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Regulatory aspects of preclinical study of xenotransplantation in us and Europe

Helly H Patel, * Dr. Jignesh S Shah, Dr. Dilip Maheshwari
L.J. Institute of Pharmacy, Near Kataria Motors, Sanand Sarkhej Circle, S.G. Highway, Ahmedabad, Gujarat, India

Abstract
The current shortage in human organs has made xenotransplantation a potential source of organ transplant in humans. Xenotransplantation attracted interest from regulatory authorities, particularly after the demonstration of pig-to-human transmission of porcine endogenous retrovirus (1996). This added to the risk of a product, resulting in a Guidance of the US Food and Drug Administration (2003). The European Medicines Agency issued a Guideline on xenogeneic cell therapy products (2009). These addressees the full flow chart starting with the designated pathogen free status of the source animal and special aspects regarding preclinical safety and monitoring toxicity, also archiving of records from the donor and recipient as well as storage of the sample. This article presents an overview of the regulatory framework with the special focus on the preclinical study regulation in US and Europe.

Keywords: xenotransplantation, porcine endogenous retrovirus, xenogeneic cell therapy products, pathogen free status

Introduction
Human Transplantation is a relatively new field of medicine that is now facing the significant challenge. Because of its clinical success, the need for these procedures exceeds the availability of donor organs. Each year Fewer than half the people on transplant waiting lists receive organ transplant. Approximately 10 people die each day waiting for organs to become available. Even if all potential donors elect to donate, the supply of human organ donation will continue to fall short of the need. One solution along with pharmaceutical and biotechnology companies is investigating to end this acute shortage is "Xenotransplantation". Xenotransplantation is the transplantation of living cells, tissues or organs from one species to another. Such cells, tissues or organs are called Xenografts or Xenotransplants. Xenotransplantation to humans is defined as any procedure that involves direct transplantation, implantation or infusion of live cells, tissues or organs from a non-human animal source. This term is also applied when human body fluids, cells, tissues or organare used that had come into contact with live non-human animal cells, tissues or organs and might be contaminated by an infectious agent from another species.

Preclinical study of Xenotransplantation in US [1, 2]
In the United States, several scientists who attended the public conference in January 1998, "Developing U.S. Public Health Policy on Xenotransplantation," urged the FDA to ban cross-species transplantation research until ethical issues and health risks are resolved. They specifically discussed the potential risk to public health from a viral transfer across species that could result in a new disease epidemic. In April 1999, the FDA released a guidance document stating that any clinical protocols proposing the use of nonhuman primates should include sufficient clinical evidence addressing the risks of such use.

The U.S. Department of Health and Human Services is developing several important mechanisms to facilitate participation by the public, scientists and industry in the progress of xenotransplantation. The DHHS is establishing the Secretary's Advisory Committee on Xenotransplantation (SACX) to review clinical protocols, conduct discussions and make recommendations about the appropriate conditions for use of nonhuman primate organs. In October 1999, the DHHS announced the creation of the SACX and requested nominations for the committee membership. The U.S. Public Health Service is also planning to develop a national patient registry and a biological specimen repository for tissues. The biotechnology industry continues to work closely with the government in the responsible development of regulations and guidelines on the appropriate safeguards for xenotransplantation.

In US, a general framework for the preclinical testing of xenotransplantation products is provided before use in clinical trials. Pre-clinical studies should support the safety characterization of xenotransplantation agents. Pre-clinical studies should focus on the intended alteration to the human pathophysiologic state (i.e., activity) as well as unintended effects (i.e., toxicity) to the host system. Such studies serve to assess the potential for clinical risks. It constitutes an important component of an FDA regulatory application. Preclinical studies are particularly valuable for gaining detailed information regarding safety issues which cannot be evaluated in human recipients due to risk involved.

