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POINTS TO CONSIDER ON XENOGENEIC CELL THERAPY MEDICINAL PRODUCTS

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1. GENERAL INTRODUCTION

1.1 Introduction

Xenogeneic cell therapy is the use of viable animal somatic cell preparations suitably adapted for: (a) the transplantation/ implantation/ infusion into a human recipient or (b) extracorporeal treatment through bringing (non-human) animal cells into contact with human body fluids, tissues or organs. The principal objective is reconstitution of cell/tissue/organ functions. The genotype and/or phenotype of the cells may have been modified, e.g. by isolation, culture, expansion, pharmacological treatment or combination with various matrices. The use of animal cells, tissues and organs has religious, ethical and legal implications that are not discussed in this document. This points to consider document lays down some principles that can be used if a marketing authorisation application for a xenogeneic cell therapy product is developed for submission to regulatory agencies within the EU. The document should not be interpreted as promotion of the use of animal cells in clinical practice.

The human use of xenogeneic cells is associated with difficult obstacles, including management of the risks of transmitting known and unknown pathogens. Importantly, there is the potential risk of introducing new infectious diseases into the general population through adaptation in an immuno-suppressed host.

The risk of immunological rejection of animal cells is yet to be prevented and overcome, and there is the challenge of maintaining the survival and functions of the xenogeneic cells in the long term. The use of immunosuppressive treatment carries substantial risks due to a weakening of host defence mechanisms. In addition, the administration of the xenogeneic cell product may require difficult surgical procedures.

These risks may be minimized – but not completely ruled out - by careful choice of donor animals, reproducible manufacturing process, accurate pre-clinical and clinical testing and monitoring as well as a risk management programme with regard to infectious agents.

Moreover, there are uncertainties with the ability of the administered cells to provide the desired function in the long term. The documentation should address risks at all stages of the procedure starting from the selection of donor animals to the administration of the product.

For an individual patient, the benefit/risk of treatment with xenogeneic medicinal products is influenced by the available therapeutic alternatives and the seriousness of the conditions to be treated. Because of the range of possible *in vivo* and extracorporeal uses and choice of animal species, the risk assessment should be made on a case-by-case basis. However, the risks of the treatment must also be regarded from the public health point of view.

1.2 Scope of the document

Xenogeneic cell therapy medicinal products contain animal cells as the active substance.

To determine whether xenogeneic cell therapy products fall within the definition of a medicinal product, reference is made to the points set out in the current medicinal product legislation.

This document is intended to provide general principles to be taken into consideration for the development and assessment of xenogeneic cell therapy products without prejudice to medical practice or national legislation, which may be applicable.

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. Attention is also given to principles of animal health and welfare in the processes of sourcing cells as active substances for the medicinal products intended for human use. The main scientific and technical issues so far identified concerning the sourcing and testing of animals, manufacture, quality control, as well as the pre-clinical and clinical development of xenogeneic cell therapy medicinal products are addressed. Relevant public health aspects are discussed and measures to ensure a proper surveillance for infections, including zoonoses are highlighted.

Xenogeneic cells may be used in different ways - e.g. by transplantation/ implantation/ infusion into a human recipient of live cells or by bringing them to *ex vivo* contact with human body fluids, cells, tissues or organs - and therefore the products may have different levels of risks that impact on the application of the principles and criteria set out in this points to consider document.

The extent and design of the non-clinical testing programme as well as of the clinical and pharmacovigilance programmes will be based on an analysis of the numerous variables that have an impact on the benefit/risk of these products.

Those variables include:

- the presence or not of barriers isolating the cells from the immune system of the recipient,
- the mode of administration – *in vivo* or *ex vivo* use -,
- route of administration: intravenous or intra-arterial infusion, surgical implant, external topical grafting, extracorporeal perfusion,
- the nature, seeding ability and productive capacity of the cells used,
- expected pharmacodynamic and therapeutic activity
    - cells replacing human cells - without production of specific main bioactive molecules acting systemically
    - cells synthesising bioactive molecules acting systemically such as proteins, hormones or neurotransmitters
- duration of the active treatment (temporary bridging or permanent replacement of functional cells, seeding of population to expand in a permanent given lineage etc.).

2. SOURCING OF ANIMALS

2.1 Selection of the animals

Source animal species may be those typically reared 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[1] and source animals should be healthy and should, at minimum, be Specific Pathogen Free (SPF) and raised in SPF conditions, including health monitoring and barrier systems[2]. 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, or are euthanised, 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 therapy medicinal products should be produced only from animals that have been bred in captivity (barrier facility) specifically for

[1] Founder animals are the animals from which source animals are initially bred.

[2] In principle, the level of microbial control in animals can be set on three different levels:

- Germ-free gnotobiotic animals. The establishment of gnotobiotic animals requires delivery by hysterectomy and maintenance in isolators under positive pressure for their entire life span. These animals are devoid of all infectious agents except for those that are transmitted in the germline, e.g. endogenous retrovirus (ERV) or via intrauterine or transplacental pathways, e.g. herpes virus.
- Specific pathogen free (SPF) animals. The establishment of SPF animals can be achieved by hysterectomy of the dams and maintaining SPF breeding units of the descendent animals under barrier conditions to produce source animals.
- Animals free of designated pathogens/Qualified pathogen free animals. Source animals are from closed herds or colonies with documented health screening programmes. All infectious agents known to infect the species have to be considered.

this purpose and under no circumstances should cells, tissues and organs from wild animals or from abattoirs be used. Tissues of founder animals similarly should not be used.

Genetically modified animals

Cells to be used in xenogeneic cell therapy 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, where conducted in the EU, operations affecting both the source animals and the cells should comply with Directive 2001/18/EC and/or with Directive 90/219/EEC (as amended by Directive 98/81/EEC), as appropriate.

Cells from genetically modified animals must be fully characterised and confirmation of the nature of the inserted or deleted gene must be given. The manufacturing process should be established taking into account the content of the relevant CPMP guidance documents (published in *The rules governing medicinal products in the European Union*, or any future update). When using "humanised" animals as source animals, the infectious status must be checked before usage in case of latent infections.

2.2 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 SPF status of the herd.

SOPs should be present for:

- Detailing the housing of animals and containment 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

Veterinary control

Protocols for monitoring the herd for disease and infectious agents should exist. Specific screening procedures should include appropriate physical examination and laboratory tests. All infectious agents known to potentially infect the source species have to be considered including viruses, bacteria, mycoplasma, fungi, TSEs and parasites. The herd health surveillance system should include comprehensive documentation of all veterinary care received. The use of antibiotics and vaccination of source animals is not recommended. If the treatment of animals with any medicines is necessary for animal welfare reasons, an evaluation of the situation should be performed, and discussed with the competent authority. Any use of vaccines must be justified.

