

Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.

No vector is stored on site in DSB 6009. It is brought on the day of injection and any remainder is disposed of in Clidox in DSB 6009.

Please see attached SOP for injection for more details.

Please include a one page research summary or teaching protocol.

Metachromatic leukodystrophy (MLD) is a fatal neurodegenerative disorder which typically begins in late infancy. It is caused by a deficiency of the enzyme arylsulfatase A which normally breaks down the glycolipid sulfatide. Sulfatide is a component of myelin (white matter) in the central and peripheral nervous systems. Myelin acts to insulate neurons to properly conduct nerve impulses. In individuals with MLD sulfatide accumulates in the cells of the nervous system resulting in the degeneration of myelin. This causes a plateau in development, the progressive loss of skills, mobility, vision, speech and hearing, and eventually death by the age of about 5.

There is currently no therapy for MLD. A mouse model of MLD has been obtained. These mice have mutated arylsulfatase A genes such that no functional arylsulfatase A (ASA) enzyme is produced.

The purpose of this project is to evaluate the efficacy of lentivirus-derived vectors (LV) to mediate the transfer of the arylsulfatase A gene (As2) into the brains of these mice. LV vectors have the advantage of being able to infect both dividing and non-dividing cells. In these experiments we will co-infect, either in the same vector or in 2 LV vectors the cDNA for SUMF1. SUMF1 is an enzyme that post-translationally modifies the arylsulfatase A enzyme which is required for enzyme activity. Without concurrent overexpression of the SUMF1 cDNA, a large amount of the ASA produced will not be enzymatically active. In order to evaluate the value of LV vectors as a gene therapy vehicle we will carry out direct injection into the brains of As2 knockout and control mice of LV vectors carrying 1) the arylsulfatase A (As2) gene, 2) the Sumf1 gene, 3) both the As2 and Sumf1 genes, 4) the marker gene green fluorescent protein (GFP) (to visually assess, by fluorometry, the success of injection method).

We will test 3 different parameters in the evaluation of the use of LV-mediated transfer of these genes and subsequent expression in the brain:

- 1) different ages at time of injection
- 2) different lengths of time post infection.
- 3) injection into different areas of the brain

We will also determine if there are any acute and chronic negative immunological effects from the injection, infection and sustained expression of ASA and SUMF1.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? YES NO
(non-pathogenic and pathogenic biological agents including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? YES NO

If YES, please give the name of the species. _____

What is the origin of the microorganism(s)? _____

Please describe the risk (if any) of escape and how this will be mitigated:

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/Supplier	PHAC or CFIA Containment Level
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No			<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? YES NO

If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	<input type="checkbox"/> Yes <input type="checkbox"/> No		Not applicable
Rodent	<input type="checkbox"/> Yes <input type="checkbox"/> No		
Non-human primate	<input type="checkbox"/> Yes <input type="checkbox"/> No		
Other (specify)	<input type="checkbox"/> Yes <input type="checkbox"/> No		

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	<input type="radio"/> Yes <input type="radio"/> No		
Rodent	<input type="radio"/> Yes <input type="radio"/> No		
Non-human primate	<input type="radio"/> Yes <input type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input type="radio"/> No		

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required 1 2 2+ 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? YES NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	Source/Supplier /Company Name	Is Human Source Material Infected With An Infectious Agent? YES/NO	Name of Infectious Agent (If applicable)	PHAC or CFIA Containment Level (Select one)
Human Blood (whole) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Blood (fraction) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Organs or Tissues (unpreserved)		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? YES NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? YES, complete table below NO

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection

* Please attach a Material Data Sheet or equivalent if available.

** Please attach a plasmid map.

Will genetically modified viral vectors be used?

4.3 Will genetic modification(s) involving viral vectors be made? YES, complete table below NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction
Made at CPRI (under different BARF)	LV-ASA-V5, LV-ASA-V5-IRES-SUMF1, LV-eGFP	CPRI lab	hASA-V5, Sumf1, eGFP	-expression of these proteins in cells of mouse brain (see CPRI BARF for more details on vectors)

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- ◆ HIV YES, please specify gag, pol, rev NO
- ◆ HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens YES, specify _____ NO
- ◆ SV 40 Large T antigen YES NO
- ◆ E1A oncogene YES NO
- ◆ Known oncogenes YES, please specify _____ NO
- ◆ Other human or animal pathogen and or their toxins YES, please specify _____ NO

4.5 Will virus be replication defective? YES NO

4.6 Will virus be infectious to humans or animals? YES NO

4.7 Will this be expected to increase the containment level required? YES NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? YES NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 6.0

5.2 If YES, please specify which biological agent will be used: _____
Please attach a full description of the biological agent.