Preclinical safety data for submission in IND application
Safety [2]
1. The animal source for the xenotransplantation product
   - Animal donor organ should resist human disease especially viral. Animals should be inexpensive to feed

and breed. Animal species should be with short gestation time

2. The tissue anatomical and physiological similarity to its human homologue
   - Animal model should be compatible similarities indented to functions well in humans.
3. The determination of function of the xenotransplantation product
4. The animal model system (eg; pig, monkey, cow)
5. The integrity of the Xenotransplant components (eg; Filters, Membranes) and Device biocompatibility:
   - A number of products for therapeutic use are combinations of Xenotransplantation products and device components, either for use as implants or extracorporeal. Xenotransplantation products also warrant further preclinical characterization for bioreactivity and biocompatibility of the device components.
   - Safety assessment needed for device components to isolate animal tissue is important aspect to be considered. e.g. Membranes with pores partially isolate xenogeneic tissue housed in devices from attack by host immune cells, but proteins and pathogens from the xenogeneic tissue may still be released into the host.
6. Route of administration: Site of implantation/ injection, extracorporeal or ex vivo use
7. The study duration: It is related to potential animal exposure.
8. Reactions between source animal and host immune system
9. Interspecies extrapolation: Cross species activity of secreted proteins/ hormones at receptors

Animal Model

General criteria for selection of animal [8]

- Animal model should be of compatible anatomy and physiology for the indented to functions well in humans
- No possibilities of cross species (i.e. animal to human) infection should exist.
- Animal donor organ should resist human disease especially viral
- Animals should be inexpensive to feed and breed.
- Animal species should be with short gestation time.
- Animal should also present no immunologic barriers to transplantation into humans.

The Source Animal Facility (SAF), production process, and records are subject to FDA inspection under section 704 of the Act (21 U.S.C. 374) and section 351(c) of the Public Health Service (PHS) Act (42 U.S.C. 262(c)).

Animal used [1]

Animals that fulfil the above criteria should be used for Xenotransplantation.

So, the U.S. Public Health Service advice those animals such as pigs and cows are considered as potential tissue and organ sources before nonhuman primates, such as monkeys, because they fulfil all ideal donor criteria. Pigs are preferred because they mature very quickly, produce large litters and have organs of comparable size and function to human organs in both infancy and adulthood. They also can be bred to high health standards in microbiologically controlled environments. Now a days genetically modified pigs are widely used as a source animals because of anatomical and physiological similarities.

Animal welfare concerns by regulatory authority [2]

Procedures for animal husbandry, tissue harvesting, and termination of animals should be approved by an appropriate Institutional Animal Care and Use Committee, in accordance with the Animal Welfare Act (7 U.S.C. 2131, *et seq*.). In cases where funds are received from the PHS, procedures must also comply with the PHS Policy on Humane Care and Use of Laboratory Animals, according to section 495 of the PHS Act (42 U.S.C. 289(d)). We recommend that the SAF be accredited by the AAALAC. Standards for accredited facilities for when funds are received from the National Institutes of Health are provided in the National Research Council's Institute for Laboratory Animal Research, Guide for the Care and Use of Laboratory Animals.

Animal health and husbandry [2]

i) Facilities

Animal Facilities built and operated in accordance with recommendations described in the National Research Council's Institute for Laboratory Animal Research, Guide for the Care and Use of Laboratory Animals and accredited by the AAALAC. Source Animal Facilities(SAF) are subject to the regulations in 21 CFR Part 600, Subpart B, on establishment standards, including the requirements regarding animals and personnel in 21 CFR 600.10 and 600.11. SAFs also are subject to the regulations in 21 CFR Part 600, Subpart C, regarding inspections. These facilities are subject to inspection by designated representatives of the clinical protocol sponsor and public health agencies. Detailed description of the facilities and procedures for housing source animals with the FDA submission (e.g., IND or Master File) should be included. The information provided should include plans for the shelters, the feeding areas, the washing areas, the fencing, air handling systems (particularly in quarantine areas), lighting, temperature, and other physical attributes of the animal environment. Facility descriptions should also include information on physical barriers and operational measures intended to eliminate or minimize exposure to insects, birds, or other animals that may transmit disease to the source animals. These descriptions are also cover the procedures and schedules followed for cleaning and other routine maintenance of the animal enclosure. Procedures for elimination of animal waste are included. Include in the description how qualified source animals will be housed and the methods used to decontaminate the housing after the source animals are used. The SAF staff should include veterinarians with expertise in the infectious diseases and agents prevalent in the particular animal species being raised in the facility. Staff should also include adequate numbers of caretaker personnel with appropriate training in the care and health of the species being housed (e.g., 21 CFR 600.10 and 600.11). Developers of xenotransplantation products should consider becoming ISO 9001:2015 certified.