Quarantine

All animals entering the facility have to be put under quarantine for a defined period to allow completion of screening procedures. Individual quarantine periods depend on the animal species and characterisation and surveillance of the animal herd.

2.3 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 animals' exposure to infectious agents. All material entering a facility should be sterilised or decontaminated. Feed and bedding of a predefined quality should be obtained from a controlled source or vendor and should be stored under appropriate and controlled conditions. Environmental conditions, such as air flow (HEPA-filters, positive pressure) and water, should be 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.

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, of them 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.

2.4 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

2.5 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 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 should be tailored to the source animal species and the manner in which the xenogeneic cell product will be used clinically. Programme testing protocols should be updated periodically to reflect advances in the knowledge of infectious diseases. Whenever applicable, guidelines related to human and veterinary medicines should be consulted (such as the CPMP Note for Guidance on the Production and Quality Control of Animal Immunoglobins and Immunosera for Human Use - CPMP/BWP/3354/99).

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 putative 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 exercised.

It is critical that adequate and validated diagnostic assays and methodologies for surveillance of known infectious agents from the source animal are available prior to initiating clinical trials.

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 humans
- 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

Consideration also should be given to:

- the commensal populations
- the possibility of transmission of latent infectious agents via the intrauterine pathway (herpesviruses)
- the usage of sentinel animals to screen for subclinical infections.

Founder and source animals should be free of known TSE-diseases and the feeding history since establishment of the source animal herd should be documented and should not raise concerns regarding possible transmission of a TSE agent. In the use of cattle, goat and sheep, the requirements of the CPMP/CVMP Note for Guidance on minimising the risk of transmitting animal spongiform agents via human and veterinary medicinal products (EMEA/410/01- rev. 1 or any future revision) should be applied.

2.6 Archiving

Long term archiving of tissue samples, cell preparations and paper records will be necessary. The archiving time will be much longer than what is currently used in the pharmaceutical industry and may be as long as 20 to 40 years. Manufacturers should present to the authorities their plan for such long term archiving at the animal facilities as well as in the manufacturing plant. This is essential for proper monitoring of medicinal product quality and safety evaluation of exposed individuals in look-back procedures.

A protocol for archiving tissue samples should be established and validated to ensure traceability and the possibility for look-back. All samples to be archived must be collected carefully and should be as representative as possible. Archiving should be arranged in appropriate storage conditions and be protected from fire or flooding. There should be restricted access and nominated person/persons who is/are responsible for the archives.

Sampling should be planned so that various samples for different methods (e.g. pathology, hybridisation, antibody-testing, PCR) are available. Samples should include (at least) the tissue concerned (e.g. spleen, liver, bone marrow, CNS, lung), body fluids, and leukocytes. If sentinel animals are used, samples from them must also be archived in a similar manner as from the actual source animal. Samples must be stored either at -70°C (e.g. plasma) or in a cool dry dark place (e.g. for paraffin-embedded samples) depending on the method of collection, storage and further processing. Paraffin blocks are recommended for long-term storage.

All batches of xenogeneic cell therapy products should be labelled so that the corresponding samples in the archives can be traced. Archived samples should not be used for any other purposes, such as research.

All records (e.g. herd feeding and health records, source animal health documentation) should be archived for a period at least equal to that of the archived tissue samples. This paper archive can be kept separately from the tissue sample archive. For electronic archiving, the computer systems need to be validated and appropriate precautions should be taken to allow retrieval of the electronic data up to the end of the archiving period (see chapter 6.4).

3. MANUFACTURING

3.1 Introduction and general considerations

For the purpose of this section, the active substance can be defined as the defined number (pool) of viable xenogeneic cells and the finished medicinal products as the pool of xenogeneic cells formulated for the intended use.

The active substance will be derived from a manufacturing process that starts either:

- at the level of an organ or tissue. The pool of cells will be prepared from primary cells (cells isolated from fresh organs/tissues/biological fluids). The manufacturing process may also involve "intermediate cell batches". These "intermediate cell batches" are prepared from primary cells or after a limited number of passages and stored. Aliquots of these intermediate cell batches are then used to prepare the drug substance. They are likely to be prepared from fresh organ or tissue at regular intervals.

or from a well-defined cell bank system generally consisting of a master cell bank and a working cell bank. Cell banking may be possible in some cases, where the primary cells have the capacity of proliferation with or without transformation.

The manufacturing area should be physically separated from the animal facility and the area where organs or tissues are obtained. If diverse tissues and cellular products are collected, processed and stored in the same manufacturing area there is an increased risk of cross-contamination during each step of the procedure, e.g. via processing equipment or in storage containers such as liquid nitrogen tanks, and therefore, adequate control measures to prevent cross-contamination should be put in place.

3.2 Description of the manufacturing process

Flow chart

A flow diagram of the entire process from organ/tissue or from cell banks should be prepared indicating critical steps and intermediate products (e.g. intermediate cell batches), as well as operating parameters and in-process controls.

Organ/tissues

Various organs and tissues may be the starting material for xenogeneic cell therapy products. The techniques used to collect such materials should avoid contamination by the environment or by the operator.

When organs and tissues are to be transported from the place of extraction from the animal to the manufacturing facility, the conditions should be validated in order to guarantee a quality that is suitable for the whole manufacturing process.

Quality parameters aimed at the definition of acceptance criteria for a given organ or tissues should be set, taking into consideration shipment and storage conditions. In particular, functional parameters of the starting material should be defined and specifications given. When the process is based on a well defined cell bank system (MCB and WCB), information should be provided as mentioned in the CPMP document "Points to consider on the manufacture and quality control of human somatic cell

therapy medicinal products" on the source, history, generation of cell banking (MCB, WCB), characterisation and testing.

Cell manipulation procedure

After appropriate controls as mentioned above have been performed/implemented, the organ/tissue processing undergoes one or more of the following steps:

- organ/tissue dissociation
- isolation of the cell population of interest
- cell culture
- cell transformation (by physico-chemical agents or gene transfer)

- *Organ/tissue dissociation*

The procedure to obtain the cells from the organ/tissue has to be described (type of enzyme, media, etc.). Viral safety and freedom from prions should be considered for all animal derived materials (see below). Consideration should be given to the degree of disruption applied to the tissues in order to preserve functional integrity of the cellular preparation and minimize cross contamination of infectivity from other cell types.

- *Isolation of the cell population of interest*

Any procedure used for the isolation of the cell population of interest should be described. Its effectiveness should be addressed in relation to the homogeneity of the cells.

