A European PTC Document on Xenogeneic Cell Therapies

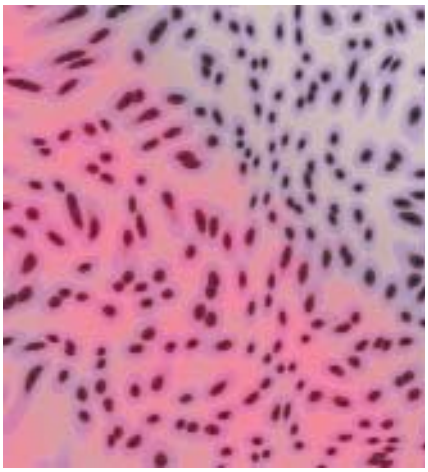
by Gail Sofer

Back in 2000, the European Committee for Proprietary Medicinal Products' biotech working party (CPMP–BWP) issued a concept paper describing the need for a points-to-consider (PTC) document on xenogeneic cell therapy medicinal products. After discussion at a rapporteurs' meeting in 2002, just such a document was transmitted to the CPMP (now Committee for Medicinal Products for Human Use or CHMP) and the equivalent committed for veterinary products (CVMP) (1). The document was released for consultation, and after further discussions it was adopted in December 2003. It came into force this past summer, in June 2004.

Xenogeneic cell therapy is defined in this PTC as

> 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.

Not surprisingly, the concerns expressed are similar to those found in the FDA's guidance on xenotransplantation (2). The risk of introducing new infectious diseases into the general population is a public health concern. The potential for zoonotic diseases necessitates development of long-term surveillance plans in advance of administering xenogeneic cellular therapies. This European PTC document addresses animal sourcing, archiving, manufacturing, and nonclinical testing. Highlights of these topics are discussed below. Clinical testing, pharmacovigilance, and special surveillance methods are not covered in this overview — but they are addressed in the PTC.



BRETT SPARKS (WWW.ISTOCKPHOTO.COM)

SOURCING OF ANIMALS

Animals used as sources for xenogeneic cell therapies must be bred in captivity specifically and exclusively for this purpose. No cells, tissues, or organs from wild animals or abattoirs can be used. Furthermore, the founder animals must be tested and should not be used for direct production of therapeutics.

If animals are genetically modified, cells from those animals must be fully characterized with the nature of the inserted or deleted gene provided. Testing protocols should be updated periodically to reflect advances in our knowledge of infectious diseases. Of particular importance are tests for pathogenic, xenotropic endogenous retroviruses (ERVs) and persistent viral infections. Animals used should be free of known transmissible spongiform encephalopahy (TSE) agents. The "Testing" box lists some infectious agents and examples that should be considered in testing for them.

In addition to testing the founder animals, sponsor companies should monitor their herds according to approved protocols. Monitoring for all infectious agents known to infect the source species will be essential. Viruses, bacteria, fungi, mycoplasma, TSEs, and parasites all should be considered.

ARCHIVING

Records on the source animals' feeding history should be maintained and full necropsies performed when they are sacrificed or die of other causes. Companies must keep archival samples from those animals, as well. The archives for tissue samples, cell preparations, and paper records will typically have to be maintained for 20–40 years. Archiving plans for both the animal facilities and manufacturing plants should be described to the authorities. The archiving of tissue samples requires validation to ensure traceability and reviewing possibilities. Sample preparation is another consideration. Multiple

types of sampling and storage may be required to allow for analysis by different methods, such as antibody-testing and PCR. If sentinel animals are used, samples must also be archived from them.

Manufacturing

Raw and Source Materials: The manufacture of cell therapies does not allow for harsh purification methods, so testing of raw materials must be very stringent. Any live cells, tissues, organs, or cell banks used as starting materials for xenogeneic cell therapies must be tested by direct culture for bacteria, fungi, and mycoplasma. Appropriate screening for viruses is also essential.

Assays for virus testing should include those capable of detecting a range of infectious agents as well as those that will pick up species-specific agents. Cocultivation assays that enhance sensitivity should be used for xenotropic ERs and other xenogeneic viruses capable of infecting humans. If viral agents are present, routine testing will be necessary. Nucleic acid amplification tests are preferred. In addition, potential latent viruses should be sought: herpesviruses, retroviruses, and papillomaviruses.

