2. Utilizing Animal Studies To Evaluate the Safety of Organ Preservation Devices and Solutions

While the national transplant waiting list continues to grow, rates of donation and transplant remain stagnant. On average, 22 people die each day waiting for a transplant. The dire deficit in organ transplants has propelled a new wave of innovation in perfusion-based organ preservation technologies. With such innovation also comes the challenge of demonstrating that these new technologies, when evaluated in animal models, are sufficiently safe for early clinical experience.

After animal organs undergo preservation using a new organ transport device or solution, there are generally two models to assess post-reperfusion injury: (1) An in vivo model in which the organ is transplanted into a surrogate recipient animal and (2) an ex vivo model in which the organ is reperfused under simulated transplant conditions. FDA intends to develop guidance to provide recommendations for utilizing both in vivo and ex vivo models to evaluate emerging organ preservation technologies. Prior to drafting our recommendations in a future guidance document, FDA invites comments on the following questions:

a. What are the potential limitations of an ex vivo model in assessing reperfusion injury, and how can these limitations be mitigated? In addition to markers for cell injury and function, histology, and the use of allogeneic blood during reperfusion, what measures can be taken to improve the data generated in an ex vivo model?

Comments:

The major focus of any new preservation technology is to reduce the impact of ischemia/reperfusion injuries on the donor organ. We believe that an ex-vivo simulated transplant model is a more robust model in isolating the relevant variables and provide the most comprehensive organ specific data on the impact of preservation condition on the graft. The in-vivo animal transplant model is extremely complex and adds significant variability that could influence the interpretation of the data. In addition, the in-vivo model uses mostly animal survival as a sign of success without providing detailed organ perfusion/hemodynamic data that are critical to assess the condition of the graft after preservation using the new preservation technology being evaluated. It also is heavily influenced by multiple variables that are unrelated to the evaluation of the organ preservation technology, like surgical technique, immunology, intraoperative, and postoperative management of the animal species.

That being said, we believe that the following points are critical to successful implementation of an ex-vivo simulated transplant model:
Ex-vivo simulated transplant should be conducted using a controlled perfusion system that provides near physiologic conditions (pulsatile flow, dual flow if need be, ventilation etc.) for the solid organ being evaluated (heart, lung, liver, kidney, etc.);

- The system must continuously capture all perfusion parameters and hemodynamics to provide a comprehensive focused assessment on organ function after preservation; as well as high-level of assurance in the results;
- The use of unmodified whole blood from a different animal must be used to simulate the condition of reperfusion after transplantation;
- Standard clinical assessment techniques should be applied at regular intervals during the simulated transplantation (i.e. bronchoscopy, ECHO when applicable, Bile production, urine production, etc.)
- The ex-vivo simulated transplant procedure should be carried out for at least 12 hours to allow for monitoring of any reperfusion syndrome occurrence and should be limited to 24 hours maximum to minimize the impact of hemolysis and other side effects of extracorporeal circulation on the blood used for the ex-vivo simulated transplant model.

b. In an in vivo model, what are strategies to limit confounding factors, such as immunological responses and hemodynamic instability, from affecting the assessment of device-related reperfusion injury?

Comments:

All reported in-vivo orthotopic animal transplant models are limited to academic institutions1-4 and are only limited to liver and single lung transplants in Swine models. Heart in-vivo orthotopic transplant model is limited to only 4 hours of reperfusion in the recipient swine due to the significant complexities of surviving the animal long-term post transplantation. These facts are a significant challenge for any developer of new preservation technologies to replicate, especially given the fact that some of these published scientific research models were developed by scientists that are no longer practicing these studies (PhD students, etc.)

In addition, we believe it would be extremely difficult to limit the confounding variables associated with animal orthotopic transplantation. More importantly, it would be impossible to discern the impact of these confounding variables on the interpretation of the results given the significant nature of the below confounding variables:

- The control of animal hemodynamics during and post transplant is extremely challenging to ensure adequate post-transplant reperfusion due to the extremely labile hemodynamic condition of the animals under general anesthesia. In addition, the high degree of pain associated with an orthotopic transplantation surgical procedure would further compromise hemodynamics management;
- Given that immune-histocompatibility matching and ABO blood typing is clinically impossible to perform in animals, immunologic rejection adds another layer of confounding variability to the outcomes and interpretation of the data of these studies. For example, it could be extremely difficult to isolate the clinical reasons for a graft dysfunction in animal
organ transplants; i.e. is it due to immune rejection or ischemic injury to the graft during preservation;

- Controlling infection in an animal transplant model is a significant hurdle given the habitat of the animal. This could further add a confounding variable to the condition of the transplanted organ. For example, it would be difficult to isolate the cause of any post-transplant infection and the relationship to preservation methodology being studied vs. post transplant contamination due to animal environment/habitat;
- Finally, the surgical procedure of transplanting organs is extremely challenging. This could further add confounding variables to the outcomes. Surgical complications could result in organ failure during/after transplantation, which is completely independent of the preservation method at question here.

c. Is there a perceived hierarchy of evidence regarding data obtained from an ex vivo model and those obtained from an in vivo model? Or rather, is it more judicious to view the two models as complements of each other?