ii) Maintenance of source animals

a) General: Source animals in accordance with standard operating procedures appropriate to the species, xenotransplantation product, and the intended clinical application should be maintained. SOPs should provide for admission of new animals to the SAF and source animal pool, for quarantine, and for removal, isolation, or elimination of diseased animals. Animals that have been removed from the source animal pool due to illness or infection should not be reintroduced. Procedures to identify incidents that negatively affect the health of the herd or colony should be developed.

b) Health Screening: Appropriate experts, such as infectious disease consultants, virologists, microbiologists, accredited microbiological laboratories, and veterinarians should be consulted to generate a list of agents for which all source animals should be screened and a list of appropriate diagnostic tests. Feces from source animal herds on a regular basis for evidence of parasitic infections should be examined. If infectious agents, including normal flora that could potentially be infectious in an immunosuppressed recipient, have been identified in source animals, the use of such animals is avoided. Techniques for introducing new animals, such as artificial insemination, caesarean section, cloning, or novel genobiotic techniques, is fully described. Source animals in barrier facilities that are considered free of designated pathogens should be maintained. For the purpose of this document, such facilities are termed "Designated Pathogen Free" (DPF), and animals derived from them are termed "DPF animals." Protocols for monitoring the herd for disease and infectious agents should exist. A copy or a summary of the SOPs in the FDA submission requesting investigational use (e.g., IND) should be included. Subclinical infections of source animals may not be apparent at the time of harvest of the nonhuman live cells, tissues, or organs and may be identified only retrospectively.

c) Healthcare: The herd health surveillance system should include comprehensive documentation of all veterinary care received by source animals. This includes documentation of all illnesses, medical care, procedures, drugs administered, vaccinations, routine physical exams, and any treatments received by each animal. Use of antimicrobial agents due to potential risk to allergic recipients receiving unprocessed nonhuman animal live cells, tissues or organs should be carefully documented. Exclusive use of killed vaccines generally is warranted both in the source animal and in the herd with which it is associated. Live vaccinations should only be used when alternative immunogens for vaccinations are not available. Live vaccination should used only if scientific evidence exists to support that the live cells, tissues, or organs from the vaccine-treated animal will not pose a risk of infection for the human recipient.

Aseptic techniques and sterile equipment for all parenteral interventions is used including vaccinations, treatment with drugs or biologics, and biopsies. Records showing the treatment of animals with drugs for any reason, including the withdrawal period following drug treatment is documented and maintained. Procedures for disposal of dead animals are developed.

d) Feed: The storage and delivery of feed, water in the application to FDA for investigational use (e.g., IND) is described. Records should include manufacturer, batch numbers, and other pertinent information, and a recordkeeping procedure in an SOP is described. Individual source animal's records and contents of feed given to a source animal for use as a source for live cells, tissues, or organs used in xenotransplantation is recorded. Natural, non-sterile foods, such as hay, to minimize potential risks of exposure to pests or infectious agents should not be used. Water should be of sufficient quality to prevent unnecessary exposure of animals to infectious or adventitious agents, drugs, pesticides, herbicides, and fertilizers. Pasteurized milk products in feeds should be included.

iii) Individual source animal qualification

a) Testing of infectious agents: All individual source animals for presence of the same infectious agents used for herd qualification should be screened. When fetal or neonatal animals will be used as source animals testing of mothers should be conducted. Biopsy of the live animal cells, tissue, or organ or other relevant tissue is examined by histopathology and tested for infectious agents by appropriate assays. All the tests for the harvest of live cells, tissues or organs are performed but which allows the results to be obtained before their use. If more than 3 months have elapsed since the initial testing or biopsy of the source animal, test before harvest should be repeated.

b) Quarantine

Individual source animals for a minimum of three weeks before harvest of their live cells, tissues, or organs should be generally quarantined. It may be appropriate to modify individual quarantine periods. This period is depended on the characterization and surveillance of the source animal herd, the design of the facility, and the clinical indication. During the quarantine period, in addition to tests for infectious agents, source animals should undergo physical examination by a veterinarian, including complete blood count, peripheral blood smear, and fecal exam for parasites.

Toxicity

Toxicity is the inherent capacity to produce harm or adverse effect on living systems and tissues.

Objectives

- To estimate safe starting dose for clinical studies.
- To assess toxic effects on target organs (clinical and histopathological) to guide patient monitoring.
- To determine balance between safety, tolerability and efficacy of given product in one single study.

