**Transplant Date: June 2, 2021**

**Protocol Number:** AMI-018-006

**Study Number:** AMI-018-006-112

**Title of Study:** Transplantation with PHH lot PTC using one of our standard NTBC cycles and two new NTBC cycle tests to evaluate engraftment and early pHH expansion and survival at ≥ 70 days post-transplant.

**Primary Investigators:** Lisa Wilson

**Number of Animals:** 40. **Species:** Rat. **Strain:** SD FRG. **Gender:** Male & Female. **Age or weight range: 5-**6 weeks

**Purpose**:

In IACUC protocol AMI-018-006, the focus of the set of experiments is to utilize the immunodeficient rat model of hereditary tyrosinemia type 1 (HT1) - which are deficient in the enzyme fumarylacetoacetate hydrolase (FAH) – as the model for expanding human hepatocytes. Hepatocytes that are FAH+ have a selective growth advantage over FAH- hepatocyte; therefore, we hypothesize that FAH+ human hepatocytes will grow and repopulate the FAH-KO rat liver. Proof-of-concept has already been demonstrated in the mouse model of HT1 (see Azuma et al. 2007 PMID: 17664939).

A pre-IND enabling safety study using the FRGN mice as recipient for huFRG rat derived human hepatocytes. The objective of the study is to provide a comprehensive data package that characterizes the FRGN mouse model for the life of the animal. These data will form the background information of the model and to better understand the pathological and toxicological consequences of transplantation of FRG rat derived human hepatocytes.

The characterization consists of two phases: Phase 1 uses FRGN mice transplanted with cadaveric primary human hepatocytes (pHH) and Phase 2, will use FRGN mice transplanted with FRG bioreactor derived human hepatocytes of the same two donors. In Phase 1 cadaveric human hepatocytes from were a commercial lot, BioIVT PTC, and a Ambys lot AM0005. In order to initiate the second phase using huFRG derived human hepatocytes we will need FRG rat livers with expanded human hepatocytes from each of the same cadaveric donor.

The purpose of study AMI-018-006-112 is to transplant FRG rats with AM005, apply the newest NTBC cycle, 2mg/L(3d)>0.05mg/L(5d) to promote high level of engraftment and robust expansion of the human hepatocytes. Those animals that achieve hAlbumin levels of ≥ 2500µg/mL at ≥ 70 days post-transplant will undergo liver perfusion for AM005 human hepatocyte isolation and cryopreservation.

All FRG rats will be on 5LJ5

All FRG rats will begin on 16 mg/L NTBC

**Experiment Outline:**

N=40 FRG rats

Animals will be held for at least one week to acclimate following arrival.

Animals will be socially housed by Group assignment. On the rare case where one animal remains in a cage without a Group partner, additional cage enrichment (non-food) will be provided.

Using body weights collected the same day as preconditioning the rederived FRG rats from Envigo will be dosed with Ad-uPA 24h ± 2h prior to transplant and placed on the drinking water as specified in the NTBC cycling protocol located on the Ambys G-drive. On the day of transplant, the cryopreserved PHH Lot PTC will be prepared following the standard protocol used for transplantation.

Blood draw schedule

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Days post-tx | 28 | 56 | 73 | 90 |
| Date | 6/30/21 | 7/26/21 | 8/9/21 | 8/29/21 |



2.0mg/L

0.05mg/L

**Day -1:** In the AM, ALL rats will be dosed with Ad-UPA by IV tail injection. Ad-uPA will be provided by a to the IVS team for dosing. The NTBC drinking water will be changed as indicated in the above chart.

**Day 0:** Hepatocytes (5 million per 100g of rat) will be prepared in hepatocyte media (2.5 million per 100 µl media). Hepatocytes will be in 1.5ml Eppendorf tubes and transferred on ice. Hepatocytes will remain on ice until time of injection. At time of injection, hepatocytes will be gently pipetted up/down x3 with P1000 pipet and sterile P1000 tip to get the cells in suspension (hepatocytes are large and will quickly pellet to the bottom of the tube). Hepatocytes will be drawn into a sterile 1cc syringe with a 27G needle and injected into the spleen via laparotomy method.

**Day 1 onwards:** Animals will be put on the short NTBC cycle as indicated above in the chart and as indicated in the NTBC water cycle sheet located on the Ambys’ G drive.

**Day 0-125:** Body weights and clinical scoring will be measured and documented up to three times each week**.**

**Day 30 – 90**: 5µL whole blood for human albumin measurements will be collected by tail vein, tail or saphenous vein nick at the specified dates on the chart above and the NTBC water cycle sheet located on the Ambys’ G drive.