Comments:
In a well controlled/defined ex-vivo simulated animal transplant model, the variables are standardized to the relevant impact of the preservation technology being evaluated and the results are directly related to preservation and reperfusion injury. Based on the above scientific reasons, a well controlled ex-vivo simulated transplant model is the most appropriate model to use to provide FDA with sufficient pre-clinical evidence of safety to initiate human studies of a new preservation technologies.

The orthotopic transplant model adds significantly more challenges and variables that make the data difficult to interpret, especially without any organ perfusion/hemodynamic data obtained from the transplanted animal. Animal survival alone may not be sufficient endpoint to evaluate impact of preservation technologies.

d. What role does the risk of the device play in the utilization of in vivo and ex vivo models? Regarding specific experimental parameters (e.g., length of preservation, total ischemic time), under what circumstances is it appropriate to test the worst-case scenario?

Comments:
Except for cold static storage, which is the current standard of care for solid organ preservation for transplantation and has built a long track record of safety, all new preservation technologies should be evaluated for pre-clinical safety using unified approach. Cold perfusion could result in significant organ damage despite using cold temperature. It is important to subject all new organ preservation technologies to similar standard of pre-clinical evaluation.

Regarding the length of preservation, it would be advisable to design pre-clinical testing for two time points:
- Clinically relevant: assuming the average clinical use scenario for the new technology;
- Worst-Case Scenario: Using exaggerated times (12 hours), to provide a safety margin for assessing impact of the new preservation modality.
e. What are the organ-specific challenges in developing in vivo and ex vivo models to assess reperfusion injury?

Comments:

Heart:
- In-vivo transplant challenges: the reported orthotopic transplant model is limited to only 4 hours of reperfusion(ref). The cardiac function of the transplanted graft is subject to the hemodynamic instability of the animal due to anesthesia, etc. In addition, please see the in-vivo transplant model challenges listed below.
- Ex-vivo simulated transplant challenges: the working heart model after-load is standardized to 100 cm of water as compared to the variable afterload in an in-vivo model.

Lung:
- In-vivo transplant challenges: The reported in-vivo animal transplant model is limited to single lung transplantation with the second native lung pulmonary artery and bronchus clamped to divert 100% of the Cardiac Output (CO) and ventilation to the transplanted lung. This is a significant challenge since it subjects the transplanted lung to severe non-physiologic condition of supporting the entire CO. In addition, please see the in-vivo transplant model challenges listed below.
- Ex-vivo simulated transplant challenges: The need for an ex-vivo technology platform that can provide near physiologic pulsatile flow to artery.

Liver:
- In-vivo transplant challenges: Please see the in-vivo transplant model challenges listed below. In addition, All reported in-vivo orthotopic animal transplant models are limited to academic institutions\(^1\text{-}\(^4\)\), thus creating a significant challenge for any developer of new preservation technologies to replicate, especially given the fact that some of these published scientific research models were developed by scientists that are no longer practicing these studies (PhD students, etc.)
- Ex-vivo simulated transplant challenges: The need for an ex-vivo technology platform that can provide near-physiologic, pulsatile flow to the hepatic artery and non-pulsatile flow to the portal vein circulation.

The following challenges of in-vivo transplant model applies to ALL solid organs:
- The in-vivo model uses only animal survival as a sign of success without providing detailed organ perfusion/hemodynamic data that are critical to assess the condition of the graft after preservation using the new preservation technology being evaluated.
- The control of animal hemodynamics during and post transplant is extremely challenging to ensure adequate post-transplant reperfusion due to the extremely labile hemodynamic condition of animals under general anesthesia. In addition, the high degree of pain associated with an orthotopic transplantation surgical procedure would further compromise hemodynamics management;
- Given that immune-histocompatibility matching and ABO blood typing is clinically impossible to perform in animals, immunologic rejection adds another layer of confounding variability to the outcomes and interpretation of the data of these studies. For example, it
could be extremely difficult to isolate the clinical reasons for a graft dysfunction in animal organ transplants; i.e. is it due to immune rejection or ischemic injury to the graft during preservation;

- Controlling infection in an animal transplant model is a significant hurdle given the habitat of the animal. This could further add a confounding variable to the condition of the transplanted organ. For example, it would be difficult to isolate the cause of any post-transplant infection and the relationship to ex-vivo perfusion vs. post transplant contamination due to animal environment/habitat;
- Finally, the surgical procedure of transplanting organs in animal models is extremely challenging. This could further add confounding variables to the outcomes. Surgical complications could result in organ failure during/after transplantation, which is completely independent of the preservation method at question here.

The challenge for ex-vivo simulated transplant model is standardizing the ex-vivo perfusion platform to provide the most physiologic conditions to the organ being evaluated.

**f. What approaches would improve the in vivo and ex vivo study designs to ensure the generation of sufficient, meaningful data while limiting the number of animals used in such studies?**

**Comments:**
The ex-vivo simulated transplant animal model is a robust and focused model that provides the most comprehensive and direct evidence to assess the safety of a new organ preservation technology. Using a technology that can perfuse all solid organs ex-vivo and maintain them in a near physiologic environment would standardize the procedure. Using a technology that monitors and records all relevant perfusion parameters and hemodynamics of the preserved organ at question, will provide the highest degree of confidence and quality assurance of the endpoints being evaluated for that organ.

**References**