Manufacturers should test all other raw materials for sterility, absence of contaminating agents, and the presence of endotoxins. The PTC document specifically advises ensuring a low level of endotoxin in ancillary products. It also references existing guidelines that should be evaluated when human- or animal-derived raw materials are used. It is noted that, whenever applicable, the use of animal reagents should be replaced by nonanimal-derived reagents of defined composition. One other important consideration for raw materials is the avoidance of reagents with sensitization potential.

Manufacturing Process: Each manufacturer of cell therapies must provide a flow chart of its entire process. Animal organ and tissue collection must be demonstrated to prevent contamination by operators or the environment. Transportation from the collection site to the manufacturing facility must be validated. Functional parameters for the starting materials should be specified. If a defined cell bank system is used, then the European PTC on human somatic cell therapies applies (3).

Manipulation procedures may include dissociation of an organ or tissue, isolation of a cell population of interest, cell culture, and cell transformation. Process developers must consider preservation of functional integrity, viral safety, and freedom from prions or cross-contamination. Cell culture requires testing for bacteria, yeast, fungi, and mycoplasma, so an appropriate virus testing program must be established. Limits should be drawn for viability, cell density/confluence, purity, and culture duration. Cell population integrities must be measured, with consideration given to the transformation potential of those cells in culture.

Validation: Manufacturers should validate their entire cell manipulation processes using cell preparation methods that are fully comparable to those intended for clinical use. The PTC document suggests performing process validation every six months. The "Validation Parameters" box lists parameters to measure.

Characterization: Cell populations intended for cell therapies must be extensively characterized. Identity, purity, potency, and suitability for intended use are essential. In some cases, other tests may be useful: tumorigenicity and karyology, for example. Characterization will determine which tests should be used routinely for in-process analysis and release of the drug substance and final product.

For cells that grow attached to a substratum, morphological analysis can be a useful tool. Isoenzyme analysis is usually sufficient to confirm their species of origin, but alternative methods are discussed in the characterization section of the PTC. Manufacturers should test for bacteria and fungi according to requirements of the *European Pharmcopoeia*. Mycoplasma tests also should be performed. Viral clearance studies are probably difficult to apply in this case; therefore, screening drug substances for xenotropic ERs and other viruses capable of infecting humans is highly recommended. Manufacturers should establish valid biological assays that measure potency of their products. Appropriate determination of biocompatibility and durability of polymers or other delivery agents should be performed to ensure suitability for use in clinical settings. If the active substance is very similar to the finished medical product, then it is acceptable to reduce testing at one level or the other.

Testing for Infectious Agents

Recognized species-specific infectious agents and parasites — depending on the source animal.

Endogenous retroviruses (e.g., porcine ERV)

Known zoonotic agents transmissible to humans (e.g., rabies)

Other agents not usually considered zoonotic (e.g., *Toxoplasma gondii)*

Infectious agents of humans relating to receptors expressed by transgenic animals (e.g., cell-surface measles virus receptor)

Infectious agents known to have a high mutation rate or recombination potential (e.g., influenza virus)

Antibiotic-resistant bacteria

Geographically important infectious agents (e.g., African swine fever, *Trypanosoma cruzi)*

Validation Parameters

These parameters should be validated regularly:

Absence of mycoplasma

Absence of adventitious viruses

Cell activity

Cell identity

Cell proliferation

Cell viability

Gene transduction efficiency (if applicable)

Purity

Sterility

Batches must be defined for active substances and finished medical products. Definitions may include size, number of passages, pooling strategies, and information related to a batch numbering system. Each manufacturers needs to describe the container-closure system used and demonstrate its compatibility with the product. Specifications for lot-release testing must be provided: identity, purity, homogeneity, microbial safety, potency, cell viability and metabolic indicators, and cell number. A valid shelf life and expiry date should be assigned based on experimental data regarding cell integrity maintenance and product stability. Finally, storage conditions will need to be specified and, if relevant, appropriate methods for freezing and thawing documented.

Nonclinical Testing

Coverage of preclinical testing in the PTC is focused on pharmacology, toxicology, and other toxicity studies.

