February 25, 2016

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Center for Devices and Radiological Health  
Food and Drug Administration  
10903 New Hampshire Ave.  
Bldg. 66, rm. 5456  
Silver Spring, MD 20993-0002

Re: Docket No. FDA-2015-N-1021 for “Medical Device User Fee and Modernization Act; Notice to Public of Web site Location of Fiscal Year 2016 Proposed Guidance Development.”

Dear Center for Devices and Radiological Health:

The following comments in regards to the title: "Utilizing Animal Studies to Evaluate the Safety of Organ Preservation Devices and Solutions" are submitted by the International Society for Heart and Lung Transplantation (ISHLT) in conjunction with our Scientific Councils on Pulmonary Transplantation, Basic Science and Translational Research, and Heart Failure and Transplant Medicine. ISHLT is the leading professional organization representing over 3000 members dedicated to improving the care of patients with advanced heart or lung disease through transplantation, mechanical support and innovative therapies via research, education and advocacy.

**Question:** 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?

**Response:** Ex vivo perfusion/ventilation strategies are broadly considered as having two goals:  
1) to deliver therapies that ameliorate reperfusion injury and  
2) to assess graft function with experimental reperfusion and ventilation.  
To assess the efficacy of the first objective, treated grafts must be transplanted. To assess the safety and efficacy of the second objective ex vivo methodology should replicate in vivo conditions. That currently is not the case. Several limitations for EV models may potentially limit application of results for translational use in humans. These limitations will be design specific but are related to composition of perfusate, lack of metabolism/elimination of wastes or generated biologics (hormones, cytokines, etc), lack of biologic homeostatic mechanisms (pH, osmolarity, sodium, etc) and conditions in which the organs is assessed. These potential limitations could be mitigated by using perfusates that approximate fresh whole blood, protocols that intermittently exchange perfusates or at the least monitor wastes and biologic readouts and designs that more accurately simulate in vivo conditions (100% cardiac output flows and physiologic or near physiologic ventilator parameters.
**Question:** 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?

**Response:** In vivo testing is required to normalize for heterogeneity among different animal and ex vivo models. By transplanting grafts treated or evaluated ex vivo, the full effects of blood reperfusion can be tested.

Rigorous study design detailing the immunologic heterogeneity, hemodynamic instability, and other potential confounders should be considered prior to undertaking these studies. When blood is used as the perfusate or added to perfusate in an ex vivo perfusion system, it is heparinized, platelet, and leukocyte depleted which blunts the effects of the coagulation cascade activation on graft reperfusion. Ideally, if randomization is possible, it would be the most robust statistical method to ensure balance between groups. If randomization is not feasible, then all potential confounders must be identified and effects estimated in order to determine the sample size required to have sufficient power to determine whether the endpoint has been achieved. Device-related variables should be collected to ensure that device malfunction or deviation of protocol occurred to ensure that neither the device nor the method have intrinsic deficiencies or methodological peculiarities that make safe application unlikely or hazardous.

**Question:** 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?

**Response:** In our opinion, no data generated ex vivo can overcome the advantages of in vivo testing to study the effects of ischemia/reperfusion injury. Although the ex vivo models can use human organs and potentially demonstrate efficacy and applicability of this technology to the clinical environment, the ex vivo perfusate or therapy does not adequately represent the complexities occurring after transplantation. The use of in vivo animal models is preferred. It is inappropriate to proceed directly from ex vivo data to human trials. However, data generated from ex vivo perfusion of human organs provides complementary data to support applicability.

**Question:** 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?

**Response:** The initial testing of an ex vivo perfusion technology should demonstrate that the device does not damage lungs that are suitable for transplant. The safe duration of ex vivo perfusion and total ischemic time should be demonstrated by adequate physiologic function and full body support in an in vivo model.

A new method tested in a bad scenario produces data that cannot be interpreted. It is difficult to know under such circumstances whether the method or the animal model that caused things to fail. Device malfunction should be collected as part of all studies and should be considered a negative outcome. Such failures should be exceedingly rare.

Worst case scenarios should be assessed after safety and effectiveness has been demonstrated as discussed above. The initial use of ex vivo assessment to demonstrate efficacy of physiologic function prior to initiating in vivo studies would be appropriate.
**Question:** What are the organ-specific challenges in developing in vivo and ex vivo models to assess reperfusion injury?

**Response:**

*Heart*—must be loaded and working in a closely simulated physiologic perfusate for any meaningful assessment. Blood perfusates are required due to their higher oxygen demand.

*Lung*—should be have 100% cardiac output flow in a closely simulated physiologic perfusate (whole blood like). It is also necessary to ventilate at physiologic levels while avoiding mechanical ventilation injury. Gravity also exerts a static effect on flow distribution ex vivo that should be accounted for or corrected with constant rotation or oscillation of position.

*Kidney*—this is probably the organ most readily amenable to ex vivo perfusion

*Liver*—in addition to the need for a blood based perfusate, capturing dynamic parameters for metabolic function would be challenging and continuous heparinization of the perfusate would be required to counter the effects of coagulation factor production.

**Question:** 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?

**Response:** Three categories of approach could be considered:

1) Use of human organs from donors consented for research.
2) Use of ex vivo perfusion as platform to test therapies without need to transplant until best working condition are identified.
3) Testing best conditions in a survival animal transplant model to generate significant data.

Animal models of EVLP offer the opportunity to investigate mechanisms of donor lung injury and test novel therapies that may ameliorate ischaemia reperfusion injury or other forms of graft injury after lung transplantation. Consideration should however be given to the advantages and disadvantages of different animal models of EVLP when considering its appropriateness to particular experiments.

Large animal models including pigs and sheep offer the closest physiology to human lungs and can be perfused using the same protocols and equipment used for human lungs but the high cost of the animals and reagents limits the number of experiments that can be performed for a given cost. In addition there are more limited assays for porcine or ovine molecular targets. These animals also differ with respect to the resident macrophage population, particularly the pulmonary intravascular macrophages which are not present in large numbers in humans. The physiologic responses are therefore different.

Small animal models of EVLP including rats, rabbits and guinea pigs are less expensive to run and allow more repeats and more conditions to be investigated for the same resource. There are however significant limitations with these models which limits the duration of perfusion to short periods of up to 1 hour which do not mimic the duration of perfusion that can be readily achieved with human lungs.

Finally murine models of EVLP have also been developed recently and have the attraction of a large range of murine antibodies and molecular assays being available as well as access to genetically modified animals that will allow specific mechanisms to be studied.
It is therefore likely that experiments performed using small animal models of EVLP may be more useful for exploratory mechanistic studies but that selected experiments will need to be reproduced in larger animal models or in human lungs not for clinical use to demonstrate applicability of their findings.

We appreciate the opportunity to provide comment on these important issues.

Sincerely,

Duane Davis, MD, MBA
President, ISHLT