- *Cell culture*

During *in vitro* cell culture, consideration should be given to ensuring optimal growth and manipulation of the isolated cells. The processing steps should be properly designed to preserve the integrity and function of the cells. The procedures for such manipulation should be documented in detail and closely monitored according to specific process controls. Microbiological control is a pivotal aspect of process control and quality evaluation. Monitoring of *in vitro* cell culturing must include verification of absence of adventitious agents, such as bacteria, yeast, fungi or mycoplasma, at selected stages of the production. The culture should be examined for any microbial contamination in accordance with the culturing procedure and growth characteristics of the cells. A specific viral testing programme should be established. Consistency/reproducibility of the cell culture process should be demonstrated. Adequate limits should be set for critical parameters such as viability, cell density/confluence, purity, total duration of culture time and maximum PDLs.

- Cellular identity and retention of integrity of heterogeneous cell populations

During the process and/or on the final cell population, a series of tests need to be performed, as mentioned below, in order to determine the cellular identity and retention of integrity of heterogeneous cell populations.

Consideration should be given to the transformation potential of cells in response to growth factors since transformed cells may gain a growth advantage over those non-transformed under defined *in vitro* culturing conditions.

- Duration of cell culture

The principal genotypic and phenotypic traits of the primary cell cultures, of the established cell lines and the derived clones should be defined and their stability with respect to culture longevity determined. The culture conditions including duration should be optimised with respect to the function of the cells suitable for the intended clinical use.

- Cell viability

Cell viability analysis should be performed to guarantee integrity and quality of the cell therapy product, and batch consistency.

- Cell transformation

Various treatments (physical or chemical) can be applied to xenogeneic cells.

In the case of transformation dealing with genetic modification of xenogeneic cells, requirements set up in the CPMP/BWP/3088/99 Note for guidance on « Quality, preclinical and clinical aspects of gene transfer medicinal products » should be followed, which gives details on the quality control, characterisation and pre-clinical testing of gene transfer vectors.

Cell populations, which are genetically engineered should be assayed for appropriate and reproducible expression of the newly acquired characteristics. As far as applicable and practicable, this should be quantified and controlled.

Process validation

The entire cell manipulation process should be validated in all instances. It is recommended that such validation - using a cell preparation process fully comparable to those intended for clinical use - be performed on a regular basis, e.g. 6 monthly interval, for sterility, absence of mycoplasma and adventitious viruses, cell identity, cell activity, cell viability, cell proliferation, purity, gene transduction efficiency if applicable. A set of essential tests (e.g. viability, bacterial contamination, phenotype, cell number per dose), in compliance with the pre-determined specification limits must be performed before the cell product is released for clinical use.

3.3 Quality criteria for materials used in the manufacturing process

Screening for infectious agents in starting materials (see also 3.5)

The starting materials of xenogeneic cell products (live cells, tissue, organs, cell banks) should be screened by direct culture for bacteria, fungi and mycoplasma. A specific virological screening programme should be in place, with assays capable of detecting a range of infectious agents as well as species specific agents with appropriate sensitivity.

Whenever possible, samples from the animal live cells, tissues or organs should be tested with co-cultivation assays that include a panel of appropriate indicator cells, in order to amplify and detect xenotropic endogenous retroviruses and other xenogeneic viruses which may be capable of initiating infection in humans. The selection of indicator cells should be determined by the xenogeneic cell product and its clinical applications. Serial blind passages and observation for cytopathic effects and focus formation in combination with reverse transcriptase assays and electron microscopy may be appropriate.

If cultures demonstrate the presence of viral agents, direct or indirect virus detection methods should be routinely employed. Universal nucleic acid amplification-based detection strategies whenever available would be preferred. Agents that may be latent or are known to establish persistent latent infections in the absence of clinical symptoms, e.g. herpesviruses, retroviruses, papillomaviruses, are of particular concern and their detection may be facilitated by using chemical or irradiation techniques.

Media and reagents

Various materials are needed for genetic or phenotypic modification, such as enzymes, antibodies, cytokines, sera, and antibiotics. Exposure to such materials can also compromise the quality, safety and efficacy of the final therapeutic product. As a consequence, each substance used in the procedure should be clearly specified and evaluated as to its suitability for the intended use. Materials involved in the collection, selection and manipulation of cells should be described in detail. The sterility, absence of contaminating agents and low endotoxin level of ancillary products should be ensured.

It is recommended to avoid the use of reagents with sensitisation potential. Where appropriate, the

Note for Guidance on the "Production and Quality Control of Medicinal Products Derived by Recombinant DNA Technology" and the Note for Guidance on the "Production and Quality Control of Monoclonal Antibodies" should be taken into account.

The manufacturing process of xenogeneic cell therapy products does not include stringent purification steps or any virus removal or inactivation steps and, therefore, acceptance criteria for all materials derived from human or animal origin should be very stringent.

- *Human derived materials*

Reagents of human origin (e.g. albumin, immunoglobulins) used during the manufacturing process should be evaluated for their suitability in a manner identical to that employed in the recommendations of the CPMP Note for guidance on plasma-derived medicinal products (CPMP guidance 269/95, rev. 3). Clinical selection of donors should be in compliance with relevant European legislation and guidelines.

- *Animal derived materials*

Animal derived reagents may harbour infectious agents and may increase undesirable immunological responses in the recipient. When applicable, the use of animal reagents should be avoided and replaced by non-animal derived reagents of defined composition.

The use of bovine, ovine or caprine derived reagents should conform to the CPMP and CVMP Note for Guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMEA/410/01, rev.1 or any future revision).

When bovine serum is used, the recommendations of the Note for Guidance on the "Use of Bovine Serum in the Manufacture of Human Biological Medicinal Products" (CPMP/BWP/1793/02) should be followed. The use of irradiated sera and/or alternative synthetic media is encouraged and should be considered.

3.4 Characterisation of the active substance

An extensive characterisation of the cell population of interest should be established in terms of identity, purity, potency and suitability for the intended use. In some cases, additional testing such as tumorigenicity or karyology may be useful. This characterization will allow setting up of the routine controls that will be applied for release of the drug substance and drug product as well as those to be performed at several steps of the process. It is the responsibility of the manufacturer to define the control strategy that will be applied at each step of the process and those applied for the release of xenogeneic cell therapy products so that a product of consistent quality is obtained.

The type of tests to be performed are listed below.

Identity

Appropriate tests should be performed to determine that the cell population is what it is intended to be. Either phenotypic or genotypic characteristics may be used in identity testing. It is not considered necessary to perform all possible tests.

