are normal responses to infection and injury. An
38 example of these processes at work in human-to-human transplantation is the severe reaction that
39 occurs when organs are transplanted across ABO blood group types. These events are typically
40 down-regulated by other proteins that are secreted into the serum by endothelial cells. However,
41 some of these down-regulatory proteins do not work uniformly well between species. For this
42 reason, the complement and coagulation cascades may be more powerful in xenotransplantation
43 than in allotransplantation across human blood group barriers.

44
45 Antibody-mediated rejection can also affect non-vascularized transplants (e.g., islets), but not in
46 the same rapid, dramatic manner. Moreover, islets from adult pigs do not express the aGal

1 carbohydrate. As is discussed later in the report, genetically engineered pigs have recently been
2 generated that lack the expression of the aGal sugar.

3 4 ***Delayed Vascular Rejection***

5
6 Major advances have been made in the prevention of hyperacute rejection by using organs from
7 pigs that are genetically engineered to express proteins that regulate human complement and
8 transplanting these organs into non-human primates. With these advances, however, another
9 immunological barrier to successful xenotransplantation, termed delayed vascular rejection, was
10 revealed.^{21,22} This form of rejection occurs over days to a few months and involves the vascular
11 system of the xenotransplantation product. Pig kidneys and hearts transplanted into non-human
12 primates stimulate progressive destruction of the pig blood vessels. Antibodies and inflammatory
13 immune cells are found in these vascular lesions. The exact immune mechanisms responsible for
14 delayed vascular rejection are not yet fully known. Anti-aGal antibodies (despite absorption
15 procedures to remove them), along with other types of anti-pig antibodies responding to the
16 xenotransplant, attack the graft by localizing to its blood vessels. Still other cellular components
17 of the innate immune system rapidly attack and remove invading organisms. This system
18 includes macrophages, natural killer cells, and primitive types of T-cells. Some of these
19 elements are activated by the products of antibody binding and complement activation just
20 described, or they may be activated independently by xenogeneic cells. Because delayed
21 vascular rejection has limited the maximum survival time of most organ xenotransplants to 2–3
22 months, the future success of these procedures will require an improved understanding of the
23 mechanisms of this reaction and the formulation of effective therapeutic strategies.

24 25 ***Acute Cellular Rejection***

26
27 Acute cellular rejection^{7,23} occurs over weeks or months after transplantation. This phenomenon
28 is mediated predominantly by T-cells, which constitute the cellular arm of the immune system.
29 Acute cellular rejection can target blood vessels but usually involves infiltration of attacking host
30 T-cells (and other immune cells) into the transplant tissues and destruction of the epithelial cells
31 that are responsible for the function of the xenotransplanted organ.

32
33 Cellular rejection, though a major problem after allotransplantation, has not yet presented a
34 major barrier to the survival of experimental xenotransplants. This may be due to the high doses
35 of immunosuppressive drugs that are currently used in xenotransplantation studies to block
36 activation of the cells that mediate this process. A number of studies have nonetheless provided
37 evidence that the T-cell immune response to xenogeneic (including porcine) antigens is stronger
38 than the response to non-self antigens of the same species. This may be because human anti-pig
39 T-cell responses appear to involve a greater number of antigenic disparities than do pig anti-pig
40 T-cell responses. The problem is compounded by the fact that many of the molecular interactions
41 between T-cells and foreign cells that express antigens seen by the T-cells seem to function
42 perfectly well between pig and human. This makes the human anti-pig T-cell response highly
43 effective. Since massive doses of immunosuppressive drugs will not remain acceptable as long-
44 term management of this vigorous human anti-pig response, its continued prominence will
45 necessitate other strategies to make widespread xenotransplantation feasible. Thus acute cellular
46 rejection may yet become an issue in future xenotransplantation studies.

1
2 ***Chronic Rejection***
3

4 Chronic rejection is the progressive and relentless destruction of a transplant over months to
5 many years. As one of the major causes of late graft loss from human donors, chronic rejection
6 is currently regarded as a major hurdle in allotransplantation. Antibodies induced by the organ
7 graft may play a role in the process. For some transplanted organs (e.g., hearts), a poorly
8 understood, distinct pathological process results in such severe narrowing of blood vessels that
9 the underlying tissue is starved of essential nutrients. This process, as well as direct attacks on
10 the cells that create and maintain the structure and functions of the transplant, may eventually
11 result in large areas of dead tissue, which are ultimately replaced by fibrotic scars.
12

13 Chronic rejection has not been widely observed in xenotransplantation because of the generally
14 short survival of xenotransplants. Because the mechanisms of chronic rejection are incompletely
15 understood and there is no effective therapy at present, this problem is likely to become more
16 prominent when the initial barriers are surmounted and graft survival is extended.
17

18 ***Additional Species Differences That May Affect Xenograft Function***
19

20 Other differences between species are also of concern in xenotransplantation. In addition to their
21 relevance to organ function, physiologic differences between species also compound
22 immunologic incompatibilities. Species differences in responses to hormones and growth and
23 other regulatory factors are additional potential barriers to xenotransplantation, which would
24 ordinarily require little consideration in allotransplantation. Current knowledge of these
25 potential challenges is extremely limited.
26

27 ***Complement and Coagulation Systems***
28

29 An important advance in elucidating the critical roles of the complement, coagulation, and
30 clotting systems^{24,25} came with the transgenic engineering of pigs to express human complement
31 regulatory proteins on their blood vessels. Although pig complement regulatory proteins that are
32 broadly homologous to their human counterparts are normally present on pig blood vessels, the
33 porcine versions of these molecules are sufficiently different so as to be unable to fully regulate
34 the activation of human complement. The transgenic addition of the human proteins to pig
35 endothelial cells increases the total amount of complement regulatory protein that is expressed
36 and adds molecules that may be more effective than their porcine counterparts in inhibiting
37 human or non-human primate complement.^{26,27,28,29}
38

39 Other incompatibilities in the mechanisms by which the complement and coagulation system is
40 activated in pigs and primates are also likely to be important. For example, investigators
41 performing xenotransplants from pigs to non-human primates have noted a systemic coagulation
42 disorder that begins with excessive coagulation and clotting, leads to consumption of clotting
43 factors and platelets, and ends in a bleeding disorder.¹⁷ The endothelial cells that line blood
44 vessels produce several types of anti-coagulation molecules that are critical to normal blood
45 flow. Patients with genetic defects in the production of these anti-clotting molecules have a high
46 risk of clotting and tissue injury. If pig anti-clotting proteins show similar incompatibility with

1 human blood coagulation factors, the increased risk of clotting might contribute to
2 xenotransplant rejection. This process appears to occur along at least one anti-coagulant
3 pathway, and other, undiscovered incompatibilities are also likely to exist in this complex
4 system.

5 6 ***Adhesion Molecules, Cytokines, and Growth Factors***

7
8 The normal function of any organ or tissue requires the regulation of its mass and architecture in
9 addition to repair of injury. These processes involve dynamic interactions at the cellular level
10 among factors that are carried in the circulation from a distance or are produced locally. It is
11 therefore critical to consider potential incompatibilities between growth and regulatory factors,
12 adhesion molecules, and their receptors in humans and animal organ sources.

13
14 These types of interactions may come into play in the transplant of non-vascularized tissue grafts
15 (e.g., cartilage, neural tissue, thymus), which require the growth of new blood vessels from the
16 recipient to maintain their nutrition and survival. In addition, the survival of intravenously
17 injected cellular transplants (e.g., islets, bone marrow cells) depends on their ability to “home” to
18 the right microenvironment, a process that depends on the interactions of specific adhesion and
19 chemoattractant molecules and their ligands as well as the ability to respond to growth factors
20 once they reach that microenvironment. Some researchers have already identified adhesion
21 molecules and growth factors that affect pig bone marrow homing and function but that do not
22 show normal interactions between pigs and primates.³⁰

23 24 ***Organ-Specific Physiologic Considerations***

25
26 Critical physiologic and/or metabolic functions are performed by the cells of every organ. The
27 cells of the heart do not merely constitute a biological pump, however, nor do the cells of a
28 kidney merely constitute a biological filter. The complexity of organ function presents a
29 challenge to both xenotransplantation and the development of artificial organs.^{31,32,33} For
30 example, cells in the liver, lungs, and kidneys take up and process drugs and body toxins.
31 Failure of a porcine graft to perform these functions normally could have profound and
32 unexpected side effects. Kidneys and other organs maintain normal levels of circulating
33 electrolytes, water, sugar, and other biochemical products, and these levels may be subtly
34 different in pigs and primates. In addition, because they have an upright posture, humans’ heart
35 valve size, pulmonary circulation, and other physiologic functions are different from those of
36 four-legged animals.

37
38 Human organs that are frequently transplanted (i.e., kidney, liver, heart) also produce a variety of
39 growth and metabolic factors that are largely unrelated to their primary function. For example,
40 in addition to fulfilling its primary role as a filter, the kidney produces the hormone
41 erythropoietin, which regulates normal blood cell production in the bone marrow and prevents
42 life-threatening anemia. Some evidence suggests that pig erythropoietin may not work or may
43 be destroyed by an immune response in non-human primates. This has resulted in severe anemia
44 in non-human primates maintained on porcine kidney xenografts.³⁴ This limitation could
45 theoretically be overcome by using transgenic pigs that produce human erythropoietin, but the

1 requirements for generating many such organ-specific factors could impose significant
2 constraints on the applicability of xenotransplantation.