These studies are most often performed in *vitro* models.

1. Repeat dose toxicity studies: [7]
In general 2 mammalian species, rodent and non rodent
e.g; Rat and dog
Determine clinical formulation
Measure Schedules like those planned for clinical study

Table 1

Clinical schedules	Example of preclinical treatment
Once q 3-4 weeks	Single dose
Daily 5 q 3 weeks	Daily 5 times
Daily 5 q 2 weeks	Daily 5 times alternative weeks
Weekly3/4 times	Once/ week
Daily	4 weeks
Weekly	Weekly 4-5 times

- **Repeat dose studies parameter assessed**

Table 2

	Rat	Dog
Clinical observation Food/Water consumption	Daily; starting pre study	Daily; starting pre study
Body weights	Daily; starting pre study	>twice weekly from pre study
Opthalmoscopy	Pre study week 4 and end of recovery(week 8)	Pre study week 4 and end of recovery(week 8)
ECG/BP	N/A	Pre study week 4 and end of recovery(week 8)
Clinical pathology	Weeks 2 and/or 4 and end of recovery(week 8)	Pre study, Weeks 2 and/or 4 and end of recovery(week 8)
Toxicokinetics	Daily 1 and 28 (steady state)	Daily 1 and 28 (steady state)
Necroscopy, histopathology	Main test week 5 and recovery kill (week 9)	Main test week 5 and recovery kill (week 9)

These studies have of most concern are:
1. Toxicities that are irreversible, if crucial organ (eyes, liver, heart etc)
2. Dose-independent toxicities
3. Toxicities those are not amenable to monitoring (for example, CNS toxicities)

2. Tumorigenicity [2, 7]
The tumorigenicity is the process by which neoplastic cells growing in tissue culture form tumors when inoculated into animal.The tumorigenic potential of the xenotransplantation product, due to altered cell growth regulation or to immunosuppression of the host, is an important consideration. Tumorigenicity is an important part of preclinical testing for some xenotransplantation products; Xenotransplantation products may be tumorigenic in a new species because of various factors, such as transgenic manipulations, endogenous viruses, ex vivo culture, and immunosuppression of the host. Therefore, for xenotransplantation products intended for implantation, consider evaluation of tumorigenicity in vivo and in vitro.

Evaluation of tumorigenicity *in vivo* models
Single dose assay
Animals used - mice, rat
Inoculum - 10^7 cell/animal
Possible Host - 10 nude mice; 10 new born rats, new born mice, or new born hamsters immunosuppressed with antithymocyte globulin (ATG); 10 mice thymectomized, irradiated and reconstituted with bone marrow from healthy mice
Observation period - 3 weeks for half and 12 weeks for half, unless tumor growth intervenes, with necropsy/histopathology of injection site, tumors, lymph nodes-organs for metastases
Endpoint - Tumor incidence (*i.e*.. No. with tumors/No. surviving)
Preclinical experiments should include attentive evaluation of controls, background tumor growth rates, tumor occurance and type, location, and time of appearace of tumors over an extended period. These should make use of histopathologic evaluation as a primary endpoint.

***In vitro* model**
Colony formation in soft agar (clonogenic assays) and growth in organ culture may be useful in vitro assays of the tumorigenic potential, specially for cell lines. These tests may provide information on stability or abnormal characteristics of cell lines and may substitute for testing in animals if indicated that the tests have equivalent sensitivity.

Preclinical study of xenotransplantation in Europe [4, 6]
European guideline is an appropriate to the guideline on cell-based medicinal products and deals specifically with requirements unique to xenogeneic specificities. This document is intended to provide general principles to be taken into consideration for the development and assessment of xenogeneic cell-based products without prejudice to medical practice or national legislation, which may be applicable. The main scientific and technical issues so far identified concerning the sourcing and testing of animals, manufacture, quality control, as well as the non-clinical and clinical development of xenogeneic cell-based medicinal products is addressed. Relevant public health aspects are discussed and measures to ensure a proper surveillance for infections,

including zoonoses are highlighted. Attention is also given to principles of animal health and welfare in the processes of sourcing of xenogeneic materials for the medicinal products intended for human use. These general principles may apply to a range of products using animal tissues as the starting material, as the key objective is to ensure that the product to be administered is of acceptable quality and standard, and free from contamination. Non-clinical testing programmes should be performed, wherever possible, in relevant animal models, in which the xenogeneic cells, including their bioactive molecules are active and can be compared to the human situation.