For cells that grow attached to a substratum, morphological analysis may be a useful tool in conjunction with other tests. In most cases, isoenzyme analysis is sufficient to confirm the species of origin, although other technologies may be substituted to confirm species of origin, including, for example, banding cytogenetics or use of species-specific antisera. An alternative strategy would be to demonstrate the presence of unique markers, for example, by using banding cytogenetics to detect a unique marker chromosome, or DNA analysis to detect a genomic polymorphism pattern (for example, restriction fragment length polymorphism, variable number of tandem repeats, or genomic dinucleotide repeats). Either confirmation of species of origin or presence of known unique cell line markers is considered an adequate test of identity.

Microbial safety

A critical aspect is to establish that xenogeneic cell products are free from adventitious microbial agents and adventitious cellular contaminants. The impact of selective agents and antibiotics on the detection of adventitious microbial contaminants should be considered when planning and performing these tests.

Tests for the presence of bioburden (bacteria and fungi) should be performed with the current methodologies described in the European Pharmacopoeia (Ph. Eur.). Tests for the presence of mycoplasma should be performed.

Viral contamination may arise from the starting material (see above), or from adventitious introduction during the manufacturing process or the activation of a latent virus during the manufacturing process. The viral safety programme should address the risk of contamination arising at these various steps. Due to the limited number of cells often used in xenogeneic cell therapy and the minimal purification steps involved, viral clearance studies may be difficult to apply.

Screening, whenever possible, at the level of the drug substance for xenotropic endogenous retroviruses and other viruses capable of infecting humans is highly recommended (see above).

Although xenogeneic cell therapy products are excluded from the scope of the ICH guideline on viral safety ("Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnology Products Derived from Cell lines of Human or Animal Origin CPMP/ICH/295/95), applicants may consult this guideline. The current World Health Organization (WHO) documents for use of animal cells may also be consulted.

Potency

A valid biological assay to measure the desired biological activity should be established by the manufacturer.

Karyology and tumorigenicity

Karyology and tumorigenicity testing of cells derived from a cell banking system may be required. Reference in made to the ICH Q5D "Derivation and characterisation of cell substrates used for production of biotechnological/biological products" (CPMP/ICH/294/95) and to the WHO document "WHO Requirements for Use of Animal Cells as *in vitro* Substrates for the Production of Biologicals" in WHO Expert Committee on Biological Standardization, 47th Report, Geneva, World Health Organization (WHO Technical Report Series).

3.5 Development pharmaceutics (formulation)

Xenogeneic cells can be administrated in several ways. The cells can be infused intravenously, injected site-specifically, or they can be surgically implanted.

When medical devices are used in combination with xenogeneic cells (e.g. bio-artificial livers) they should comply with the European directive 93/42/CEE. When matrices of bio-compatible polymers are used in combination with xenogeneic cells (e.g. microencapsulation of Langerhans islets cells) they should be characterised with respect to their physico-chemical properties, and defined and controlled by a satisfactory specification. Their suitability for use in a clinical setting in terms of biocompatibility and durability should be properly evaluated. Other auxiliary components, such as fibres or beads introduced along with the cells for structural, biological, or immuno-isolation purposes, should be viewed as part of the final product and evaluated accordingly.

3.6 Active substance and finished medicinal product lot release testing and stability

In some cases, the active substance and the finished medicinal product will be very similar for example when only a filling step occurs. With regard to specifications, it would be acceptable to have reduced testing at one level provided an exhaustive control is performed at the other one.

For the active substance and the finished medicinal product, the following information needs to be provided:

Batch definition

A definition of a production batch should be given (i.e. size, number of passages, pooling strategies, batch numbering system).

Container closure

A description of the container closure should be provided. Compatibility with the product should be demonstrated. It should be indicated if the container closure has a CE marking under the Medical Devices Directive 93/42/CEE. Information on the sterilisation procedure needs to be described.

Specifications

The specifications retained for the release of the active substance and finished medicinal product batches are selected on the basis of the characterisation section. Selection of tests is product specific and has to be defined by the manufacturer.

Specifications for lot release testing should be set for identity, purity (limits for product and process related contaminants), homogeneity, microbial safety, potency, cell viability and metabolic indicators, cell number

Stability

The xenogeneic cell therapy medicinal product should be assigned a valid in-use shelf life. This should be supported by experimental data with regard to the maintenance of cell integrity and product stability during the defined period of validity.

Storage conditions including temperature range need to be determined.

If relevant, appropriate methods for freezing and thawing should be documented.

4. NON-CLINICAL TESTING

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 homing of cells into tissues/organs, effects induced by rejection or encapsulation of xenogeneic cells and effects like graft versus host disease (GvHD) in immuno-suppressed animals.

The recommendation of the *ICH Document S6 on the safety of biotechnology-derived pharmaceuticals* should be considered. The number of animals, the genders and frequency and duration of monitoring should be appropriate to detect toxic effects.

4.1 Pharmacology

4.1.1 Biological activity / pharmacodynamics

The biological activity of xenogeneic cells or the expression of a xenogeneic cell product should be first evaluated *in vitro* and subsequently *in vivo* as part of proof of concept. *In vitro* studies may also provide information on cell morphology, proliferation, phenotype and the level of differentiation. The results of these *in vitro* studies are reported in Module 3 of the submission and should be considered in the context of non-clinical *in vivo* studies.

Non-clinical *in vivo* studies should provide the "proof of concept" for subsequent clinical trials. Non-clinical studies may provide valuable data to support the posology and concomitant (immunosuppressive) treatment chosen for human clinical trials.

4.1.2 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. Dosages should be given that are in relation to exposure in the therapeutic range, if known.

Effects on central nervous system endpoints should be studied if applicable. Further secondary pharmacodynamics should be considered on a case-by case basis depending on the character of the excreted bioactive molecule, if present.

For further guidance see ICH S7A Note For Guidance On Safety Pharmacology Studies For Human Pharmaceuticals (CPMP/ICH/539/00), when applicable.

4.1.3 Pharmacokinetics

Studies for pharmacokinetics should be performed for xenogeneic cells synthesising bioactive molecules. The survival, the general functioning and stability/functioning of the cells at appropriate tissues/organs should be studied. When the xenogeneic cell therapy consists of cells whose biologic activity is not dependent of well-defined molecules, pharmacokinetic studies will be impossible and may be omitted.

The survival and adequate function of the administered xenogeneic cells, as well as the corresponding host tissues or organs grafted should be studied during a period of at least 6 months. Shorter study periods should be scientifically justified. The influence of immunological effects on the measurements in the different animal systems should be considered.