3
4 Liver xenotransplantation poses the greatest challenge in regard to organ-specific differences
5 between species.³³ The liver produces innumerable proteins that are involved in normal clotting,
6 complement function, drug metabolism, metabolism of normal biological waste products,
7 digestion, lipid metabolism, and more. It will be critical to determine which of the normal
8 human homeostatic mechanisms can and cannot be maintained by the pig liver by elucidating the
9 interactions of pig and human molecules that regulate these functions and characterizing the
10 similarities and differences between pig and human physiology. It may be prudent to undertake
11 such research even before long-term organ graft survival has been achieved in non-human
12 primates in vivo, when these problems might come to light. Early initiation of molecular and
13 comparative physiologic studies would allow investigators to anticipate potential dysfunction
14 between species and to potentially circumvent problems by genetically engineering the source
15 animal.

16 ***Size Considerations***

17
18
19 Adult pigs typically weigh 1,000 pounds and obviously have organs that are too large for use in
20 humans. The use of very young porcine source animals has resolved the problem of size in non-
21 human primates. However, studies have not yet involved orthotopic, functioning heart
22 transplants (i.e., organs transplanted in place of the recipient's original organ), and survival of
23 heterotopic transplants (organs transplanted alongside the recipient's original organ) has not
24 exceeded a few months. It is unknown whether the growth potential of these porcine organs
25 could ultimately result in physiologic problems in recipients. A potential solution to this
26 problem would be to use adult miniature swine, whose maximum weight of about 250 pounds
27 more closely approximates adult human size.

28 29 **Current Approaches to Xenotransplantation Challenges**

30
31 The issues described in the preceding sections would have to be successfully resolved in order
32 for xenotransplantation to be considered as a human therapy. It is likely that a combination of
33 strategies will be needed to address these challenges. Some of the most promising strategies are
34 genetic modification of source animals, development of devices to encapsulate or otherwise
35 isolate the transplant, creation of tolerance to the xenotransplantation product, gene therapy,
36 targeted molecular therapy, and several others.

37 38 ***Genetic Modification of Source Animals***

39
40 One of the major advantages of the use of pigs as source animals for xenotransplantation
41 products is their excellent breeding capabilities in captivity. These breeding characteristics
42 facilitate genetic engineering and allow the rapid transmission of introduced genetic
43 modifications into the herd, as well as their combination with other genetic modifications.
44 Several human genes have already been introduced into pigs as transgenes, and advances in
45 nuclear transfer techniques have permitted the recent development of pigs that do not express
46 aGal on cell surfaces (referred to as *aGal knockout pigs*; see "Other Host Treatments" under

1 “Current Approaches to Xenotransplantation Challenges”). It is likely that the optimal pig for
2 xenotransplantation will require multiple genetic manipulations, which could be facilitated by
3 increasing experience with the techniques for porcine genetic modification, developing more
4 efficient techniques for genetic modification, and sharing of proprietary genes or genetically
5 modified pigs to allow their combination in a single animal.

6
7 **Transgenic source animals.** Investigators have produced several strains of transgenic pigs that
8 express human proteins that down-regulate activity of the human complement cascade involved
9 in hyperacute rejection. These human proteins were selected because some of the corresponding
10 proteins in pigs do not fully down-regulate human complement activity. It is thought that the
11 expression of human complement regulatory proteins at higher than physiologic levels might
12 significantly diminish antibody-mediated destruction of the graft. Some of these proteins have
13 been introduced into pigs, and all showed some efficacy. In some cases, the proteins reduced or
14 prevented hyperacute rejection of porcine solid organs that had been transplanted into non-
15 human primates.^{26,27,28,29,35,36,37} Another transgenic approach involves the expression in porcine
16 source animals of an enzyme that glycosylates porcine glycoproteins and glycolipids in a
17 manner that masks the expression of the aGal carbohydrate on these molecules.^{35,38}

18
19 Survival of up to approximately 135 days has been achieved for genetically engineered pig
20 organs implanted into non-human primates. Organs from these transgenic pigs may function
21 even better in humans, because the humanized pig organs may induce immune responses in non-
22 human primates that would not occur in humans. Similarly, the human proteins may not interact
23 optimally with the non-human primate complement components that they are expected to
24 regulate.

25
26 Although these studies have demonstrated the efficacy of the transgenic approach in preclinical
27 models, they have also highlighted the fact that hyperacute rejection is only one of several
28 rejection processes that can rapidly destroy solid-organ xenografts. Transgenic pig heart and
29 kidney grafts have been lost in days to months to the process of delayed vascular rejection,
30 probably because of the binding of antibodies to the endothelium of the xenogeneic organ.
31 Delayed vascular rejection may also involve cells of the innate immune system, such as
32 macrophages and natural killer cells, and these may also play a role in graft loss.

33
34 Islets from the tilapia fish have been shown to be capable of maintaining normal blood sugar in
35 mammals. These animals are an attractive source of islets in part because of their abundance and
36 ease of isolation. They can also be genetically modified to improve their suitability as a
37 xenograft source and, indeed, their insulin genes have been effectively “humanized” to make
38 them less likely to incite immune responses in humans.¹⁸

39
40 Researchers have recently developed a technique of nuclear transfer in which a genetically
41 modified donor cell nucleus can be used to replace the nucleus of a germ cell (an oocyte). This
42 development provides an approach to making site-specific genetic modifications. The technique
43 has been used successfully to generate transgenic animals and may be more efficient than earlier
44 strategies.

1 Additional transgenic strategies are also under consideration or development. One of these is
2 genetic modification to protect the source organ's endothelial cells from activation and death in
3 the face of antibody and complement activation. Another is engineering pigs to express
4 molecules on the cell surface that are inhibitory of immune responses. Still other transgenic
5 modifications that are currently being considered would make porcine bone marrow cells more
6 receptive to adhesion molecules and growth factors in the human marrow microenvironment,
7 where bone marrow homing and function are regulated. Such modifications would be aimed at
8 making porcine bone marrow cells more effective for the induction of immune tolerance to the
9 source animal (see "Tolerance").

10
11 **Knockout pigs.** Until recently, the development of genetic modifications in which specific
12 genes are targeted for mutation has been an elusive goal in the field of xenotransplantation.
13 Researchers consider the most important target of gene knockout strategies to be aGal
14 transferase, the gene that leads to the production of the ubiquitous aGal carbohydrate that is the
15 target of hyperacute rejection and delayed vascular rejection of porcine xenografts in primates
16 (see "Scientific Challenges in Xenotransplantation"). A major advance in this direction has been
17 made with the recent success of a nuclear transfer approach to develop knockout pigs that lack
18 the aGal transferase gene.^{39,40} The viability of pigs with both copies of the gene knocked out is
19 very encouraging.⁴¹ Pig-to-primate transplant studies using these knockout source animals are
20 now in progress, and promising preliminary data provide proof of principle that porcine renal
21 xenografts can survive in non-human primates for longer than 80 days with no evidence of any
22 type of rejection (public presentation to the SACX). Although antibodies to aGal are currently a
23 major challenge in pig-to-primate xenotransplantation, it should be borne in mind that the
24 absence of aGal in knockout pigs may expose other antigens that may evoke an immune
25 response.

26 27 *Encapsulation and Other Bioartificial Isolation Devices*

28
29 Encapsulation of xenotransplantation products represents an approach to protecting cellular
30 transplants, such as islets, from destruction by proteins and cells of the immune system. In this
31 technique, the transplant is encapsulated in a material that is impermeable to destructive factors
32 but allows the diffusion of nutrients from the recipient's body fluids into the encapsulated cells,
33 as well as the diffusion of the desired product of the encapsulated cells (e.g., insulin) into the
34 recipient's bloodstream.⁴² This approach has been under investigation for many years in the
35 allotransplantation field. Although no product with demonstrated clinical utility is yet available,
36 research in both academia and the biotechnology industry may ultimately yield useful
37 technologies.

38
39 Another approach to protecting cellular xenografts includes the in vivo production of a
40 bioartificial isolation environment. This could be accomplished by, for example, implanting a
41 tubular scaffold that would become coated with collagen from the recipient and neovascularized.
42 The xenograft would then be implanted inside this device.

43 44 *Tolerance*

1 Although numerous approaches to inducing tolerance between individuals of the same species
2 have been developed in rodents, only a few of these methods have been successfully applied in
3 large animals and in xenotransplantation models. The approaches that have shown success in
4 these latter, more difficult settings are hematopoietic cell transplantation and thymic
5 transplantation. In addition, “costimulatory blockade” has shown some success with islet
6 xenotransplantation in rodents. However, costimulatory blockade alone and other approaches,
7 including a variety of monoclonal antibodies, donor antigen infusions, and certain drugs, have
8 not yet been successfully applied in more stringent large-animal or human transplantation
9 settings, even within a species. In contrast, hematopoietic cell transplantation has shown to
10 successfully achieve tolerance in humans.^{43,44}

11
12 Because of the potency of the human anti-pig antibody and T-cell responses, some researchers
13 believe that the induction of tolerance will be necessary for the clinical success of
14 xenotransplantation. Without tolerance, highly toxic, broadly immunosuppressive drugs would
15 be needed to prevent rejection.

16
17 Hematopoietic cell transplantation has been shown to induce simultaneous tolerance among all
18 classes of antibody-producing cells as well as of T-cells in xenogeneic combinations.^{45,46,47} An
19 advantage of this approach over those that aim to remove aGal from the source animal or to
20 specifically suppress the immune response to aGal is that cell transplantation tolerizes to all
21 donor antigens expressed by hematopoietic cells, including those that might become important
22 targets of rejection in the absence of aGal. Xenogeneic bone marrow transplantation to aGal-
23 negative recipients (aGal knockout mice) has been shown to lead to tolerance not only of cells
24 that make antibodies to aGal, but also to those recognizing other antigens on the xenogeneic
25 source animal.