Expression levels, routes of administration and dosages should reflect the human situation to the highest possible degree. Standard toxicological testing in animals, where the material is or is not active might add information on general effects of xenogeneic cells, such as production of unintended proteins/hormones, unintended pinpoint of cells into tissues/organs, effects induced by rejection or encapsulation of xenogeneic cells and effects like graft versus host disease in immuno-suppressed animals.

Safety [6]

1. Bioactivity/Pharamacodynamics

The biologic activity of Xenogeneic cells or the expression of xenogeneic cell product should be first evaluated in vitro and subsequently *in vivo*. *In vitro* studies may also provide information on cell morphology, proliferations, phenotype and level of differentiation. The results of these in vitro studies are reported in module 3 of submission and should be considered in context of non clinical *in vitro* studies. Non clinical studies may provide valuable data to support the posology and concomitant treatment chosen for human clinical trials.

2. Safety Pharmacology

Studies on cardiovascular and respiratory endpoints are needed in an appropriate animal model to investigate the potential undesirable effects of xenogeneic cells including their bioactive products. Effects on central nervous system endpoints should be studied. Secondary pharmacodynamics should be considered on a case by case basis depending on the character of the excreted bioactive molecules.

3. Pharmacokinetic

The survival and adequate function of the administered xenogeneic cells including synthesis relevant bioactive molecules should be studied.

Cells from xenogeneic cell-based products may migrate within the host, thus presenting clinical concerns regarding adverse reactions deriving from displaced, possibly differentiating bioactive cells or unexpected anatomical obstruction. This should be evaluated in animals using histopathology complemented by an appropriate method for specific identification of the xenogeneic cells.

Animal model [4, 6]

Animal welfare concerns

The Council of Europe issues the European Directive Regarding the Protection of Animals Used for Experimental and Other Scientific Purposes.

Procedures for animal husbandry, tissue harvesting, and termination of animals should be approved by Committee for Medicinal Products for Veterinary use.

1. Selection of the animals

Source animal species may be those typically beared for consumption or conventional laboratory animals. The origin and derivation of source animals should be fully described considering possible infectious agents and diseases of the particular animal species. Founder and source animals are healthy and are Specific Pathogen Free (SPF) and raised in SPF conditions, including health monitoring and barrier systems. External stresses on the barriers should ideally be minimised. Information should be available on the feeding history (e.g. the nature of manufactured feedstuff) of each source and founder animal. When source animals die, a full necropsy should be performed to identify clearly the cause of death and, where appropriate, archival samples should be obtained for storage. Herd records should be kept pertaining to the source animals and facilities. When the source animal is sacrificed to harvest the organs/tissues, a full necropsy should be conducted including histopathological and microbiological evaluation. Samples should be archived for future examination. Cells, tissues and organs intended for the manufacture of xenogeneic cell-based medicinal products should be produced only from animals that have been bred in captivity (barrier facility) specifically for this purpose and under no circumstances should cells, tissues and organs from wild animals or from stockyard be used. Tissues of founder animals similarly are not used.

Genetically modified animals

Cells/tissues to be used in xenogeneic cell-based products may be obtained from genetically modified (transgenic or knock-out) animals, or may be obtained by *ex vivo* genetic modification. The modification might have been introduced either to express new properties in the cell, e.g. expression of human complement-regulatory proteins, or to modify specific antigenic structures, e.g. carbohydrate antigens like α 1-3 galactose terminal sugar residues, in order to reduce or minimise the risk of xenogeneic cell rejection. In either case, genetically modified animals from which cells are obtained, have to comply with applicable European legislation.

2. Animal Husbandry

Procedures should be developed to identify and prevent incidents that negatively affect the health of the herd or colony, or that could negatively impact on the barrier facility or the Specific Pathogen Free status of the herd.