Pharmacology: Relevant animal models should be used for nonclinical testing whenever possible. Standard toxicological testing in animals can provide information on the general effects of xenogeneic cells: production of unintended proteins and/or hormones, unintended homing of cells into tissues or organs, effects induced by rejection or encapsulation of the xenogeneic cells, and immune system effects such as graft-versus-host disease in immunosuppressed animals. Biological activity of the xenogeneic cells or expression of their products should be evaluated in vitro and subsequently in vivo as part of the overall proof-of-concept studies. Safety pharmacology studies (using cardiovascular, respiratory, and central nervous system endpoints) should be performed in appropriate animal models. Pharmacokinetics should be examined for cells synthesizing bioactive molecules, but when biological activity is not dependent on well-defined molecules, such studies will be impossible.

Migration and persistence of xenogeneic cells can lead to adverse reactions or unexpected anatomical impediments. This potentiality should be evaluated in animals using histopathology complemented by an appropriate method for specific identification of the introduced cells. However, biotransformation studies and tests of absorption, metabolism, and excretion are unnecessary.

Toxicology: Single- and repeated-dose toxicity studies are performed to confirm whether a dose range of xenogeneic cells required for exerting desirable pharmacodynamic effects is tolerated — rather than to define the maximum tolerated dose, as is typical with such testing. Relevant animal models should be used, of course. If the xenogeneic cells are not immediately rejected, toxicology studies can be combined with safety, pharmacology, local tolerance, immunotoxicity, or proof-of-concept and efficacy studies. Because xenogeneic cells are intended to function for long periods, the testing duration might be much longer than in standard single-dose studies.

Genotoxicity studies are unnecessary unless the expressed products could interact with DNA or other chromosomal material. Carcinogenicity studies may be necessary, and manufacturers should discuss the need for them with the authorities. The need to perform reproductive performance and development toxicity studies will depend on each type of product, area of implantation, clinical indication, and patient population. Local tolerance studies may be unnecessary if the proposed clinical formulation and route of administration have been examined in other animal studies.

Other Toxicity Studies: Immunological and immunotoxicity studies will be critical because xenogenic cells are likely to induce vigorous immune responses. When relevant, both immunosuppressed and nonimmunosuppressed hosts should be evaluated. Manufacturers must present approaches for controlling immunogenicity.

The final nonclinical study mentioned is a viral mobilization study that addresses the possibility of mobilizing replication competent and pathogenic ER elements. Also, reactivation of latent viruses should be investigated with in vitro studies. Despite the fact that viral mobilization might vary among species, it is expected that in vivo studies will be carried out with immunosuppressed animals to best mimic the intended clinical situation.

A Must-Read

This PTC document is a must-read for anyone planning to produce xenogeneic cell therapies. It will aid in understanding the concerns related to the use of nonhuman animal cellular sources for therapeutic applications and provide insights into current expectations for use of such products in humans. It addresses animal sourcing, archiving, manufacturing, and both clinical and nonclinical testing requirements. Although the sections on clinical testing are not addressed here, they also provide significant information on the concerns related to this type of therapy. Of particular importance there is the need for pharmacovigilance and special surveillance methods to protect not

Continued on p. 65

A Few Terms

abattoir: slaughterhouse

biotech working party: a group within CHMP that concerns itself with vaccines, blood products, and biologically derived therapeutics that involve genetic engineering

CHMP: Committee for Medicinal Products for Human Use, a focused committee within the European medicines agency (EMEA)

rapporteurs: officials charged with drawing up and presenting reports; CHMP's rapporteurs are appointed by the committee

zoonotic: of or relating to a disease capable of passing from animals to humans under natural conditions

The Regs *continued from p. 20*

only those receiving and administering such therapies, but also the general public.

References

1 CPMP/1199/02. *Points to Consider on Xenogenic Cell Therapy Medicinal Products.* June 2004; www.emea.eu.int/pdfs/human/regaffair/119902en.pdf.

2 Center for Biologics Evaluation and Research. *Guidance for Industry: Source Animal, Product, Preclinical, and Clinical Issues Concerning the Use of Xenotransplantation Products in Humans.* US Food and Drug Administration, April 2003; www.fda.gov/cber/gdlns/clinxeno.pdf.

3 CPMP/BWP/41450/98. *Points to Consider on the Manufacture and Quality Control of Human Somatic Cell Therapy Medicinal Products.* 31 May 2001; www.emea.eu.int/pdfs/human/bwp/228901en.pdf.

Gail Sofer *is director of regulatory compliance at GE Healthcare in Piscataway, NJ; gail.sofer@amersham.com. Along with John Dougherty, she is a coordinator of our "Focus on the Regs . . ." article series.*