26
27 The use of hematopoietic cell transplantation has previously been limited by the requirement for
28 very toxic host treatments to achieve successful engraftment. However, investigators have
29 recently developed animal models of allogenic and xenogeneic transplantation, and even clinical
30 approaches to allogenic bone marrow transplantation, that achieve marrow engraftment with less
31 toxic treatment of the recipient. An immunodeficient mouse model has recently provided proof
32 of principle that porcine marrow engraftment tolerizes human T-cells developing in a human
33 thymus.⁴⁸ However, success has not yet been achieved in non-human primate models of pig
34 hematopoietic cell transplantation. The reasons for this lack of success are largely because the
35 barriers to engraftment posed by the immune system have not been fully addressed, and because
36 physiological incompatibilities limit porcine marrow function in primate recipients.¹⁷ This
37 approach would be moved forward by the development of better reagents for temporarily
38 depleting cells of the immune system in primates, as well as more information on the nature of
39 the physiologic incompatibilities described here (see “Physiologic Issues”).

40
41 Another promising approach for the induction of T-cell tolerance involves the transplantation of
42 porcine thymus tissue. T-cells develop in the thymus, and studies have shown that pig thymus
43 grafts can induce tolerance among developing T-cells of other species, including humans.^{49,50}
44 This approach, however, would have to be used in combination with some other strategy for
45 overcoming the antibody problem, because antibody-producing cells are not tolerized by thymic
46 transplantation. In addition to hematopoietic cell transplantation, another approach that has

1 induced tolerance of the B cells that produce natural antibodies to a xenograft in rodents is the
2 infusion of donor antigens along with treatment with certain drugs.⁵¹

3 4 ***Gene Therapy***

5
6 Gene therapy involves the transfer of genetic material (DNA or RNA) in order to provide a copy
7 of a gene to alter cells in a manner that is beneficial. This technique could be used to modify
8 either source animals or recipient cells. An example of the former is the in vitro transfection of
9 porcine neural stem cells with a gene to protect the cells from immune destruction in the
10 xenogeneic environment. Many technical hurdles must be overcome, however, before this
11 technique will be practical.

12
13 Recipient cells could also be modified to facilitate xenotransplantation. Recipient bone marrow
14 can be engineered to incorporate the aGal transferase gene. After a conditioning procedure to
15 prepare the recipient, reimplanted recipient marrow will express aGal and induce tolerance of
16 anti-aGal antibody-producing cells.⁵² Although this approach is promising as a component of a
17 xenotransplantation strategy, it is limited to tolerization to known antigens for which a gene can
18 be identified. It is unlikely on its own to permit the induction of tolerance to the wide variety of
19 the many unknown foreign antigens expressed by the pig. In addition, gene therapy has its own
20 set of safety issues, often associated with viral vectors used for gene transfer (see “Gene
21 Therapy” under the section “Parallel or Alternative Strategies to Xenotransplantation”).

22 23 24 ***Targeted Molecular Therapies***

25
26 A number of new drugs are being developed to inhibit the activation of the complement and
27 blood clotting systems that follow antibody binding and complement activation. These agents
28 include soluble inhibitors of complement activation-some of which are quite promising-and
29 inhibitors of coagulation.¹⁷ Further knowledge of species incompatibilities in the regulation of
30 coagulation and clotting could permit the development of additional, useful agents of this type.

31 32 ***Other Host Treatments***

33
34 Removal of anti-aGal antibodies before xenotransplantation has been used to prevent hyperacute
35 rejection.¹⁷ This can be accomplished by perfusion of pig organs with the non-human primate
36 recipient’s blood, perfusion of extracorporeal columns bearing aGal sugars that can absorb
37 natural antibodies from the plasma, or infusion into the recipient of conjugated aGal sugars or
38 substitutes that absorb the anti-aGal antibodies in vivo. Success in preventing hyperacute
39 rejection and even delayed vascular rejection in some non-human primate studies has been
40 tempered by the inevitable occurrence of delayed vascular rejection once the antibody removal
41 procedure is discontinued and antibodies are allowed to recover. This rebound of anti-aGal
42 antibodies has been a recurring problem in all such studies.

43 44 45 **INFECTIOUS DISEASE RISKS ASSOCIATED WITH XENOTRANSPLANTATION**

1 Allotransplant procedures have associated risks caused by known human pathogens. Infectious
2 disease risks associated with xenotransplantation, however, have unique features that could pose
3 a threat to public health. Pathogens in source animal organs, tissues, cells, or body fluids could
4 result in the undetected spread and establishment of novel infections in the humans.

5
6 Xenotransplantation may allow infectious agents from source animals to circumvent natural
7 barriers, such as mucosal surfaces and skin, through the surgical placement of an animal organ in
8 a human. If an infectious agent from a source animal is transmitted to a human recipient, the
9 agent may then be passed on to other humans. Risks from xenotransplantation include both
10 acute and chronic persistent viral infections. The infectious agents may not have caused disease
11 in the source animal. Infections that are asymptomatic in the animal but pathogenic in humans
12 have occurred. HIV-1, for example, originated from chimpanzees, where it causes no disease,
13 but the virus induces AIDS in humans.

14
15 Parasitic, bacterial, and mycotic infections also pose a risk but are of less concern because of the
16 availability of diagnostic screening methods, effective animal husbandry practices, and
17 antimicrobial treatments to eliminate known potential pathogens. Nevertheless, oversight of the
18 emerging field of xenotransplantation requires vigilance in preventing all infections.

19
20 The magnitude of the risk associated with xenotransplantation would be influenced by a number
21 of factors:

- 22
- 23 • The requirement for immunosuppressive agents
- 24 • The species of the source animal and the applicable husbandry practices
- 25 • The type of tissue or organ used and the duration and type of recipient exposure
- 26

27 Many of these issues have been addressed in earlier publications,^{53,54,55,56,57,58,59} including peer-
28 reviewed manuscripts, chapters, and FDA and PHS guidelines. This report highlights the areas
29 considered most critical and in need of further research and action.

30 **General Properties of Infectious Agents Relevant to Xenotransplantation**

31
32
33 The microbial agents that are of concern in the transplant setting can be broadly classified as
34 exogenous and endogenous. Exogenous agents are transmitted from an infected individual or
35 animal to a susceptible host. Examples include viruses (e.g., HIV, herpes simplex virus, and
36 viruses that cause the common cold); bacteria (e.g., *Streptococcus pneumoniae* and
37 *Mycobacterium tuberculosis*); fungi (e.g. *Aspergillus*); and parasites (e.g., *Toxoplasma gondii*).
38 Some exogenous microbes can be transmitted across the placenta. The organisms that pose the
39 greatest hazard to public health in xenotransplantation are those that persist asymptotically in
40 quiescent or latent phases in source animals. Active screening for agents such as herpesviruses,
41 arenaviruses, and retroviruses will be essential. Although fungi and parasites show similar
42 persistence and transmission patterns, these exogenous microbes are more visible and well
43 recognized and can be more readily excluded or removed from the chain of transmission by
44 screening and husbandry techniques.

1 In contrast to exogenous viruses, endogenous agents are essentially embedded in the host
2 genome and therefore are considerably more difficult to eliminate from source animals. Many
3 endogenous viruses are defective, but a few do replicate and may be capable of infecting other
4 animal species, including humans. These endogenous viruses have been detected in all animal
5 species and have not yet been eliminated from source animals by either classical husbandry
6 techniques or more sophisticated knockout technologies.

7
8 There are many known infectious agents in source animals and many diagnostic tests and
9 husbandry practices capable of reducing the risk. Although these agents represent a major risk
10 in unregulated xenotransplantation, they can be largely controlled by measures discussed
11 elsewhere in this report (see “Viral Persistence, Latency, and Species-Specific Virulence”).
12 However, there is no doubt about the future emergence of unknown exogenous and endogenous
13 infectious agents, for which no means of detection currently exist. Examples include recent
14 outbreaks of severe acute respiratory syndrome (SARS) and Nipah and Hendra viruses, which
15 were transmitted from animals to humans. As yet undiscovered, novel transmissible agents
16 constitute a potential risk to the transplant recipients themselves, their intimate contacts, health
17 care workers, and the population at large.

18 **Non-Human Primate and Porcine Source Animals and Infectious Disease Risk**

19
20
21 Current strategies in xenotransplantation focus on swine as source animals, although several
22 other species have been proposed. Heightened concerns over the potential transmission of
23 infection from apes and monkeys to humans have further focused xenotransplantation studies on
24 porcine cells, tissues, and organs. Concern about the use of non-human primates comes partly
25 from prior experience with xenografting of their organs and cells.⁶⁰ In experiments carried out in
26 the 1960s and 1970s, for example, humans with transplanted chimpanzee kidneys survived for as
27 long as 9 months. However, the compelling reason to assess the risk of infection in these
28 recipients was not appreciated before the identification of latent retroviral infections in non-
29 human primate source animals. In 1993, simian retroviruses and herpesviruses were detected in
30 two human recipients of baboon livers.⁶¹ Because both patients survived only briefly after
31 transplantation, the ability to determine the level of risk from these infections was limited.
32 Evidence of simian foamy virus (SFV) was detected in several tissues taken from both autopsied
33 patients; however, whether retroviral transmission from non-human primate cells to human cells
34 occurred could not be resolved.⁶² SFV is an apparently nonpathogenic retrovirus found in most
35 non-human primates but represents a risk of uncertain magnitude in xenotransplantation. A
36 second simian virus, baboon cytomegalovirus, was also isolated from the blood of one of the two
37 liver recipients.⁶³ Human cytomegaloviruses are routinely transmitted from human donors to
38 recipients and can cause serious complications, and although cytomegaloviruses have generally
39 proven to be species-specific with regard to pathogenicity, concern remains that the baboon
40 homologue might result in human disease.