Migration and persistence of Xenogeneic Cells

The tissue distribution and duration of expression of xenogeneic cells should be studied when appropriate.

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

Metabolism

Biotransformation studies and studies on Absorption, Metabolism and Excretion are not considered necessary.

4.2 Toxicology

4.2.1 Single/repeated dose toxicity studies

The toxicity studies have the main purpose to confirm whether the dose range of xenogeneic cells required for exerting the desirable pharmacodynamic effect is tolerated, rather than to define the Maximum Tolerated Dose (MTD).

Toxicity studies due to the nature of xenogeneic cell products, should be performed in relevant animal models and, if the xenogeneic cells are not immediately rejected, may be combined with safety pharmacology, local tolerance, immunotoxicity or proof of concept and efficacy studies.

The duration of observations in such studies might be much longer than in standard single dose studies, since the xenogeneic cells are supposed to function for long times, which should be reflected in the design of the studies. The duration of those studies should be considered also in the clinic (see

ICH M3 and S4A). e.g. if a chronic clinical effect is sought, then a 6 month observation in the animal study should be the aim (if no rejection!).

In toxicity studies, the route and dosing regimen should reflect the intended clinical use.

4.2.2 Genotoxicity studies

As for biotechnology-derived pharmaceuticals, genotoxicity studies are not considered necessary for xenogeneic cell/tissue products unless the nature of the expressed products indicate an interaction directly with DNA or other chromosomal material. The omission of genotoxicity studies should be discussed.

4.2.3 Carcinogenicity studies

Potential mechanisms involved in carcinogenicity may be transgenic manipulations, endogenous or exogenous viruses, *ex vivo* culture, immunosuppression, or a direct action of the xenogeneic cell / tissue product. For the testing of the carcinogenic potential of the xenogeneic cells both *in vitro* and *in vivo* methods may be appropriate and should be considered. The need for carcinogenicity studies should be discussed in the light of the intended use and the type of the product. The choice of the models and the design of the studies should be discussed on a case-by-case basis.

4.2.4 Reproductive performance and developmental toxicity studies

The need for reproductive/developmental toxicity studies is dependent upon the xenogeneic cell / tissue product, area of implantation, clinical indication and intended patient population and should be discussed on a case by case basis. The results of previous studies with isolated substances, that are produced by xenogeneic cells should be considered.

4.2.5 Local tolerance studies

Local tolerance studies may be required in an appropriate species. However, if the proposed clinical formulation and route of administration have been examined in other animal studies then separate local tolerance studies are not necessary. Most often local tolerance can be evaluated in single or repeated dose toxicity studies thus obviating the need for separate local tolerance studies.

4.3 Other toxicity studies

4.3.1 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 segregation of the cells or control of the immune response, e.g. by immunosuppressive drugs, natural antibody purging, xenogeneic antigen deletion/modification (genetically modified animals) and tolerance induction. These methods raise different concerns that should be addressed in appropriate non-clinical studies.

The level of immune response will depend on the nature and modality of cells used and the testing programme should be adapted accordingly (see introduction).

Ex vivo use with a barrier (extracorporeal use); encapsulated cell products with non-inert material may induce tissue reactions. The compatibility of the animal cells may be also increased by immune-isolation. Any encapsulating device used for protecting the administered cells from the recipient's immune system should be considered as part of the final product. Therefore, it should be characterised, quality controlled and lot released. In this situation, immunological studies may be useful to support the integrity of the barriers.

Another approach is the introduction into the animal cells of human genes coding for proteins that ameliorate the hyperacute rejection. In practise of course a complete tolerance is very difficult and potentially risky.

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, i.e. of antibodies, immune complexes, complement activation and antibody dependent cytotoxic cells and subsequent immunogenic cellular reactions.
- Infiltration of immune/ inflammatory cells into the xenogeneic cells or encapsulation of xenogeneic cells resulting in necrosis or impaired function or production of xenogeneic cell products i.e. proteins/hormones.

Graft versus Host Reaction in animals with and without immuno-suppression should be investigated when appropriate.

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

Effects of immunosuppressive substances in order to maintain the xenogeneic cells and the direct immunoactivation or immunosuppressive effects originated by the xenotransplant should be differentiated, if possible.

The survival of the xenogeneic cells and the underlying mechanisms responsible for the rejection, encapsulation or necrosis should be studied.

4.3.2 Studies on viral mobilisation

The possibility that replication competent and pathogenic endogenous retroviral elements in the xenogeneic cells could be mobilised should be addressed also in relevant non-clinical studies.

The possibility of reactivation of other types of latent viruses (e.g. herpes zoster, Epstein-Barr virus and cytomegalovirus) should also be investigated when appropriate.

In vitro studies on viral mobilisation after xenogeneic transplantation to human tissue, with and without addition of immunosuppressants, might be useful. It should be considered if a model using SCID mice transplanted with human tissue could be applied (e.g. NOD/SCID mice for diabetes type 1 evaluation).

In the *in vivo* studies, part of the tested animals should be immuno-suppressed to mimic the intended clinical situation, in order to study whether immunosuppression induces viral mobilisation. It should however be taken into consideration that viral mobilisation might differ between different species.

5. HUMAN EFFICACY AND SAFETY

5.1 Claims and patient selection

The use of xenogeneic cell therapy may be attractive because of the limited availability of human cells and a relatively reduced risk of immune rejection compared with solid organ xenograft.

There are at least three types of possible claims: temporary/bridging/extracorporeal use, treatment of last resort, treatment where other alternatives are available. Because of the potentially serious public health risks arising from possible transmission of infectious agents, xenogeneic cell therapy should be limited to patients with serious or life-threatening disease for whom adequately safe and effective alternative therapies are not available, and where there is a potential for a clinically significant benefit.

5.2 Xenogeneic cell therapy team/site

A multidisciplinary team with appropriate competence and infrastructure support is mandatory. As a result, the use of xenogeneic cell therapy should be restricted to specialised centres, usually hospitals, with access to experience and expertise in this type of cell therapy, testing of infectious agents and other relevant monitoring procedures as well as in management of adverse events. A crisis management plan identifying risks and appropriate response measures should be available.

5.3 Endpoints

Both safety and clinically relevant efficacy endpoints are required, as appropriate for the clinical condition. The therapeutic intervention (implantation, infusion etc) and concomitant treatments used to enhance the safety and efficacy of xenogeneic cell therapy should be documented. Because of limited understanding on the control and subsequent development of xenogeneic cells within human recipients, the efficacy and safety of such therapy will require regular long-term assessment and the duration of follow-up has to be justified. The possible impact of the disease process itself on the transplanted xenogeneic cells should also be clarified.