SOPs should be present for:

- Detailing the housing of animals and regulatory conditions
- Water
- Bedding
- Performance and monitoring of health screening
- Removal from production and disposal of the animals and their by-products
- Identifying individual animals and recording their movements to, through and out of the facility
- Entry and exit of the animals
- Animal transportation

- Disposition of animal tissues and dead animals

Source and handling of feed, including feeding:
- Isolation and quarantine

3. Animal Facilities

A separate facility should exist for founder and source animals. Animal facilities should be isolated from each other to prevent cross-contamination and should be operated in such a way, including the use of biosecure barriers, as to minimise the exposure of the animals to infectious agents and to prevent cross-contamination both among animals and humans. In the preparation of feed, precautions should be taken to avoid chemical, physical and microbiological contamination. All feeding, bedding, water and utensils should be sterilized and animals disinfected (e.g. the outer surface of boxes). All feeding and bedding supplier should be approved and certified. Environmental conditions, such as air flow (HEPA-filters, positive pressure) and water should be routinely controlled and analysed. Programmes for cleaning, disinfection and sterilisation of the animal cages and pens after usage, and for disposal of waste including animals, feed, bedding, equipments, reagents, etc., should be established. The necessary microbiological quality control tests should be carried out. An adequate number of staff should be available and should include veterinarians, either permanent or available on consultation. Animal caretakers should participate in a documented training programme and health monitoring of them, including vaccination history, should be recorded. SOPs on tasks and responsibilities of animal caretakers should be established. Air treatment and handling and gowning procedures for personnel should prevent the transfer of animal diseases into humans and vice versa.

4. Transportation

Transportation of source animals exposes them to risks not encountered in closed herds and should be avoided. In exceptional cases where transportation is necessary, barriers equivalent to, or better than, those in place at the facility, should be maintained during transit to avoid source animal contamination. Transportation should use dedicated vehicles in which the animals are not exposed to any other animals and the method has to be documented. Quarantine facilities should exist at the destination to allow for clinical evaluation upon arrival prior to acceptance for further processing.

For transportation of organs, tissues or even primary cells, procedures should be in place for appropriate shipping conditions in order to maintain the integrity of the materials and to avoid shipping errors and contamination.

5. Testing for infectious agents in source or founder animals

Source animals may carry known or unknown infectious agents. The acceptability of the source animal as a donor for tissues/organs or cells depends equally on prevention of infections and on thorough testing of the source animals. Programmes for screening and detection of known infectious agents are modified to the source animal species and the manner in which the xenogeneic cell-based product will be used clinically. The selected assays should be capable of detecting a broad range of infectious agents, as well as species-specific agents in the source animal. Appropriate *in vivo* and *in vitro* assays should be in place to characterise the potential of identified human pathogens. The assumed pathogenicity of xenotropic endogenous retroviruses (ERV) and persistent viral infections in source animal cells, tissues and organs is of particular importance. Assays used for the screening and detection of infectious agents should have well defined and documented specificity, sensitivity, reproducibility and validity in the setting in which they are to be used. Appropriate laboratory quality assurance standards must be used.

Consideration needs to be given to screening the animals for the following infectious agents:
- Their own recognised infectious agents and parasites
- endogenous retroviruses (ERV e.g. porcine ERV)
- known zoonotic agents transmissible to humans (e.g. rabies) and other zoonotic agents such as *Toxoplasma gondii* which are usually not considered zoonotic but which may infect through the therapy
- known infectious agents of human
- infectious agents of humans relating to receptors expressed by transgenic animals, e.g. human complement-regulatory protein CD46 (membrane cofactor protein, MCP-1) as the cell-surface receptor for measles virus
- infectious agents known to have a high mutation or recombination potential such as influenza virus
- antibiotic-resistant bacteria
- geographically important infectious agents such as *Trypanosoma cruzi*, African Swine Fever

Toxicity [6]

1. Single/ repeated dose toxicity studies

The toxicity studies have main purpose to confirm whether the dose range of xenogeneic cells required for applying the desirable pharmacodynamic effect is tolerated. Toxicity studies due to nature of Xenogeneic cell products should be performed in relevant animal models and if xenogeneic cells are not immediately rejected may be combined with safety, pharmacology, local tolerance, immunotoxicity or efficacy studies. The duration of observation in such studies might be much longer than in standard single dose studies, since xenogenic cells are supported to function for long times which should be reflected in design of the studies.

2. Genotoxicity studies

For biological derived pharmaceutical, genotoxicity studies are not considered necessary for xenogeneic cells/tissue products unless the nature of the expressed products indicate an interaction directly with DNA or other chromosomal material.