41
42 A 1996 baboon-to-human bone marrow xenotransplant highlighted uncertainty about
43 transmission of infectious disease.⁶⁴ The recipient was a patient with AIDS whose bone marrow
44 transplant survived for only 13 days. The source material contained two known simian viruses
45 (baboon endogenous virus and baboon gamma herpesvirus), but there was no evidence of
46 recipient infection. The patient may have been spared from infection because the transplanted

1 baboon cells were present only transiently. With knowledge of these and other simian viruses
2 harbored by non-human primates, along with ethical considerations on rearing animals in closed
3 environments, the FDA published *Guidance for Industry* in 1999 urging researchers not to
4 consider non-human primates as source animals for human xenotransplantation.⁶⁵
5

6 Another source of concern regarding transmission of simian viruses to humans is the historical
7 record of the development of the childhood viral vaccines in the 1950–1970 time frame, in which
8 monkey kidneys were used as a source of cell substrates for the propagation of vaccine viruses.
9 Dozens of novel viruses were identified as exogenous contaminants of cells used for vaccine
10 production and represented actual or potential contaminants of early viral vaccine preparations.
11

12 Swine also carry several infectious agents that are transmissible to humans. Although transfer of
13 viruses from pigs to humans has been documented and has public health implications (e.g., the
14 worldwide influenza epidemic of 1918 is thought to have originated in pigs), these types of
15 infections would be excluded from an FDA-approved source animal colony. Accordingly, many
16 experts believe that the pig generally poses less of a risk of infection than does the non-human
17 primate.
18

19 The anxiety about transmission of pig viruses to humans through xenotransplantation was
20 recently rekindled by studies demonstrating that a virus called porcine endogenous retrovirus
21 (PERV) could infect human cells in culture.^{66,67,68} As with any zoonotic infections, the risk of
22 PERV transmission from carriers to contacts (e.g., sexual contacts, health care workers) presents
23 a potential risk. Current efforts have focused on understanding the determinants of PERV
24 infection and disease in transplant recipients, but determining the potential risk from endogenous
25 retroviruses remains a daunting task. The overall lower risk from porcine xenografts can be
26 minimized through good animal husbandry practices, construction of barrier-contained breeding
27 facilities, appropriate controls for surgical procedures to harvest organs, and screening of source
28 animals for known infections. It is also possible that an infected blood donation or other
29 recipient contact with an infected individual could spread new, unidentified pathogens.
30 Infectious risks from animal sources will continue to warrant more intensive investigation as the
31 field of xenotransplantation matures.
32

33 **Other Sources of Xenotransplantation Products** 34

35 As discussed earlier, pigs are currently considered the most likely sources for
36 xenotransplantation products. Other species, however, may also prove useful, including the fish
37 tilapia as a source of islet cells, and mice as a source of primary cell feeder layers for some stem
38 cell applications.⁶⁹ FDA guidance specifies that even autologous transplants of human tissues
39 are considered xenotransplants when feeder layers employ cells of a different species.⁶⁷
40

41 The principles for preventing contamination of the porcine-derived xenotransplantation products
42 by endogenous and exogenous infectious agents are essentially the same as those for products
43 derived from other source animals and their production processes (e.g., reagents, facilities,
44 equipment, staff, product containers). Details of the control procedures may require adjustment
45 for the specific properties of the endogenous and exogenous infectious agents associated with the
46 source species, their mode of transmission, and the most efficient means for their monitoring and

1 control. However, appropriate methods to control potential infectious agents in the environment,
2 in the reagents and raw materials, and harbored by staff who handle the animals and animal-
3 derived materials remain largely the same. These controls are needed because the organ, tissue,
4 or primary cells often cannot be characterized in detail before the product is used. The use of
5 source animal procedural and environmental controls should help to limit or prevent
6 contamination of the transplant product.

7
8 Some products in use in humans are actually xenotransplantation products, as defined by the
9 FDA and the PHS, because they use animal cell feeder layers (e.g., autologous human skin cells
10 grown on murine 3T3 feeder layers) based on characterized banked cells. Xenotransplantation
11 products could use characterized banked cells either as a direct source of transplanted cells or as
12 feeder layers for autologous human tissues. Xenotransplantation products based on cell lines that
13 can propagate and maintain their differentiating characteristics (e.g., murine embryonic stem
14 cells) may be developed in the future.

15
16 Appropriate controls rely on characterization of the banked cells as well as on procedural and
17 environmental management of the application process (e.g., scale-up of cells from the
18 characterized cell bank and their harvest for intended use). Decades of experience with
19 producing viral vaccines, recombinant DNA products, and monoclonal antibodies in cells from
20 characterized cell banks testify to the success of rigorous characterization efforts in keeping
21 these products free from the risks of infectious agents.^{70,71} The specific tests used for
22 characterization depend on the species of origin for the non-human cells, the exogenous and
23 endogenous viruses found therein, and the most appropriate microbiological methods for their
24 detection. In general, products made from characterized banked cells have significantly lower
25 risks of contamination and are safer to use than products using primary cells or other
26 uncharacterized cells or intact organs, due to the ability to perform more extensive testing and
27 characterization on banked cells.

28 29 **Viral Persistence, Latency, and Species-Specific Virulence**

30
31 Acute symptomatic viral infections can usually be identified and eliminated from source animals.
32 In addition, if a source animal infection is transmitted and leads to a rapid clinical course in the
33 patient (e.g., within days or weeks of exposure), the main risk posed is to the patient and health
34 care workers in the hospital setting. Hospital containment practices are generally useful in
35 restricting the spread of most acute infections.

36
37 Some viral infections, however, can remain in a latent state within the source animal and,
38 potentially, in transplant recipients for prolonged periods, even decades.⁷² These persistent or
39 chronic infections are of concern because they may not be recognized during the post-transplant
40 recovery period. Because infections by viruses in their natural host species commonly produce
41 little or no disease, it is difficult to make predictions about the relative virulence of an animal
42 virus in human recipients on the basis of its virulence in the natural host. Several examples exist
43 of asymptomatic infections in non-human animals that have become important emerging
44 diseases in humans. One is HIV, where natural infection of African nonhuman primates does not
45 lead to disease, but transmission to humans led to the establishment and spread of HIV-1 and a
46 global AIDS epidemic.⁷³ Source animals may harbor other known and unknown infectious

1 agents that might elude attempts at identification, possibly threatening public health.

2
3 Many new, unidentified infections could be avoided by establishing closed colonies of source
4 animals through cesarean derivation. Considerable attention has been given to intensive
5 breeding practices and maintenance of closed herds to minimize infections carried by pigs. Pigs
6 may carry viral infections that pose a measurable risk in xenotransplantation, and they may also
7 carry undefined viral infections with unknown potential consequences to public health.
8 Although many of these risks can be reduced with good herd management, the endogenous
9 infection PERV remains a current concern. To date, subjects who have received experimental
10 xenotransplants have not been found to be PERV infected. Although this is reassuring, some of
11 these studies were limited in the types of samples that could be evaluated. Recently identified,
12 genetically distinct strains of pigs appear to be incapable of producing replication-competent
13 PERVs.⁷⁴ A greater understanding of the biology of infectious PERV could lead to strategies for
14 avoiding such infections in human recipients of xenotransplantation products. Because of its
15 ability to replicate in human cells and the possibility that transplanted organs and cells harbor
16 and express PERV, efforts have been directed at establishing a basic understanding of the
17 infectious nature of PERV and its risk to humans in the transplant setting.

18 19 **Studies to Address the Risk of PERV in Humans**

20
21 Several studies have assessed the risk of infection with PERV by examining archived samples
22 from humans who had received porcine xenografts, had been exposed to porcine cells by ex vivo
23 perfusion of their blood through porcine liver or spleen, or had been exposed through a
24 bioartificial device containing porcine cells. More than 160 patients with various degrees of
25 exposure to pig cells have been studied.⁷⁵

26
27 To date, PERV infection in humans exposed to porcine cells or tissues has not been
28 demonstrated by either molecular or antibody-based methods. However, porcine cellular DNA
29 and PERV have been detected in human recipients exposed to porcine spleens.⁷⁵ In some cases,
30 porcine cells have persisted in these patients for as long as 8 years after transplantation. Since
31 PERV, and possible other porcine infections, could be associated with porcine cells circulating in
32 human patients, those patients may be at continual risk for PERV infection. Other studies have
33 failed to demonstrate PERV transmission to human recipients of pancreatic islet cells or to
34 patients exposed to extracorporeal perfusion from either transgenic porcine livers or bioartificial
35 devices containing pig hepatocytes.⁷⁶

36
37 These studies provided no evidence for transmission of PERV or other porcine virus to human
38 xenograft recipients. Although these results are reassuring, the studies were performed on
39 archived samples from recipients who had various degrees of exposure. Furthermore, no patients
40 have survived whole-organ porcine xenotransplants, which may confer a greater risk of infection
41 than other forms of xenotransplantation. Further work is necessary to assess the risk of PERV
42 transmission from porcine xenografts, especially with tissue expected to survive for long periods.

43 44 **Animal Models to Assess the Risk of PERV**

45
46 Animal model systems for xenotransplantation represent an important strategy for determining

1 the risk of PERV infection in the transplant setting. In a seminal study, mice implanted with
2 porcine pancreatic islet cells became infected with PERV.^{77,78} Genetic immunodeficiencies in
3 these mice may have facilitated their acquisition of PERV. (Mice do not generate the anti-aGal
4 antibodies that occur naturally and that neutralize PERV in humans and non-human primates and
5 thus would be more likely to become infected.) The study illustrates that PERV is actively
6 expressed during xenografting and can be infectious to other animal species. Further in vivo
7 testing and long-term xenograft survival will be needed to determine whether xenografts from
8 pigs to non-human primates and humans would have similar consequences.