5.4 Non-clinical data and the clinical development

This embraces both preclinical and clinical development. Proof of concept in suitable large non-rodent animal models is required before proceeding to studies in humans. Justification is required that knowledge gained from the chosen animal models can be extrapolated to the clinical situation.

Non-clinical studies

The amount of clinical data will be limited since xenogeneic cell therapy will be, at least initially, restricted to small patient populations. Studies in healthy volunteers are not feasible and the establishment of dose and kinetics will be difficult in the clinical trials. Thus, non-clinical studies will be decisive for the "posology" and method of administration of xenogeneic cells. The viability, functions and kinetics of transplanted xenogeneic cells in animals as well as in human blood should be demonstrated. Rejection reactions should be characterised, including complement and platelet mediated damage, as well as cellular rejection. Ancillary measures including immunosuppressive treatment and immunoisolation devices, if required, should be studied and selected on the basis of preclinical studies and relevant clinical experience for further study.

5.5 Allogeneic cell therapy

Developments using allogeneic cell therapy for similar indication should be taken into account. Justification must be given before proceeding with clinical trials using xenogeneic cells for indications where corresponding allogeneic cell therapy is available.

5.6 Techniques for manipulation and administration

The development of optimal techniques for the manipulation and administration of xenogeneic cells needs to be shown because they are critical to subsequent success.

5.7 Pharmacodynamics

The biochemical and physiological effects of the xenogeneic cells in the recipients should be evaluated. In general, the pharmacodynamic endpoints for xenogeneic cell therapy should be the same as those applied for the allogeneic cell therapy or other relevant alternatives.

5.8 Pharmacokinetics

There must be method(s) to trace the xenogeneic cells in clinical samples. The distribution, proliferation and survival of the xenogeneic cells need to be characterised within the recipients. The methods of measurements and duration of assessment should be justified. Possible approaches may include biochemical/clinical endpoints, imaging studies, or flow cytometry.

5.9 Dose finding

The quantity of cells required to achieve the intended clinical endpoint(s) should be determined. The methods for quantification and dosage calculation should be justified.

5.10 Concomitant treatment

A number of measures may be used to enhance safety and efficacy. These may include immunosuppressive agents, anticoagulants, antiviral agents or even vaccination. Concomitant treatment schedule should be tested rigorously, including the monitoring procedures for therapeutic effects and adverse events.

5.11 Pivotal clinical trials

The design of the clinical trials should be appropriate for the clinical condition in question. Reference should be made to existing relevant guidelines.

The added considerations are:

- Informed consent and counselling; this will require detailed information on the risks and alternatives, the need for long-term follow up with regular efficacy and safety assessments, archiving of plasma and tissue samples for future analysis, possible access of the recipient's medical records by public health agencies, a request for autopsy and confidentiality issues.
- The duration and schedule for follow up efficacy and safety assessments should be justified.
- Adoption of optimal techniques for the manipulation and administration of xenogeneic cells.
- Interventional techniques should be clearly described. Feasibility of repeat procedures and the consequences should be evaluated. Justification for the techniques is required, particularly if the techniques are new to clinical practice.
- The Sponsor should commit to updating the recipients with new information on risks, benefit and the need for additional treatments as part of the long-term follow up.
- The Sponsor must demonstrate that the entire chain of procedures beginning with cell harvesting, through to cell preparation, storage and transport, and administration, is clinically feasible. The SPC should provide sufficient information on the handling and administration of the cells, as well as specification on how to determine the viability and functions of these xenogeneic cells immediately prior to their use.
- Public health implications.

5.12 Clinical Safety

The safety assessment includes the risks incumbent on the recipients, those in close contact with the recipients, health care providers and the public health implications. Acute and long-term risks should be considered.

5.13 Interventional procedure

The risks associated with the procedure and repeat procedures should be assessed.

Meticulous adoption of optimal interventional techniques should be ensured.

5.14 Immunological complications

Safety issues arising from immune rejection, immunosuppression, and breakdown of immunoisolation devices should be considered. The development of late complications, such as cancer and opportunistic infections should also be considered.

5.15 Infections

The risk of transmission of infection in the course of xenogeneic cell therapy procedures represents one of the most important issues to consider.

Infections may be acute or chronic. It is anticipated that recipients are at highest risk for infections in the first few months after the procedure. However, there may be a significant delay in clinical manifestations of infection. As with allogeneic transplantation, recipients of xenogeneic cell therapy are at risk for infections due to immunosuppression. A recipient may also be at risk for infections caused by agents contained in the xenogeneic cell product. These could broadly include zoonoses, endogenous retroviruses and other unknown animal pathogens. It is not possible to predict clinical symptoms of all possible infections. Thus, when the source of a recipient's post-treatment illness remains unclear, testing should be performed in appropriate samples. Long-term safety assessment of the recipients is mandatory.

Apart from the risk of transmission of zoonotic infections to the recipient, there are also the risks of transmission of infectious agents into the close contacts and even into the general population after adaptation in an immunosuppressed recipient. A system to monitor close contacts of the recipients and health care providers is required (see pharmacovigilance section).

An assessment of the risks, methods of detection for acute or chronic infections and periodicity of screening for pathogens is clearly required. This system must be adapted to the type of xenogeneic cells, duration of expression, and the epidemiological situation. The monitoring system must deal with both known human, relevant animal and unknown pathogens, including new pathogens evolving through recombination The applicant must present a rigorous system for the testing of infections in the recipients as well as in their close contacts. (For further discussion, see 6.1).

6. PHARMACOVIGILANCE AND SPECIAL SURVEILLANCE METHODS

There is a need to guarantee continuity of information gathering and look-back procedures throughout sourcing, manufacturing, development and clinical use of the xenogeneic cell therapy products.

The special requirements set out in this document should be considered taking into account possible different levels of risk associated with each individual product and the sought therapeutic use.

The requirements are to be considered also in the light of relevant national and EU legislation.

6.1 Groups at a (potential) risk

Xenogeneic cell therapy has potential risks that are not seen with the current medicinal products. These risks may affect not only the patient but also his or her close contacts as well as the public in large.

6.2 Surveillance for possible risks

Zoonoses, both known and unknown may be difficult to recognise without special surveillance methods. There should be a plan to control a possible outbreak of atypical infections.

Establishment of a special surveillance system:

In addition to the existing national and European surveillance systems there is a need of applying new tools and methodologies. The surveillance system needs to be fully operational and has to start prior to exposure of the patients to the products irrespective whether in clinical trials or in clinical practice.

