Advancing Xenogeneic Therapy

Non-clinical safety assessment is a key element in developing xenogeneic cell therapies – and progressing products from early animal models to promising clinical studies.

Although xenotransplantation has disadvantages and safety concerns specific to the product class, the availability of organs, tissues and cells from animals remains a significant advantage to the technology. As a result, there is continuing interest in xenogeneic cellular therapy, with a number of products in development – including porcine pancreatic islets and a bioartificial liver using porcine hepatocytes (1,2).

Many development issues to be addressed for xenogeneic cellular therapies are similar to those for other cellular therapies – for example, biodistribution and persistence, formation of ectopic tissue, and so on. Others, such as immune rejection and inter-species retroviral transfer, are more pronounced or specific to xenogeneic cells.

Non-Clinical Safety Assessment

In addition to the confirmation that transplanted cells remain functional following implantation, there are certain areas that should be addressed in a non-clinical safety assessment programme for a xenogeneic cell product (see Figure 1).

Guidance documents specific to xenogeneic therapies are available in Europe and the US. A single species is usually sufficient for non-clinical toxicology testing of xenogeneic therapeutics where the transplantation procedure and/or device combination requires the use of a large animal species.

Toxicity testing can often be combined with efficacy end-points by conducting the study in spontaneous, non-spontaneous or transgenic animal models of disease – for example, testing porcine islet xenotransplantation in non-human primates, or dogs rendered diabetic by total pancreatectomy (3,4).

Issues to Evaluate

Toxicology studies should assess both local and systemic toxicity at several dose levels, and examine the time of onset and reversibility of effects. Therefore, while it is possible to include other end-points – such as tumorigenicity or biodistribution – in the toxicity study, the complexity of the research and animal numbers often require separate biodistribution studies.

The evaluation of potential immune rejection should include assessment of inflammatory cell infiltration, necrosis of implanted cellular material and fibrotic overgrowth at the site of implantation by histopathology. Alteration in the number or function of immune cells in other compartments such as blood can be evaluated by routine haematology assessment, flow cytometry or immunophenotyping, and in lymph nodes local to the site of implant using histopathology.

In addition to the pathological and haematological assessment, it is important to assess the integrity, function and durability of any barrier or other encapsulation method used to protect the xenogeneic cells from the host immune system. This typically involves both a histopathological assessment of the barrier itself and a functional assessment of the cellular product as an indication of barrier integrity. Lastly, the possibility of
Cell Migration

Cells from implanted xenogeneic cell products may migrate within the host, presenting clinical concerns regarding adverse reactions from displaced, bioactive cells or unexpected anatomical impediments. This is likely to be the case where cells are incompletely differentiated, so it is important to consider not only the cells intended for transplantation, but also other cell types that may inadvertently be transplanted with the intended cell population. Evaluation is often performed in a standalone study due to the number of animals required to assess multiple time-points. Assessment of cell fate, biodistribution and persistence is determined by methods such as immunohistopathology, flow cytometry or RT qPCR.

Viral Infection Risk

One of the safety issues of particular concern to xenogeneic products is the possibility of cross-species infectious agent transfer from donor animal cells to the recipient. Addressing possible viral transfer starts with sourcing of animals; quarantine and isolation procedures; animal husbandry; and production and quality control methods. Donor animals should be screened for a wide range of potential viral infections of the source species, including endogenous retroviruses, zoonotic agents that are transmissible to humans – as well as those not normally considered zoonotic – transmissible spongiform encephalopathy diseases, infectious agents with high-mutation rates, antibiotic-resistant bacteria, and known infectious agents of humans.

Non-clinical safety assessment related to infectious agent transfer should be modified based on the source animal species and the manner in which the xenogeneic cell-based product will be used clinically. The primary aim of safety assessment is typically testing for endogenous retroviral transfer. In vitro assessment of viral mobilisation is followed by in vivo assessment if required, typically by quantitative reverse transcriptase polymerase chain reaction (RT qPCR) assessment of viral RNA in blood and tissues.

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consideration should be given to evaluation of tumorigenicity both in vivo and in vitro. In vivo assessment, where needed, should be conducted in well-designed, robust Good Laboratory Practice studies. It may be possible to include tumorigenicity end-points in other in vivo studies with careful study design and planning.

Clinical Safety Assessment

A small number of clinical studies with xenogeneic cell therapies for the treatment of diseases such as diabetes and severe liver failure have been completed recently, with recent announcements of trials in Parkinson’s patients (5,6).

Patient populations eligible for inclusion into such studies are restricted to those with serious or life-threatening diseases for whom adequate safety and effective alternative therapies are not available, and where there is a potential for a clinically significant benefit. The advancement in such studies has identified many challenges in terms of clinical safety assessment and regulatory requirements, which are often retrospective to the treatment (see Table 1).

Leading the Way

Xenogeneic cellular therapies offer some advantages to other cellular therapies, but possess a number of potential safety issues that need to be addressed to ensure safe development.

A growing body of work now exists on the non-clinical testing strategies for xenogeneic products, and these studies have enabled the safe progression of early products from animal models into clinical studies where they are showing promise in the treatment of various diseases. As the technology and understanding of how to mitigate risks develops, it seems likely that other products will follow these first products into clinical trials.

References
1. Ekser B et al, Comparison of hematologic, biochemical, and coagulation parameters in x1,3-galactosyltransferase gene-knockout pigs, wild-type pigs, and four primate species, Xenotransplantation 19(6): pp342-354, 2012

Table 1: Challenges to clinical safety assessment, regulatory considerations and strategies

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<th>Clinical strategies</th>
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<td>Establishing suitable safety and clinically relevant end points</td>
<td>Regular long-term assessment and justification of the duration of follow-up</td>
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<td>Pharmacodynamics</td>
<td>Determining robust methods to assess biochemical and physiological effects in the recipient</td>
<td>End-points similar to those for allogeneic cell therapy can be considered for similar indications</td>
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<tr>
<td>Pharmacokinetics</td>
<td>Determining robust methods to trace the presence of xenogeneic cells in clinical samples</td>
<td>Distribution, proliferation and survival of xenogeneic cells require characteristic within recipient</td>
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<td>Pathogen transmission to host/close contacts/ healthcare provider and public health implications</td>
<td>Risk of infection due to immunosuppression or from agents contained in xenogeneic cell product</td>
<td>Acute and long-term safety assessment of recipients mandatory</td>
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<td>Concomitant treatments</td>
<td>Choice of treatment to enhance safety and efficacy</td>
<td>Treatment schedules should be tested rigorously, including monitoring procedures for therapeutic effect and adverse events</td>
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<td>Malignancies</td>
<td>Reducing risk of tumorigenicity of donor cells</td>
<td>Development of late-stage complications, such as cancer, should be considered</td>
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<td>Immunoisolation barriers and devices</td>
<td>Proof-of-concept in humans</td>
<td>Medical device regulations may be applicable</td>
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**Other Reading**