9
10 Xenotransplantation models using pig source animals and non-human primate recipients have
11 been used to develop protocols for similar procedures in humans. These models have focused
12 primarily on strategies to overcome immunologic rejection. The advantage of using Old World,
13 non-human primates is that they, like humans, produce anti-aGal antibodies. As such, porcine
14 xenografts induce hyperacute rejection in both Old World primates and humans.

15
16 Despite biochemical and physiological differences between non-human primates and humans,
17 this model remains an important source for developing methods to overcome immunologic
18 rejection and to study the risk of infectious disease. The average survival to date for whole-
19 organ xenografts in a non-human primate has been short, although one recent study demonstrated
20 a 76-day median survival with heterotopic heart transplantation from swine (MacGregor,
21 personal communication, February 2003). Several monkeys succumbed from reactivation of
22 simian cytomegalovirus, as could occur in humans undergoing immunosuppression in the
23 absence of anti-viral agents, because of widespread latent human cytomegalovirus infection of
24 the human population.

25
26 As with previous studies in humans exposed to porcine tissues or cells, there is also no evidence
27 for PERV transmission to monkeys experimentally treated with porcine tissues.⁷⁹ It is not
28 known whether baboons are susceptible to PERV, although one report suggests that PERV does
29 not replicate in non-human primate cells.⁸⁰ Long-term survival of porcine xenografts in
30 monkeys would set the stage for clinical trials in humans but may be limited in their true
31 assessment of the infectious disease risk from PERV.

32
33 Although all pig genomes carry PERV, some studies have raised the possibility that development
34 of inbred lines of pigs may lead to reduced risk for PERV transmission.⁸¹ These studies
35 demonstrate that certain inbred herds harbor PERV strains that are not infectious for human
36 cells, suggesting that these swine breeds may represent a reduced risk in the transplant setting.
37 Recent advances in understanding the biology of PERV infection of human cells raise the
38 possibility that the relevant PERV could be genetically engineered out of a source animal herd.
39 As encouraging as these findings may be, the risk from PERV in pigs has not yet been eliminated
40 and further studies will be necessary to characterize endogenous infections that are transmissible
41 to humans.

42
43 Another potential infectious disease concern may arise as a byproduct of efforts to develop
44 genetically altered pigs (e.g., aGal knockout pigs) to minimize immunologic rejection.⁸²
45 Naturally occurring aGal antibodies render PERV non-infectious by attacking the aGal
46 incorporated on the surface of the enveloped virus during budding of the aGal-positive pig cell

1 membrane.⁸³ Production of pigs that lack aGal may have the unintended effect of increasing the
2 infectious nature of PERV in humans.^{84,85} Further consideration must be given to strategies that
3 seek to overcome rejection but that may enhance associated infectious risks.

4 5 **Potential for Xenogeneic Infections Other Than PERV**

6
7 Many types of infectious agents may be transmitted from animals to humans via
8 xenotransplantation. Depending on the type of infection and the degree of risk, different
9 strategies can be adopted for prevention or control. The greatest attention should be paid to
10 whether an infectious agent is present in the source animal population and, more importantly, in
11 the graft itself. Next to be considered should be whether the microbial agent is known to infect
12 humans or human cells in vitro and, by extension, its potential risk to a transplant recipient.

13
14
15 All animals, including pigs, harbor bacteria in their upper respiratory and gastrointestinal tracts
16 as part of their normal flora. Most of these bacteria are harmless to the host animal. An example
17 is the bacterium *Pasteurella multocida*, which normally resides in the pig respiratory tract.
18 Although harmless to the pig, this organism can cause disease in humans. In the
19 xenotransplantation setting, the risk for transmission to humans via a xenograft would be
20 negligible as long as bacteria are absent from the bloodstream of a swine source animal. Control
21 measures to ensure the absence of occult bacteremia in source animal organs, tissues, or cells can
22 include blood cultures before harvest of a xenograft.^{1,51}

23
24 In contrast, porcine cytomegalovirus (PCMV), though not proven to infect humans, is a potential
25 pathogen via xenotransplantation. This virus is found in most swine tissues, can be transmitted
26 horizontally and *in utero* in swine, and leads to lifelong infection.⁸⁶ Persistently infected pigs
27 generally show no signs of disease, but like immunosuppressed humans, infected pigs treated
28 with immunosuppressive agents develop severe CMV-related disease.⁸⁷ The virus can be
29 excluded from porcine source animals by early weaning or cesarean delivery. Screening
30 procedures are available to exclude source animals that are positive for cytomegalovirus.⁶³

31
32 Other porcine herpesviruses include the porcine gamma-herpesviruses residing in cells or organs
33 that are likely to be harvested for xenotransplant. Among them are the recently identified
34 porcine lymphotropic herpesvirus (PLHV) types 1 and 2.⁸⁸ Fortunately, these viruses are
35 typically acquired postnatally. Infection should therefore be preventable by cesarean section,
36 removal of piglets from infected mothers soon after birth, and maintaining the animals in a
37 closed herd.

38
39 Other types of infectious agents that could be transmitted from source animals to humans include
40 parasites, such as *Toxoplasma gondii*, and viruses, such as hepatitis E virus, rabies, or infections
41 for which swine can act as an intermediate host (e.g., influenza viruses).^{89,90} Methods of
42 controlling these agents would vary according to the organism but would generally include
43 primary prevention of exposure of the pig to the outdoors, where *T. gondii* cysts reside, or
44 maintenance of a closed colony in which screening can exclude the agents.

45
46 For many infectious agents of animals, diagnostic tests remain in development or are unsuited

1 for testing in humans. Antibody-based assays to detect porcine infections of xenotransplant
2 recipients are important but have limitations. In some cases, immunologic profiles suggestive of
3 infection have not been validated, making verification of active infection difficult. Another
4 problem with antibody-based assays is that transplant recipients are generally given
5 immunosuppressive therapies that could obscure immune responses to possible infections.
6 Nucleic acid-based methods have not been standardized for most porcine infections and may not
7 be suitable for detecting viruses that replicate in tissues other than blood. In response to
8 concerns that tests should be standardized, in 2002 the FDA convened a workshop to address
9 problems associated with diagnostic assays to detect PERV and recommended further efforts to
10 develop standardized reagents and assays for PERV testing.⁹¹

11 **Control of Infectious Disease Risks**

12
13
14 Primary and secondary prevention of infection in source animals will be important to the safety
15 of xenotransplantation procedures. A number of methods are available to mitigate some of the
16 risks. No single method is capable of removing them all, but judicious combinations of control
17 methods are likely to provide the best possible results.

18
19 Current technology is probably adequate for the task of minimizing the known risks presented by
20 infectious agents transmitted by xenotransplantation. Differing combinations of control methods
21 will be needed for each product category. For example, the approach of controlling exogenous
22 contamination of cell-based transplants prepared from well-established cryopreserved cell lines
23 is likely to be more robust than that which is achievable with current technology for controlling
24 exogenous contamination of cells, tissues, and organs derived directly from the source animal.

25
26 Approaches to infection control in cryopreserved cell-based transplants can be summarized as
27 follows:

- 28
29 • Cell-based xenotransplantation products prepared from well-established cryopreserved cell
30 lines can be fully characterized for a spectrum of infectious agents before they are used in
31 production.
- 32
33 • Cells can be propagated by using biological safety cabinets housed in properly designed
34 rooms to prevent microbial contamination from the environment. Cell culture reagents can
35 be tested, prepared, and stored using standard technology for preventing microbial
36 contamination.
- 37
38 • If needed, existing technology for contained fermentation devices can be used to prepare very
39 large numbers of animal cells in culture.
- 40
41 • Aseptic processing technologies can be used to transfer animal cells from their growth
42 vessels to the patient for implantation.
- 43
44 • Specific tests for infectious agents can be performed at various stages of production, some as
45 soon as within half a day after harvest.
- 46

1 When appropriate measures are used, the risk of contamination by infectious agents can be
2 reduced to a very low level. This is evident from ongoing research involving cell-based
3 xenotransplantation products, produced under IND, for which no evidence of infection has been
4 found in recipients of swine or other animal products. Similar safety records exist for these
5 technologies when applied to the preparation of FDA-approved autologous human cell products
6 such as Apligraf and Carticel.

7
8 As with characterized cell lines, when cells derived directly from an animal are used to create a
9 xenotransplantation product, substantial influence can be exerted on the risk of contamination
10 during cell processing. However, control of microbial contamination is more difficult during
11 animal procurement and tissue removal. Full characterization of the cell banks may be possible
12 when a cell suspension can be created and aliquots of it can be cryopreserved for use after
13 characterization. If cryopreserved primary cell suspensions cannot be used (i.e., the product is
14 administered immediately after procurement with minimal processing), the risk of infection for
15 primary cells would approach that for organs and tissues.

16
17 Managing the risk of contamination of xenotransplanted organs or tissues used directly from
18 source animals is heavily dependent on animal husbandry techniques and conformity to federal
19 guidelines. Guidelines for selection and maintenance of source animals for xenotransplantation⁹²
20 are documented in *Xenotransplantation: Guidance for Industry: Source Animal Product, Preclinical, and Clinical Issues Concerning the Use of Xenotransplantation Products in Humans*⁵⁸
21 from the FDA Center for Biologics Evaluation and Research; the PHS *Guideline on Infectious Disease Issues in Xenotransplantation*⁴;
22 the World Health Organization's *Xenotransplantation: Guidance on Infectious Disease Prevention and Management*⁵⁶;
23 and guidelines from various countries and professional societies. Guidelines pertain to the use of
24 closed herds to exclude random source animals of unknown status. Cesarean section or animal
25 cloning to establish initial breeding colonies of source animals adds another layer of safety by
26 minimizing the possibility of perinatal transmission.