Tracking of patients and close contacts for potential development of infections that may present public health hazards is deemed to be of inherent importance. The establishment of the surveillance system shall ensure full traceability of the medicinal products, documentation of the manufacturing process of individual batches, of raw materials used and animal records Information from all relevant sources should be integrated. The system should enable rapid identification of epidemiological significant common features among recipients and provide data for the assessment of long-term safety of xenogeneic cell therapy.

Collaboration with infectious disease epidemiological surveillance systems- where existing - is considered to give important contribution to the assessment of the safety of xenogeneic cell medical products.

Key elements of the surveillance system to be considered in a risk-management plan include:

- Clinical surveillance of individuals should be in place in order to monitor for clinical diseases.
- Laboratory testing of recipients is a powerful augmentation to clinical monitoring
- Monitoring data of all recipients via registries is deemed necessary.
- Biological specimen archives ensure the ability to investigate future adverse events.
- Data should be registered in a database for identification of risk signals and common features among recipients and reviewed on a regular basis.
- Ad hoc surveillance studies including epidemiological studies may support the investigation of certain emerging issues.

An efficient system for the prompt retrieval and linkage of the reported observations with the clinical trial records, the biological specimens and the source animals should be in place and ready for use prior to marketing authorisation.

The Marketing Authorisation Holder (MAH) has to submit a detailed plan of the surveillance system prior to marketing authorisation including full scientific judgement on all elements of the clinical and laboratory monitoring. The marketing authorisation holder is obliged to submit a detailed protocol for monitoring and testing, including the time schedule and volumes of samples. The MAH is responsible for proper collection, storage and proper reporting of information to competent authorities.

Personal data concerning the recipient, close contacts and health care providers should be treated as confidential and handled in accordance with rules on personal data protection.

6.3 Clinical surveillance

Monitoring of all exposed individuals at periodic clinical visits for clinical disease is essential. Surveillance of recipients should continue life-long or as long as the technology is applied unless further data justify a limited duration of follow-up. These principles should be considered for *ex vivo* perfusion involving bioreactors, such as bioartificial liver devices, unless data demonstrate that these kind of products have different safety clauses in terms of preventing microchimerism and infections due to inherent physical barriers in the system. It is well recognised that e.g. in the *ex vivo* perfusion system, membranes will separate to various extent the animal cells from the blood of the patient. If the applicant can demonstrate by validated tests that the relevant pathogens will not be able to enter the blood, the requirements for the surveillance system may not be as extensive as for implanted/transfused medicinal products.

It is recommended that the recipient prior to the exposure to the product should agree upon the follow up measures via informed consent. The recipient should be informed about the potential risks of xenogeneic cell therapy. Information should be presented in a way that the recipient could acknowledge that these constraints are extremely important to protect the health of patients, close contacts and the public. Measures shall be put in place to guarantee the safeguard of the subjects' privacy and the freedom of consent.

In general, clinical surveillance may be more intense shortly after treatment. Intervals of clinical and laboratory surveillance may increase over time.

Laboratory surveillance

A laboratory surveillance programme should be instituted. All efforts should be made to perform the testing on all patients. Laboratory testing should include testing for infectious agents e.g. known infectious agent testing that should be in use for the source animal screening programme and endogenous retroviruses (e.g. PERV). It has to be defined prior to marketing authorisation which test should be performed on a regular basis. It may be acceptable that certain tests will only performed when clinically indicated (e.g. in the case of a suspected transmission of an infectious agents).

In the event that an acute infectious episode warrants, additional investigational testing and the appropriate testing facilities should be identified. Clinical centres should be made aware of proper procedure for sample submission.

Laboratory tests should be available for agents known potentially to be present, including agents known to be pathogenic in the source animal species and also agents that infect human cells *in vivo* and *in vitro*. The capability to test for latent pathogens should exist. In general, clinical testing programme to detect infectious agents should be individualised for the specific xenogeneic cell product in question because infectious agents may differ among cell types and among source animals species. It is important to clearly identify species specific infectious agents e.g. to distinguish between infections derived from the source animal and those present in humans. Appropriate validated state-of-the art tests should be used. Tests considered to be appropriate to ensure the safety of the product should described prior to marketing authorisation and validation documents should be submitted in the application dossier for marketing authorisation. For certain transmissible agents well-validated assays may not exist. Further assay development and research should continue to be conducted by the MAH..

Registries

It is considered that data of all recipients should be available via a register. Monitoring clinical and laboratory data of all recipients will facilitate the early discovery of significant common features and especially incidents that can suggest that an infectious agent has been transferred. The register will also facilitate the work with tracing infectious agents and implementation of retrospective as well as prospective analyses. The register will trace even rare adverse events. It is also possible to calculate incidence rates.

Due to uncertainties concerning potential risk it is recommended that specialised medical teams in an centre accredited for the manipulation of cells and treatment with cells should undertake xenogeneic cell therapy according to an approved protocol. Detection, diagnosis and effective treatment of recipients' infection, if it occurs, is only possible in a well co-ordinated team. The clinical sites should have either in-house an accredited laboratory facilities with capability for performing routine tests for pathogens or access to an accredited laboratory with this experience. Only specialised and accredited centres should participate in the register.

Surveillance of close contacts and health care providers involved in xenogeneic cell therapy

It is important to provide adequate information about possible risks to close contacts and health care professionals involved in xenogeneic cell therapy. It is not always feasible and not necessary to monitor close contacts and health care providers on a routine basis for infectious diseases related to xenogeneic cell. However, the principles of the surveillance of the close contacts must be determined. The system becomes operative if the recipient is infected and a risk of transmission cannot be excluded for close contacts and health care providers. All efforts should be made to adequately inform close contacts if transmission of infectious agents cannot be excluded. It is recommended to archive tissue/blood samples of close contacts prior to the procedure for retrospective testing. Appropriate protection of confidentiality is mandatory.

The MAH should have a safety programme in place for all clinical, laboratory and manufacturing personnel.

Education of health care workers

The MAH should be responsible for providing comprehensive information specific for the product to health care workers in order to ensure proper handling of the product, the treatment procedure and the follow up.

6.4 Screening programme

After licensure, prospective, routine screening for immune response of the recipients, functionality of the xenogeneic cells and for possible infections should be performed. Scientific justification of the protocol is warranted. Active health monitoring of recipients and their contacts should include regular clinical and laboratory information as well as other relevant medical information.

The terms of reference for the surveillance system should be clearly defined including precise description of monitoring and reporting requirements (e.g. who has to report to whom).