3. Carinogenecity studies

Potential mechanism involve in carcinogenicity may be transgenic manipulations, endogenous or exogenous viruses, ex vivo culture, immunosuppresion or a direct action of xenogenic cells/ tissue product. For the testing of carcinogeneic potential of the xenogeneic cells both in vitro and in vivo methods may be appropriate and should be

considered. The need of carcinogenicity studies is discussed in the light of intented use and the type of product.

4. Reproductive performance and developmental toxicity studies

This study is dependent upon the xenogeneic cells /tissue product, area of implantation, clinical indication and intended patient population and should be discussed on case by case basis.

5. Local tolerance studies

Local tolerance studies may be required in an appropriate species. However, if the proposed clinical formulation and route of administration (intravenous and intra artirial infusion, surgical implant, external topical grafting, extracorpeal perfusion) have been examined in other clinical studies than separate local tolerance studies are not necessary. Most often local tolerance can be evaluated in single or repeated dose toxicity studies thus removing the need for separate local tolerance studies.

Other toxicity studies

Immunological and Immunotoxicity studies

In principle, xenogeneic cells induce vigorous immune responses by the host provided that the immuno-competent cells of the host come into contact with the xenogeneic cells or their parts. Studies should address, as relevant, the immunologic response of the host with or without immunosuppression to the xenogeneic cells, including their bioactive products. Several approaches can be attempted for controlling immune responses, e.g. mechanical seperation of the cells, introduction into the animal cells of human genes coding for proteins that improve the hyperacute rejection or control of the immune response, e.g. by immunosuppressive drugs, xenogeneic antigen deletion/modification (genetically modified animals) and tolerance induction. The compatibility of the animal cells may be improved by physical separation from the host (e.g; encapsulation). In this situation, immunological studies may be useful to support the integrity of the barriers. The material used to encapsulate the cells may induce tissue reactions and should be addressed. The selection or adaptation of the animal model should reflect the effects of the product and the therapeutic procedure as a whole.

Among the effects to be monitored are:

- Induction of humoral and cellular responses and subsequent immunogenic reactions such as formation of immune complexes and complement activation.
- Immune modulatory properties of xenogeneic cell therapy, including the concomitant immunosuppressive regimen, can be addressed by studying the following parameters:
  - Evidence of myelosuppression, such as pancytopenia, anaemia, leukopenia, lymphopenia, thrombocytopenia, or other blood dyscrasias
  - Alterations in histology, including thymic atrophy or hypocellularity of immune system tissues such as the spleen, lymph nodes, or bone marrow
  - Increased incidence of infections
  - Increased incidence of tumours

Conclusion

Xenotransplantation is tremendous field for fulfilling the demanding necessities of shortage of organs by transplanting animal organs in to humans. Regulatory aspects of xenotransplantation products are complex, but do not block product development therefore stringent regulations for Xenotransplantation are necessary. In this study it is observed that countries like United States and Europe having stringent regulations to be followed. In this way, it will be possible to make new xenotransplantation products through development to a product on the market, to meet the medical need of all those patients with end-stage organ dysfunction waiting for a xenotransplantation product.

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References

1. Xenotransplantation: The and Risks of Special Organ Transplantation https://www.bio.org/.../xenotransplantation-benefits-and-risks-special-organ-transplantation
2. USFDA. Guidance for industry: Source animal, product, preclinical and clinical issues concerning the use of Xenotransplantation products in Humans. http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.
3. WHO- Second WHO Global Consultation on Regulatory Requirements for Xenotransplantation Clinical Trials http://www.who.int/transplantation/xeno/en
4. European Medical agency. Xenotransplantation Regulations,http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003893.pdf
5. Health Canada. Revised fact sheet of Xenotransplantation, https://www.canada.ca/en/health-canada/services/drugs-health-products/biologics-radiopharmaceuticals-genetic-therapies/activities/fact-sheets/revised-fact-sheet-xenotransplantation.html.pdf
6. EMEA. Points to be consider on Xenogeneic cell therapy medicinal products http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003893.pdf
7. Andrew Lewis M. Regulatory implication neoplastic cell substrate tumorigenecity https://www.fda.gov/ohrms/dockets/ac/05/slides/5-4188S1_2draft.PPT
8. Marlon Levy F. Animal organ for human transplantation: how close are we?, Baylor University Medical Centre Proceedings. 2000; 13(1):3-6.