27
28
29
30 Yet another safety measure is uniform standards for maintaining source animals in a biosecure
31 environment, requiring all source animals to have a PHS assurance for laboratory animals and
32 U.S. Department of Agriculture (USDA) registration with periodic inspections. Accreditation of
33 facilities by the Association for Assessment and Accreditation of Laboratory Animal Care
34 (AAALAC) would ensure that facilities comply with all regulations. Conformity to current
35 regulations is considered an essential requirement for conducting clinical xenotransplantation.

36
37 Among the methods available to control infectious agents among herds of source animals are the
38 following:

- 39
- 40 • Use of specific pathogen-free animals, typically derived by cesarean section and raised under
41 barrier-sustained conditions with high-efficiency particulate air (HEPA) filters
 - 42
 - 43 • Use of genetic methods to remove or neutralize endogenous retroviruses
 - 44
 - 45 • Employment of closed herds to minimize the entry of infectious agents from other animals.
46 New animals would enter the herd only after quarantine and testing for the presence of

1 specific pathogens

- 2
- 3 • Use of pest control programs and sanitation methods
 - 4
 - 5 • Use of sentinel animals and antibody monitoring programs to screen for infectious agents
 - 6 that could contaminate the herd, staff, and xenotransplant product
 - 7
 - 8 • Employment of environmental controls over potential infectious agents that might be spread
 - 9 by air, drinking water, and staff
 - 10
 - 11 • Control of human infections, including influenza, through vaccination to prevent
 - 12 contamination of herds
 - 13
 - 14 • Accreditation of animal facilities by AAALAC, approval of registration by the USDA, and
 - 15 maintenance of inspection records to ensure compliance
 - 16

17 The current PHS guidelines are generally believed to be adequate at present but should be
18 reviewed and amended on a regular basis to reflect updated scientific knowledge and experience.

19

20

21 **“XENOTOURISM”: AN EMERGING GLOBAL PUBLIC HEALTH CONCERN**

22

23 The SACX believes that xenotourism constitutes a potential emerging public health risk. The
24 SACX defines “xenotourism” as the travel of U.S. residents to foreign nations to participate in
25 xenotransplantation programs or clinics for the purpose of obtaining therapies not presently
26 available or acceptable in the United States. Such xenotransplantation programs have offered
27 various animal cells, including rabbit, goat, pig, and shark, with claims of treating a multitude of
28 disorders. Some of these programs advertise the availability of luxury accommodations and
29 travel packages, often listing U.S. addresses and contacts that can facilitate patient evaluation.
30 Ready access to unregulated or inadequately regulated xenotransplantation clinics could have
31 global infectious disease implications.

32

33 Xenotourism is likely to be associated with violation of the principles underlying U.S.
34 recommendations. The consensus reached after many years of public discussions conducted by
35 the PHS is that transmission of animal pathogens to humans constitutes an infectious disease
36 risk, including a public health risk, that warrants caution and regulatory oversight. Oversight
37 within the United States will be incomplete if individuals receiving unregulated
38 xenotransplantation products abroad can potentially introduce a xenogeneic pathogen into the
39 population upon reentry into the country. At present, information is insufficient to permit review
40 of efficacy and safety data for these clinics or to determine whether the level of regulation of
41 these practices is appropriate in many of the host countries, nor have mechanisms been
42 established to obtain this critical information.

43

44 Concerns relate to the possibility that xenotransplantation procedures might be performed under
45 circumstances that would not be allowed to proceed in the United States for lack of compliance
46 with the PHS *Guideline on Infectious Disease Issues in Xenotransplantation*. The forms of non-

1 compliance might include the absence of measures intended to ensure the safety of using the
2 source animals or of procedures for monitoring recipients for the potential to transmit
3 xenotransplantation-related infectious diseases. American xenotourists would be likely to return
4 to the United States after receiving their transplants, where they would receive the majority of
5 their health care. These patients will participate in the daily activities of society, such as school
6 attendance, commercial preparation of food, social interaction, and sexual activity, without
7 recognizing or taking precautions to reduce the risk of transmission of a potential xenogeneic
8 infection. Likewise, they might also inadvertently donate blood, tissues, and organs for
9 transplantation. Furthermore, unless the foreign xenotransplantation product is revealed by the
10 patient, there is no reliable way to recognize its importation or to ensure proper monitoring of
11 the patient, who may seek care at a hospital or medical clinic for a complication or unexpected
12 illness.

13
14 Xenotransplantation procedures offered in the context of xenotourism could be available in two
15 different forms. First, the procedure could be offered as a clinical trial. Although the level of
16 regulation, oversight, consent procedures, infection monitoring, sample archiving, and/or follow-
17 up might not meet U.S. guidelines, the procedure would be presented as an experimental medical
18 procedure. In that situation, it is possible that U.S. citizens might assume that the high level of
19 FDA oversight of the safety and potential benefit that is expected in U.S. clinical trials is
20 comparable to that of the FDA counterparts in other countries.

21
22 Alternatively, xenotourism may involve unconventional procedures described as therapies
23 offered by luxury spas or clinics, where the connection to xenotransplantation and a clear sense
24 of the potential danger to the recipient and to society are likely to be obscure to the average
25 citizen. Recipients might well assume that any “therapy” offered in the context of a clinic or
26 hospital would already have been approved as safe and efficacious by medical authorities and
27 regulatory agencies. The foregoing examples of confusion or lack of awareness on the part of
28 potential participants highlight the dangers implicit in xenotourism.

29
30 There is currently no way to determine how many U.S. citizens receive xenotransplantation
31 products outside the United States and no systematic way to track the serious health
32 consequences of these procedures. There is limited information on the number of foreign
33 xenotransplantation clinics or their location. Likewise, the actual validity or quality of what
34 patients receive in these xenotransplants, even though they are advertised as cells and tissues
35 from animals, is unknown. The SACX believes that a systematic effort should be undertaken to
36 identify these programs and to assess their use by U.S. citizens. The objectives of this effort
37 would be to determine the scope of the problem, including an estimate of the number of U.S.
38 citizens who receive xenotransplantation products abroad; to direct efforts to inform and educate
39 U.S. citizens of the potential dangers of this practice; and to develop strategies for further
40 discussions with the international community on the regulation of xenotransplantation.

41
42 Health policy issues involving foreign and sovereign nations are complex. However, the risks of
43 xenotourism to U.S. citizens merit serious consideration. Appropriate agencies should review
44 policy options and communicate their judgment to the public, particularly to the patient groups
45 most likely to seek xenotransplantation therapies. Educational materials explaining the potential
46 problems and risks of xenotourism can be developed. Screening questions about exposure to

1 xenotourism could be addressed to individuals entering the United States, and public health
2 reporting and health monitoring procedures could be considered for those who reply positively to
3 the queries. In addition, the considerable expertise in the oversight of clinical
4 xenotransplantation trials in the United States should be offered constructively to appropriate
5 governmental agencies in nations interested in developing such procedures. The SACX is
6 encouraged that both the U.S. State Department and the World Health Organization have
7 initiated efforts to promote international cooperation toward the harmonization of
8 xenotransplantation regulations.

11 **KNOWLEDGE GAPS AND RESOURCE LIMITATIONS**

13 Although increased understanding of molecular immunobiology and cell and organ physiology
14 has permitted significant progress in xenotransplantation in recent years, many challenges must
15 be addressed before organ and cellular xenotransplantation can be clinically valuable.
16 Following are several areas in which major gaps in knowledge or limitations in resources may
17 hinder the progress of xenotransplantation.

19 **Molecular Incompatibilities Between Species**

21 Currently available investigative and technological tools should allow the identification and
22 development of strategies for addressing the challenges in xenotransplantation. Expansion of
23 research resources in the form of funding and expertise would optimize the ability to exploit the
24 opportunities provided by these new tools and would accelerate the pace of discovery.

26 More research into species incompatibilities in innate immune system (e.g., recognition of
27 porcine sugars and lipids by human macrophages and natural killer cells) could generate
28 important new molecular and genetic strategies for avoiding delayed vascular rejection and other
29 deleterious outcomes. A greater understanding of the factors that limit the function of porcine
30 hematopoietic cells in primates should improve adaptation of porcine cells to the human
31 microenvironment. Efforts should be initiated to anticipate potential molecular incompatibilities
32 in the physiologic functions of xenotransplanted organs. Many of the specific physiologic
33 problems associated with each type of xenograft will not be revealed until we have succeeded in
34 achieving long-term xenograft survival in human or non-human primates. Waiting until this
35 point to discover such incompatibilities, which may then require additional years for the
36 development of new strategies to overcome them, could greatly delay the success of clinical
37 xenotransplantation. For example, a failure of interaction between a porcine apoprotein ligand
38 and its human lipoprotein receptor could impair vital steroid hormone synthesis in the liver
39 transplant recipient. Early recognition of this incompatibility through basic research could
40 stimulate earlier development of a porcine source animal that produces the human apoprotein.
41 Scientists with expertise in the physiology of specific transplantable organs, tissues, and cells
42 and their products should be encouraged to explore such potential incompatibilities.

44 **Animal Models**

1 Non-human primate models play a unique and critical role in advancing many therapeutic
2 interventions, including allotransplantation. These models are particularly important in the
3 setting of xenotransplantation, in which the unknown risk of infection from the source animal
4 imposes an unusual ethical requirement to justify clinical trials. Unlike most clinical trials,
5 xenotransplantation clinical trials entail risks that are borne by society in addition to the study
6 participants because of the potential for transmission of infectious agents to family members and
7 the community. The potential for societal risk imposes a higher standard of expected benefit.⁹³
8 Therefore, before clinical trials are undertaken, extensive preclinical data should support a high
9 expectation of significant benefit to the participants in the trial. Efforts should be made to
10 overcome the limitations of non-human primate models (e.g., clinical and biological monitoring
11 methods that are inferior to those available for humans; limited disease models in non-human
12 primates) that diminish the utility and predictive value of these models.

