



## **Addi and Cassi Cyclodextrin Infusion Bio-Sampling Protocol**

### **Extension 1.0**

#### **Summary**

Addi and Cassi Hempel will be receiving additional infusions with cyclodextrin as an experimental treatment for Niemann Pick Type C disease. The next infusion, in hospital, will be at a dose of 500 mg/kg/day at a rate of 20 ml/hr. Thereafter, each additional infusions of 8 hrs duration will occur every 3-4 days (i.e., twice/week) and examine increasing the dose of cyclodextrin by 100 mg/kg. A number of research centers are examining biomarkers in NPC disease and have agreed to receive biological specimens. This protocol summarizes the collection procedures for research and safety labs using blood, urine and feces collected during the course of the study. All biospecimen samples will be labeled with a unique label which does not identify the patient. With the exception of the lymphocyte sample, all samples will be stored frozen and shipped at the end of the study.

#### **New Baseline**

Baseline samples should be collected no more than 14 days prior to the start of infusions. Samples obtained at the conclusion of the core protocol may be used for baseline determinations if collected within 14 days of the start of the extension. Biomarker blood samples can be collected from the MediPort at the start of infusions and should be in a fasted condition. Safety clinical labs should be obtained and analyzed prior the initiation of all infusions.

#### **Clinical Labs**

The following safety labs will be collected and analyzed prior to all infusions:

- **GLUCOSE - RANDOM**
- **BLOOD UREA**
- **CREATININE - Serum**
- **CALCIUM - TOTAL**

- **URIC ACID - SERUM**
- **LIVER FUNCTION TEST**
- BILIRUBIN - TOTAL
- BILIRUBIN - DIRECT
- PROTEIN TOTAL
- ALBUMIN - SERUM
- GLOBULIN
- A/G Ratio
- ALP
- SGPT/ALT
- SGOT/AST
- GGT
- AMYLASES
- **LIPID PROFILE**
- COLESTEROL - TOTAL
- TRIGLYCERIDES
- CHOLESTEROL -LDL
- CHOLESTEROL - VLDL
- CHOLESTEROL - HDL
- 
- Total Cholesterol/HDL
- Cholesterol Ratio
- **ELECTROLYTES (NA, K, CL,**
- **& BICARB**
- SODIUM - SERUM
- POTASSIUM - SERUM
- CHLORIDE - SERUM
- BICARBONATE - SERUM
- 
- **HEMATOLOGY**
- **APTT (Automated/**
- **clotting assay)**
- **ESR**
- **COMPLETE BLOOD**
- **COUNT (Automated) (CBC)**
- HAEMOGLOBIN (Automated)
- TC ( TOTAL COUNT)
- RBC COUNT (Automated)
- PCV (PACKED CELL
- VOLUME) (Automated)
- MCV
- MCH

- MCHC
- PLATELET COUNT

#### **DIFFERENTIAL COUNT**

- **(Automated cell counter)**
- Neutrophils
- Lymphocytes
- Eosinophils
- Monocytes
- Basophils

#### **URINALYSIS**

- am spot urine Pr/Cr
- UA

#### **Biomarkers**

Blood samples will be collected in an overnight fasted condition, labeled, and identifier recorded on the attached chart as well as in the patient chart.

#### ***Lipidomics:***

1. Collect 2 ml whole blood in the provided special tube containing BHT. Tube is a vacutainer, but blood can be injected directly into the tube. Invert to mix blood. ***IMMEDIATELY*** centrifuge at 2000 RPM for 20 min or until plasma separated. Save plasma in labeled sample tube. Save and label RBC pellet. Freeze at -70C save until end of study. Send overnight to Dan Ory on dry ice.
2. As above, but send to Alfred H. Merrill on dry ice. In addition, send ca 1 ml of urine (frozen), which has preferably not been allowed to settle before aliquoting--or if it has, that was swirled to remix before aliquoting because the urine sediment often contains sphingolipids of interest.

#### ***Proteomics:***

Collect 5 ml blood in GREEN top tube, centrifuge to separate serum. Save serum in labeled sample tube. Resuspend RBC in 5 ml PBS (phosphate buffered saline), label and save. Freeze -70C and save until end of study. Send overnight to Yiannis Ioannou on dry ice.

### ***Lymphocytes:***

Collect 5 ml blood in PURPLE top tube, invert to mix. DO NOT CENTRIFUGE, DO NOT REFRIGERATE. SHIP at ROOM TEMPERATURE overnight to the UK (Fran Platt). Note suggestions:

please send at room temp in well mixed EDTA vacutainer tubes (or equivalent), normally to avoid too much temperature fluctuation we get them wrapped in a couple of sheets of tissue paper (in a sealed bag) and then placed into a polystyrene box inside a cardboard box (just please make sure it is not marked refrigerate). Also if marked store at room temp and diagnostic or clinical samples that can also help.