**Day 50 huAlb readout:** hAlbumin **≤ 250µg/mL** will be removed from study and euthanized.

**Day 70 huAlb readout:** hAlbumin **≤ 750µg/mL** will be removed from study and euthanized.

**Day 70 – 125:** animals will be selected along the study time course for examination.

**Day >125:** IVS to notify PI and Perfusion Team of any remaining animals.

**Animal care and welfare**

Refer to **the document titled “INSTRUCTIONS FOR CLINICAL REPORTING and EUTHANASIA OF FRG RATS”** for the evaluation and documentation of animal health**.**

Animals with a score of 3 may be managed with supplementation (e.g moistened 5LJ5 ration, supplemental rodent nutrition in the form of highly palatable ration and/or gels, SC warm saline or Lactated Ringers) per veterinary discretion. Maintenance on these plans will be based upon response to supplementation and will be discontinued when animal stabilizes weight loss or moves up in score. Progressive decline (or lack of improvement) in score and/or body weight will initiate request to PI or designee to remove the animal from study immediately. Submission for perfusion and/or euthanasia may be elected; tissue harvest requests to be followed as described on this approved study design.

**Humanized FRG rats for perfusion, hepatocyte isolation and human hepatocyte purification:**

All animals at ≥ 71 days post-transplant with human albumin concentrations of ≥ 2000µg/mL will be offered to the Product Development team for optimization of processes involving perfusion, hepatocyte isolation, human hepatocyte purification and in vitro characterization. Once the cannula has been inserted into the caudal vena cava or portal vein, secured and blood starts to fill the head, ≥ 500µL whole blood will be collected for sera isolation. Alternatively, the blood may be collected from the tail vein based on a 50µL aliquot of sera will be dispensed into a pre-labeled sterile 1.5 mL tube for Tyrosine and Succinylacetone quantitation. Remaining sera will be transferred to a second pre-labeled sterile 1.5mL tube. Both aliquots should be stored at -20C until shipped for analysis

Principal Investigator: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Date:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Head of Pharmacology (or alternate): \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Date:\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Appendix A: Processing blood, urine and tissue samples**

Blood collection

* Using Isoflurane, anesthetize the animal to a surgical plane of anesthesia (Induction 4-5%, maintenance 2-3%). Confirm with a firm toe pinch on both hind feet.
* Using operating scissors or other appropriately sized scissors, cut through the skin and muscle to open the peritoneal cavity.
* Using gauze, sweep the intestines out of the body cavity to expose the inferior vena cava.
* Using 5 mL (rat) syringe and 25 G needle puncture the inferior vena cava and slowly withdraw 4mL of blood from rat. Be careful to not collapse the vein by creating too much vacuum pressure.

Serum:

* Dispense ≤ 2mL whole blood volume into 4mL serum separator tube and allow to clot for at least 1 hour at room temperature.
* Centrifuge at 10,000 rpm for 5 minutes.
* Dispense 220µL into 4 X 1.5 sterile mL tube, store at -80C

Plasma:

* Dispense ≥ 1mL of whole blood in a 4mLK2EDTA anticoagulation micro tube (do not overfill or blood will clot).
* Gently invert 3 times (do not shake) to facilitate complete mixing with anticoagulant.
* Centrifuge at 10,000 rpm for 5 minutes.
* Dispense 80µL into 4 X 1.5 sterile mL tube, store at -80C

Urine during Necropsy

* Following collection of blood, collect urine (if present) via cystocentesis. Urine to be placed in labeled freezer compatible tube and store at -20oC

Collection of Liver

* Using blunt tipped scissors (curved if possible) separate the liver from the diaphragm, blood vessels and connective tissue and remove the liver from the body cavity.
* Make 4-5, 1cm nicks across the liver to facilitate fixation.
* Place whole liver in bottle of 10% NBF.

Collection of the Kidney

* Remove the adrenal glands, located in the fat cranial to the kidneys.
* Dissect the kidneys away from the fat and cut at the juncture of the kidney with the renal artery and vein.
* Transect the left kidney (transverse section) with a scalpel blade or straight razor.
* Longitudinally bisect the right kidney at the midline.
* Place into a bottle with 10% NBF

Urine From living animals

* Place animals on clean Petri dish
* Allow animals to urinate
* Collect urine using a micro-pipet and sterile tip. Transfer to a labeled freezer compatible tube and store at -20oC