13
14 The limitations of non-human primates as models for studying xenotransplantation in humans
15 include species differences in physiology, immunology, and susceptibility to infections, as well
16 as difficulty in monitoring non-human primates in a clinically relevant manner. Knowledge
17 about optimal drug levels and the ability to monitor drug levels and biochemical and physiologic
18 parameters are far less sophisticated for non-human primates than for humans. Although some
19 of these limitations are insurmountable, others could be minimized by the development of assays
20 and facilities with advanced monitoring capabilities for non-human primates. Core facilities and
21 regional centers could be developed for these purposes. Such developments would require a
22 significant financial investment and access by all investigators to the requisite knowledge,
23 facilities, and reagents. Unfortunately, even simple primate studies are extremely expensive to
24 conduct, and the funding available for them is limited. Models that are more sophisticated will
25 require resources at a level well beyond those currently available.

26
27 Improved knowledge about the ability of human transgenes in porcine source animals to perform
28 their functions in non-human primates could reveal a need for the development of transgenic pigs
29 that are more specifically engineered to function in non-human primate species. The
30 development of pigs expressing non-human primate transgenes would require a major
31 investment of resources and expertise.

32 33 **Sharing of Resources**

34
35 It is probable that pigs will ultimately be the most widely tested xenograft source animals.
36 However, it is also likely that success will require that source pigs be genetically modified in
37 several ways, perhaps differently for transplantation of different organs, cells, and tissues. This
38 expectation raises a more general concern, since different biotechnology firms have already
39 invested heavily in the development of various genetically modified pigs and are understandably
40 reluctant to share their product animals with other commercial entities that might bring
41 additional modifications to the same animal. In anticipation that additional modifications will
42 become necessary, public support of research leading to future modifications might best ensure
43 that newly developed reagents would be shared. Alternatively, creative new partnership
44 arrangements between industry and government might better ensure the sharing of genetically
45 modified pigs and other reagents. Without such initiatives, optimal genetically modified pigs
46 may never be developed. By analogy, none of the scientific advances made with the many

1 knockout mice that were generated and crossed to other knockouts would have been possible if
2 the animals were each privately owned. Similarly, the science of xenotransplantation is unlikely
3 to proceed expeditiously without sharing of reagents between investigative teams.
4

5 **Support for Xenotransplantation**

6

7 In addition to major concerns about inadequate sharing of data, animals, and reagents if industry
8 continues to be relied upon to fund technological advances in xenotransplantation, there are other
9 good reasons why the private sector can no longer be expected to play the major role. At
10 present, funding for xenotransplantation from the biotechnology industry is low. In recent years,
11 many of the major biotechnology companies involved in xenotransplantation research have
12 either discontinued or suspended their efforts in this area (e.g., PPL Therapeutics, GenVec
13 [formerly Diacrin], Circe; Novartis closed its subsidiary biotechnology company Imutran). This
14 turn of events cannot be attributed to a lack of significant advances in xenotransplantation,
15 especially as the long-awaited aGal knockout pig has only just been successfully generated and
16 shown to be viable. Unfortunately, a serious obstacle is the incompatibility of the typical short-
17 term horizons for return on venture capital with the long-term investment required for large-
18 scale, successful organ xenotransplantation to become feasible. Investor enthusiasm for
19 xenotransplantation is further diminished by concerns about product liability from potential
20 infectious risks and by disappointment over earlier failure following exaggerated expectations.
21 Many investigators who have relied on industry funding for xenotransplantation research can no
22 longer do so. If the field is to advance, imaginative new strategies for revitalizing the
23 biotechnology sector's interest in xenotransplantation or other sources of funding are needed to
24 fill the void.
25

26 Although non-profit organizations would be responsive to a potential breakthrough that would
27 benefit their constituency, these organizations are not in a position to make a financial
28 commitment of the magnitude required. The intellectual commitment needed by academia to
29 reinvigorate the field of xenotransplantation is also constrained by the level of support from the
30 traditional sources. The public has a very high stake in the products of the xenotransplantation
31 enterprise and may be the logical source of the requisite additional support. In other medical
32 advancements, the NIH has typically underwritten the basic research that has led to translational
33 efforts by foundations or industry in collaboration with basic scientists, which has often led to
34 clinical development of therapies by the industrial sector. The SACX's analysis of the state of
35 the science of xenotransplantation underlines the need for extensive additional basic research in
36 xenotransplantation. Major program initiatives in xenotransplantation, the development of a
37 reagent and information repository, core facilities to serve common technical requirements, and
38 incentives for scientists in alternative career paths to apply their expertise to xenotransplantation
39 are currently needed.
40
41

42 **PARALLEL OR ALTERNATIVE STRATEGIES TO XENOTRANSPLANTATION**

43

44 In addition to xenotransplantation, a number of other approaches are under development for the
45 treatment of conditions involving cellular, tissue, and organ destruction. These other approaches
46 include prevention of the diseases that lead to end-organ failure, and in the absence or failure of

1 prevention, gene therapy, stem cell therapy, and artificial organs. In the sections that follow,
2 these other approaches are briefly reviewed to provide a perspective on xenotransplantation
3 within the context of other potential treatments.

4 5 **Prevention of End-Organ Failure**

6
7 The best alternative to the need to treat end-organ failure is the prevention of the acquisition
8 and/or progression of the chronic diseases that lead to it in the first place. Prevention activities
9 need to be promoted by all available means. For example, the current epidemic of obesity in the
10 United States is a major factor in the increase of type 2 diabetes mellitus and contributes to risk
11 factors for end-stage renal disease, hypertension, coronary artery disease, and osteoarthritis.
12 Control of this epidemic and an increase in physical activity (e.g., Shape Up America's 10,000
13 Steps Program) could substantially reduce the incidence of renal and cardiac failure and, along
14 with it, the need for organ transplantation.

15 16 **Gene Therapy**

17
18 Gene therapy⁹⁴ remains a relatively new and highly experimental technology for treating human
19 disease. It involves the transfer of genetic material (DNA or RNA) into a patient in order to
20 provide a copy of a normal gene to compensate for a defective gene or to direct the body to
21 produce a potentially therapeutic substance. Viruses are often used as the vehicle (vector) for
22 delivering the new genetic material to human cells. The viruses are genetically engineered so
23 that they retain their ability to infect human cells (and thereby deliver the new genetic material),
24 but lose their ability to cause disease. Nonviral vectors for delivering genes into cells are also
25 being explored, including the use of plain DNA and DNA wrapped in a coat of fatty molecules
26 known as liposomes.

27
28 More than 700 gene therapy studies have been performed since the first trials began in the United
29 States in 1990. However, gene therapy is still in its early days in terms of development, and
30 most of the studies conducted to date have been Phase I studies that investigate safety rather than
31 efficacy. Less than 1% of gene therapy trials in the United States have progressed to Phase III
32 studies, which test for effectiveness by using large numbers of subjects. No human gene therapy
33 product has yet been approved by the FDA to be marketed for medical use. The field of gene
34 therapy is more advanced than xenotransplantation, but both are still considered experimental
35 procedures.

36
37 Gene therapy has shown some promise the treatment of X-linked severe combined
38 immunodeficiency, however there have been serious complications of leukemia in some
39 cases.^{95,96} It has also been successful in the treatment of a form of immune deficiency known as
40 adenosine deaminase deficiency.^{97,98} Other potential applications of gene therapy include
41 additional genetic hematopoietic deficiencies, hemophilia, heart disease, cancer, cystic fibrosis,
42 and organ transplantation. In organ transplantation, gene therapy approaches could one day be
43 useful in preventing transplant rejection, inducing tolerance, prolonging graft survival, and
44 ameliorating some of the problems associated with systemic immunosuppression.⁹⁹ In this
45 context, gene therapy could serve as a complement to xenotransplantation. However, although
46 gene therapy could replace the need for xenotransplantation for some diseases (e.g.,

1 Huntington's disease, cystic fibrosis), it could not correct all of the end-organ failure states for
2 which xenotransplantation has potential.

3 4 **Stem Cell Therapy**

5
6 Stem cells are unspecialized cells that have the ability to self-renew, divide repeatedly, and
7 develop into different specialized cell types. Stem cells offer the possibility of a renewable
8 source of replacement cells and tissues to treat a variety of diseases and disorders involving
9 tissue destruction or cellular injury and dysfunction.^{100,101,102} Two types of stem cells are being
10 studied: (1) adult stem cells, which can be recovered from tissues and blood and are multipotent
11 and potentially useful for autologous adult stem cell transplants; and (2) embryonic stem cells,
12 which are derived from 5-day-old blastocysts of human embryos and are pluripotent because
13 they have the capacity to give rise to all of the various differentiated cells of the body.