Prospective screening programme for infections

Unlike in the allogeneic context, there will be a need for long-term surveillance and tracking of transmissible diseases including routine clinical and laboratory monitoring. Routine screening for clinically apparent and unapparent infections is necessary. Active screening of infections should allow detection of a novel infection and a disease in a recipient as early as possible, even before clinical symptoms may occur. If a xenogeneic cell product known to harbour an infectious agent is used, active screening for that infectious agent should be done, e.g. in the case of porcine cell transplantation all recipients should be screened for PERV (porcine endogenous retro viruses). It is realised that the frequency of follow-up monitoring will decrease with the time post-procedure. It is reasonable to plan a tapering frequency of screening. Appropriate biosafety precautions should be followed.

It is important to perform a post-mortem examination with appropriate tissue samples including histopathology and cultures to clarify the cause of death. If possible, blood samples and autopsy samples of relevant tissues should be collected and archived. Samples for endogenous retrovirus testing should also be taken.

Health records

It should be ensured that the recipient's medical records contain all relevant information on recipients health including all screening test results, all adverse events and all results of retrospective testing as well as all relevant xenogeneic cell therapy related information including procedure, product description including information of the source animal. A tracking system should be implemented. Additional information including clinical examination and laboratory testing as well as additional information on source animals should be collected in the case of adverse events, when appropriate.

Health records of a recipients concern information generated from different databases of the surveillance programme e.g. the registry, the laboratory or archive specimen database. Information is also linked to information on the whole production chain of the product.

Health records should include:

- Product information: animal source, manufacturing facilities, clinical centres, xenogeneic cell therapy product identifiers (see quality module), MAH contacts
- Recipient information: (of note, a unique identifier e.g. a code number, patient initials and birthdate is necessary to link the recipient to relevant information in the tracking system)
- Procedure information: recipient identifiers (see above), date of procedure, clinical centre where treatment was performed, physician who performed the procedure, procedure description
- Medical history and clinical status of the recipient prior to procedure
- Medical information at each follow up: Medications and therapies of the recipient, clinical information on rejection reactions, infections, any significant co-morbidity, hospitalisations or inter-current condition,
- Adverse event reporter

- Adverse event (including time of onset, duration, outcome)
- Recipients follow up examinations. Date, testing, specimen, result of the examinations
- Measures: treatment of recipient for adverse event
- Animal health events
- Recipient deaths reports

The MAH has to take provisions for the surveillance system, all samples and records to be maintained in the event that the establishment ceases operation. The running of archives and registries can be contracted to a third party when appropriate for practical and legal reasons.

Archives of biological specimens

In addition to the active screening programme, appropriate body fluids (blood, plasma, urine etc) should be collected and archived for retrospective testing in the case of a diagnosed infection or a suspected infection. The applicant must ensure that specimens from the source animals, the xenogeneic cells, and the recipient are archived for further evaluation in the case of indications of risks and transmission of disease. Samples of the close contacts should also be archived if possible. The location and nature of archived samples should be documented in the health care records and the information should be linked to the databases when they become functional.

The schedule of the collection of such samples should be presented prior to marketing authorisation. Sample should be acquired prior to xenogeneic cell therapy, during the procedure, immediate after and at appropriate time thereafter. Biological samples should be collected and archived under conditions which will guarantee their suitability for subsequent public health purposes such as investigations to assess the public health significance of infections that occurred. The MAH is responsible for making available an accurate archive of biological specimens with the appropriate safeguards to ensure long-term storage for several decades (see also section 2.6). The MAH should consider the animal source and type of product in proposing the schedule and test to be used.

6.5 Notification of adverse events

Serious adverse events should be notified immediately to competent authorities. Reporting forms should be uniform and should include all relevant information.

Notification of the following adverse events are considered to be important

1. Potential transmission of infectious diseases: If any new information about potentially communicable diseases associated with the recipient and the corresponding source animal becomes available following the administration of xenogeneic cells (e.g. post mortem examination) it has to be communicated to the competent authorities..
2. Appearance of any infection in a recipient e.g. HIV, HBV, HCV, TBC or other infection and any evidence of pathogen transmission including all zoonoses. It should be clearly defined which kind of pathogens occurring in a close relative should also be reported.
3. Appearance of a new cancer in a recipient.
4. Primary non-function or mal-function of transplanted xenogeneic cells.
5. Rejection of the xenogeneic cells.
6. Death of the patient.
7. Other clinically relevant adverse events possibly caused by the xenogeneic cell therapy.

Information on suspected adverse drug reactions should be provided to the recipient. Information is crucial also for close contacts and the public in the case of a reasonable risk to acquire an infection. Appropriate measures should be taken to ensure persons privacy

Clear guidance should be provided how adequate actions should be taken if a recipient is tested positive for a transmissible pathogen. All provisions and procedures designed to minimise the risks to

the public health should be described in a protocol prior to the exposure of recipients.

Transparency of the data is recommended to enhance the confidence of the public in xenogeneic cell therapy.

6.6 Database

As part of the surveillance system, a unique electronic EU databases should be developed or existing databases should evolve in a network and be amended to identify and to implement EU wide compatible system design, routine data collection methods, data reporting and general start-up and to assess routine operational issues associated with a fully functional database. In this context, uniform criteria for reporting to ensure compatibility of data are necessary. Existing tools such as MedDRA can be used. The MAH shall ensure full and updated data entry as well as the transmission in appropriate format of relevant documentation.

The database should include information and extensive internal and external linkage facilities on:

- Xenogeneic cell therapy medicinal product (including identifiers for each batch)
- Clinical Facility data
- Patient information/ identifier or code number and details of the method of administration
- Adverse events
- Updated action plan sheet and information for Patient follow-up, clinical examination and laboratory testing
- Patient death report (when available)
- Testing of the biological specimens
- Contact details for implementation of measures to minimise the risk of transmission of infectious diseases
- Provisions for access to animal facility data and animal health report (depending on the product).

Such databases would enable i) recognition of occurrence of adverse (health) events, including consequences of putative xenogeneic infections; ii) linkage of those events to exposures on a national and international level; iii) notification of individuals and clinical centres regarding epidemiologically significant adverse events associated with xenogeneic cell therapy; iv) biological and clinical research assessments, and v) assessment of long-term safety.

Competent authorities of the Member States, the Agency and the Commission should have access to the database.

6.7 Reporting requirements

MAH is requested to submit a safety report including data on health records, adverse events, laboratory testing as well as animal health data and facility information data on a regular basis (quarterly for the first two years after licensure, half yearly for the following three years and annually thereafter) to competent authorities.

The MAH is responsible to forward any emerging new information (e.g. about infections in source animals), which may have impact on the well-being of the recipient and/ or contacts and/or the public immediately to competent authorities.

Transparency of clinical work and potential risks to the public including positive and negative results in xenogeneic cell therapy has to be ensured.