### ***Metabolomics:***

Thiol blocking buffer provided by Jung Suh. First, dilute 10 ml of the solution with 90 ml HPLC grade water.

Collect 5 ml blood in LAVENDAR top tube, invert to mix. Then:

- 1) Mix 3 ml of whole blood and add to a tube containing 3 ml of the diluted thiol blocking buffer and mix by inverting the tubes. **This step must occur immediately following blood draw.**
- 2) Transfer 1 ml of diluted, buffered blood into a 1.5 ml eppendorf tube. Centrifuge at 13,000RPM x 1 min (or 2000 x g for 20 min). Collect the plasma sample and transfer to a new tube. Transfer the RBC pellet to a new tube. Label both tubes with unique identifier and note identifier in patient chart. Freeze samples at -70 degrees C.
- 3) Use 3 ml of blood left from step 1 for peripheral blood mononuclear cell isolation. For this, gently overlay 1.5 ml of the diluted, buffered whole blood in step 1 on top of 3 ml of histopaque solution (provided by Jung Suh; prepare two 15 ml tubes). Then, centrifuge at 400 x g for 30 min. After centrifugation, carefully aspirate the upper plasma layer with a pasteur pipet. Carefully transfer the opaque cell layer (white cell layer) and pool them in a new tube.
- 4) Add 10 ml of PBS to the white cells from step 3 and gently invert. Centrifuge for 250 x g for 10 min. Aspirate out the PBS, label tube with unique identifier and note in patient chart, store at -70C. Label and store the cell pellet at -70 C.
- 5) With the remaining blood from the initial LAVENDAR top collection, transfer the whole blood sample to a new tube and centrifuge at or 13,000 RMP x 1 min (2000 x G for 20 min) and collect the plasma and RBC fractions and transfer to new tubes, label with unique identifier and note in patient chart. Store frozen at -70C until end of study.

Ship all samples at the end of the study overnight to Jung Suh on dry ice.

*Diaper urine and feces:*

**Preparation of the cloth diapers:**

New cloth diapers are washed several times and then weighed when dry and clean and the dry weight (grams) is written on the diaper with permanent ink. Occasionally the diapers are reweighed after multiple use and the new weight is recorded if the weight has decreased.

**Collection of Urine and Stool:**

Label a large plastic bag (use black sharpie permanent marking pen) with name of patient and dates of 24 hour collection.

Put each wet diaper in an individual ziplock with label affixed to the outside of bag. Record the date, time and dry diaper weight on the ziplock bag label. And Record this information on flowsheet.

Put all individually bagged diapers for each 24 hour period into the labeled large plastic bag. Store in freezer, -20 C.

If the stool is well formed and can easily be separated from the diaper, put it into a separate ziplock bag and label with time and date.

If diaper has stool on it that cannot be separated, please mark "Stool On Diaper" on the ziplock bag label.

It is ideal if the parents only use 1-2 diapers at a time with plastic pants, and change the diapers no more than 4-6 times in each 24 hour period when there is a noticeable amount of urine and/or stool present. If diapers only have very small amounts of urine, then the amount of water needed to extract the urine, dilutes the urine too much so that the analysis becomes inaccurate.

Continue to collect diapers during the course of the infusion period. Store frozen. Either bulk ship at the end of the study or, if space is an issue send as needed to OSHU on dry ice.

***Pharmacokinetics:***

Collect 2 ml blood in PURPLE top tube, invert to mix. ***IMMEDIATELY*** centrifuge at 2000 RPM for 20 min or until plasma separated. Save plasma in labeled sample tube. Save and label RBC pellet. Freeze at -70C save until end of study. Ship to Jean-Baptiste Roulet, Ph.D. at the end of the study.

I. Weekly safety labs

Prior to all subsequent weekly cyclodextrin infusions as per protocol the above clinical safety labs should be collected and analyzed.

II. Every 8 weeks

Collect urine and feces diapers for 24 hrs beginning with the start of the infusion. The next morning following the week 8 infusion repeat all biomarker sampling as above in a fasted condition. It will be necessary to take the patients to the Renown Hospital for sample preparation and storage.

III. Study termination

Collect urine and feces diapers for 24 hrs beginning with the start of the infusion. The next morning following the last scheduled infusion (week 15), or if the patients are discontinued for any reason, repeat all biomarker sampling as above in a fasted condition. It will be necessary to take the patients to the Renown Hospital for sample preparation and storage.

**Addresses for sample shipment**

Note: Please notify recipient of shipment with tracking number.

**Lipidomics 1**

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**Lipidomics 2**

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**Proteomics**

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**Lymphocytes**

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**Urine and Feces diapers**

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## **Pharmacokinetics**

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