14
15 Stem cells, whether adult or embryonic, have potential applications for almost every realm of
16 medicine, including the treatment of Parkinson's and Alzheimer's diseases, spinal cord injury,
17 stroke, burns, heart disease, osteoarthritis, and rheumatoid arthritis. Early-stage experiments are
18 under way with pancreatic, neural, muscular, hepatic, cardiac, and kidney adult stem cells. One
19 example is adult human pancreatic stem cells extracted from a pancreas and grown in the
20 laboratory in the presence of the hormone glucagon-like peptide-1 (GLP-1). The pancreatic stem
21 cells transform and mature into insulin-secreting cells. Although this approach is far from being
22 ready for clinical application, it could ultimately benefit patients whose islet cells have stopped
23 producing insulin.¹⁰³

24
25 Closer to reality are clinical trials with adult stem cells to treat heart failure; however, the safety
26 and efficacy of this approach is unknown, and the ability of bone marrow stem cells to
27 differentiate into other cell types, such as heart muscle cells, is still controversial.¹⁰⁴ Additional
28 research will yield more efficient methods of isolating and purifying stem cells and growing
29 them in culture, as well as a better understanding of the characteristics of stem cells, the
30 molecular signals that direct their differentiation and transdifferentiation into specialized cells
31 and tissues, and the long-term survival, fate, and function of transplanted cells and tissues
32 generated from stem cells. From this perspective, stem cell therapies offer hope for treating a
33 multitude of clinical diseases and have several advantages and disadvantages when compared
34 with xenotransplantation. Thus, with more research stem cells may have considerable potential
35 for cellular replacement and repair, but its potential for whole-organ replacement is currently
36 unknown.

37 38 **Artificial Organs**

39
40 Although left ventricular assist devices can improve cardiac function in patients with isolated
41 left-sided heart failure, many patients have biventricular failure for which a total artificial heart
42 or a transplant could provide the only replacement. An artificial heart has been developed and
43 evaluated in a small group of patients. This device (Abioco) is a totally implantable,
44 mechanical replacement heart.. Two of seven recipients lived for more than 3 months with the
45 artificial heart. At present, the device is limited to patients with end-stage congestive heart
46 failure whose other vital organs remain viable. Several obstacles remain to the more widespread

1 use of such devices. The exact costs of artificial organs and the ongoing patient care and
2 rehabilitation associated with their use are yet to be determined. Despite the very large public
3 investment that has been made in the development of artificial hearts over a period of 40 years,
4 the potential for the success of these technologies in terms of improving quality of life and
5 longevity are still unknown.

6 7 8 **FINDINGS AND RECOMMENDATIONS**

9
10 The SACX makes the following recommendations for pursuing xenotransplantation as a strategy
11 for treating a variety of medical disorders:

- 12
13 **1.** Continue to evaluate pigs as a suitable source animal for xenotransplantation. Due to
14 heightened risks and ethical concerns apparent with nonhuman primates, these animals
15 should not be considered as source animals for xenotransplantation. The establishment of
16 closed colonies of pigs will ultimately be needed to raise animals for clinical trials.
17
- 18 **2.** Support existing federal guidelines on source animals for xenotransplantation.
19
- 20 **3.** Further development of diagnostic tools, including antibody and nucleic acid-based assays, to
21 detect known and unrecognized porcine pathogens that might pose a risk to humans should
22 be supported. Continue research on the risks of zoonotic infection in xenotransplantation
23 recipients and gauging the potential for new emerging diseases is needed.
24
- 25 **4.** Initiate research studies that will use the new tools of molecular biology and genetics to
26 reveal physiologic and immunologic incompatibilities between source animals and humans.
27
- 28 **5.** Develop facilities where pig-to-non-human primate models could be used to gauge the
29 efficacy of xenotransplantation of pig organs, tissues, and cells to humans.
30
- 31 **6.** Encourage scientists from diverse disciplines to apply their expertise in the discovery of
32 solutions for successful xenotransplantation.
33
- 34 **7.** Establish repositories in which reagents, genetically modified pigs, and other valuable
35 materials can be maintained and distributed to researchers and laboratories engaged in
36 xenotransplantation research.
37
- 38 **8.** Build government-industrial-academic partnerships that ensure the sharing of reagents and
39 research animals.
40
- 41 **9.** Provide counseling to industry early in their development of xenotransplantation products on
42 issues related to compliance with federal regulatory and safety issues.
43
- 44 **10.** The problem of broad liability for the consequences of possible zoonotic infections is
45 perceived by some as a deterrent to participation by industry in xenotransplantation research.
46 Investigate this issue and identify solutions.

- 1 **11.** Periodically re-evaluate federal guidelines on xenotransplantation and institute a system of
2 review and oversight of regulations.
3
- 4 **12.** Investigate the scope of xenotransplantation in countries lacking stringent oversight and the
5 extent of risks posed by entry into the United States of persons receiving xenotransplants in
6 such countries. Appropriate federal agencies should consider the need for adjustments to
7 immigration policy and questionnaires to protect the public health.
8
- 9 **13.** Educate U.S. residents about the risks of unregulated xenotransplantation procedures and
10 discourage their participation in those lacking regulatory oversight as stringent as that in the
11 United States.
12
- 13 **14.** Work closely with international health agencies to promote regulations and guidelines for
14 xenotransplantation that are as rigorous as those developed by the PHS and assist other
15 countries in implementing them.

1 **GLOSSARY**

2
3 **Galactose(a1-3)galactose (Gala1-3Gal) (aGal):** A carbohydrate that is attached to numerous
4 proteins and lipids on cell surfaces and is expressed by most animal species other than humans
5 and Old World monkeys.

6
7 **Allogenic:** Involving, derived from, or being individuals of the same species that are sufficiently
8 unlike genetically to cause an immune response.

9
10 **Allotransplantation:** Transplantation between genetically different individuals.

11
12 **Antigen:** A substance (usually a protein or carbohydrate) recognized by the immune system and
13 capable of stimulating an immune response.

14
15 **Bacteremia:** The usually transient presence of bacteria in the blood.

16
17 **Closed colony:** A group of animals that have been raised separately from other animals in order
18 to maintain the genetic integrity of members of the group and to prevent transmission of
19 infectious organisms.

20
21 **Collagen:** An insoluble fibrous protein of vertebrates that is the chief constituent of the fibrils of
22 connective tissue.

23
24 **Complement:** A complex group of blood proteins that are activated in a cascade to form
25 structures capable of lysing cell membranes of microorganisms. In certain situations,
26 uncontrolled activation of complement can also damage host cell membranes. Certain
27 complement proteins bind antibody-antigen complexes and facilitate their removal by activating
28 complement receptors of phagocytic cells.

29
30 **Cryopreserved:** Preservation by subjection to very low temperatures.

31
32 **Down-regulation:** The process of reducing or suppressing a response to a stimulus; specifically,
33 reduction in a cellular response to a molecule (e.g., insulin) due to a decrease in the number of
34 receptors on the cell surface.

35
36 **Encapsulation:** The process of surrounding, encasing, or protecting in or as if in a capsule.

37
38 **Endogenous:** Caused by factors within the body or arising from internal structural or functional
39 causes.

40
41 **Exogenous:** Caused by factors outside the body or arising from external causes.

42
43 **Extracorporeal:** Occurring or based outside the living body.

44
45 **Gene knockout:** Having all or part of a gene eliminated or inactivated by genetic engineering.

1 **Gene therapy:** The insertion of genes into cells to replace defective genes in the treatment of
2 genetic disorders or to provide a specialized disease-fighting function (as the destruction of
3 tumor cells). Vectors such as those derived from altered, nonreplicating viruses, are often used
4 for the insertion.
5

6 **Genetic engineering:** A group of applied techniques of genetics and biotechnology used to
7 separate and join together genetic material, particularly DNA, from one or more species and to
8 introduce the result into an organism in order to change one or more of its characteristics.
9

10 **Gnotobiotic:** A controlled environment containing only one or a few kinds of microorganisms.
11

12 **Growth factor:** A biologic substance that promotes growth, particularly cellular growth.
13

14 **Hepatocyte:** A parenchymal cell of the liver.
15

16 **Heterotopic:** Alongside or adjacent; a heterotopic transplant is one in which a donor organ is
17 placed into a recipient at a location other than that of the native organ.
18

19 **Immunosuppression:** Suppression (as by drugs) of natural immune responses.
20

21 **Islet cell:** An insulin-producing cell of the pancreas.
22

23 **Latency:** The state or period of living or developing in a host without producing symptoms.
24

25 **Neovascularize:** To provide with a new supply of blood vessels.
26

27 **Orthotopic:** In place of; an orthotopic transplant is one in which a donor organ replaces a
28 recipient's organ in its original location.
29

30 **Porcine endogenous virus (PERV):** A virus that is found in all swine and is contained within
31 the pig genome.
32

33 **Porcine:** Of or derived from swine (pigs).
34

35 **Rejection:** An immune response in which foreign tissue (such as a skin graft or transplanted
36 organ) is attacked by immune system components (such as antibodies, T-cells, and macrophages)
37 of the recipient organism.
38

39 **Simian:** Pertaining to an ape or monkey.
40

41 **Stem cell:** An unspecialized cell that gives rise to differentiated cells.
42

43 **Thymus:** A gland of largely lymphoid tissue that serves as the site where T cells develop in
44 cell-mediated immunity.
45

1 **Tolerance:** The capacity of the body to endure or become less responsive to a substance (as a
2 drug) or a physiological insult with repeated use or exposure. Immune tolerance is the ability of
3 the immune system to specifically accept donor organs, cells, or tissues without requiring
4 therapies that generally suppress the immune response.

5
6 **Transgenic engineering:** A technique to produce an organism or cell of one species into which
7 one or more genes of other species have been incorporated (e.g., transgenic mice).

8
9 **Xenogeneic:** Derived from, originating in, or being a member of another species.

10
11 **Xenotransplantation:** According to the U.S. Public Health Service, “Any procedure that
12 involves the transplantation, implantation, or infusion into a human recipient of either (a) live
13 cells, tissues, or organs from a non-human animal source or (b) human body fluids, cells, tissues,
14 or organs that have had ex vivo contact with live non-human animal cells, tissues, or organs.”
15 (See footnote 1.)

16
17 **Zoonotic:** A disease communicable from animals to humans under natural conditions.
18

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