5.2 Will the biological agent be able to replicate in the host? YES NO

5.3 How will the biological agent be administered? _____

5.4 Please give the Health Care Facility where the clinical trial will be conducted: _____

5.5 Has human ethics approval been obtained? YES, number: _____ NO PENDING

6.0 Animal Experiments

6.1 Will live animals be used? YES NO If no, please proceed to section 7.0

6.2 Name of animal species to be used Mus musculus (ASA knockout and wild-type controls on C57B16 background).

6.3 AUS protocol # 2007-044

6.4 Will any of the agents listed in section 4.0 be used in live animals YES, specify: All NO

6.5 Will the agent(s) be shed by the animal: YES NO, please justify:

Replication-deficient so no new vector formed. Any vector particles that remains in body fluids are incapable of transduction, therefore are not a risk for infection, even if shed - which there is no evidence for.

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? YES No If no, please proceed to section 8.0

7.2 Please specify the animal(s) used:

- ◆ Pound source dogs YES NO
- ◆ Pound source cats YES NO
- ◆ Cattle, sheep or goats YES, please specify species _____ NO
- ◆ Non-human primates YES, please specify species _____ NO
- ◆ Wild caught animals YES, please specify species & colony # _____ NO
- ◆ Birds YES, please specify species _____ NO
- ◆ Others (wild or domestic) YES, please specify _____ NO

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? YES NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s) _____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD₅₀ (specify species) of the toxin _____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

8.6 Will any biological toxins be used in live animals? YES, Please provide details: _____ NO

*For information on biosecurity requirements, please see:

http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

9.0 Insects

9.1 Do you use insects? YES NO If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

9.5 What is your intention? Initiate and maintain colony, give location: _____

"One-time" use, give location: _____

9.6 Please describe the risk (if any) of escape and how this will be mitigated:

9.7 Do you use insects that require a permit from the CFIA permit? YES NO

If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

10.0 Plants

10.1 Do you use plants? YES NO If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _____

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree...)? _____

10.5 What is your intention? Grow and maintain a crop "One-time" use

10.6 Do you do any modifications to the plant? YES NO
If yes, please describe: _____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:

10.8 Is the CFIA permit attached? YES NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

11.0 Import Requirements

11.1 Will any of the above agents be imported? YES, please give country of origin _____ NO
If no, please proceed to Section 12.0

11.2 Has an Import Permit been obtained from HC for human pathogens? YES NO

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens? YES NO

11.4 Has the import permit been sent to OHS? YES, please provide permit # _____ NO

12.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- ◆ Biosafety
- ◆ Laboratory and Environmental/Waste Management Safety
- ◆ WHMIS (Western or equivalent)
- ◆ Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biological agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE C. A. [Signature]

13.0 Containment Levels

13.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required.

1 2 2+ 3

13.2 Has the facility been certified by OHS for this level of containment?

- YES, permit # if on-campus _____
 NO, please certify
 NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

14.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at <http://www.wph.uwo.ca/>

SIGNATURE _____ Date: _____

Signature (s)

14.2 Please describe additional risk reduction measures will be taken beyond containment level 2+ measures, that are unique to this agent.

None - the procedures in place for level 2+ work in the level 3 facility are more than adequate measures.

14.3 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury:

If a stick injury occurs the area will be wiped with Clidox, the person will leave the room and immediately clean the area with soap and water.

An appointment with staff health will be made and the procedures set up previously with staff health will be followed.

15.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: _____
Date: _____

2) Safety Officer for the University of Western Ontario
SIGNATURE: _____
Date: _____

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: _____
Date: _____

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

SOPs for Rupar mouse injection protocol (2007-044)

SOP 167-03 "Containment procedures sixth floor inclusion facility (Health Sciences)" will be followed with the following modifications:

- 1) As is also approved on the protocol, decapitation by guillotine after deep anaesthesia will also be used.
- 2) Tissues will be removed from the inclusion facility. These tissues will be perfused with formalin, and will be placed in a container that is decontaminated out of the area (Virkon).

SOP 660-01 "Health Sciences animal facility guidelines for containment level II aerosol and non-aerosol animal protocol" will be followed with the following clarifications:

- 1) Personnel allowed entry: Dr. Jiahui (James) Liu and Dr. Tony Rupar
- 2) Spill procedure:

Vector to be injected is stored in single use aliquots of less than 500 microliters. Vector tubes will only be opened in the BSC. In the event of a spill of the microtube of vector inside the BSC (for example, onto the stereotaxic apparatus) the spill will be wiped up with paper towel soaked in 1:5:1 CLIDOX solution.
- 3) Accidental exposure of personnel to vector stock:

The only possible risk of direct contact with vector stock by personnel is through accidental puncture through PPE of the syringe containing vector to be injected. This risk is extremely low. The "needle" of the hamilton syringe used is not as sharp as a "true" needle and would not puncture two layers of gloves without significant force. I tested this with an unused hamilton syringe dipped in india ink – after applying varied amounts of pressure to the back of my double-gloved hand pressure marks were apparent after I removed the gloves, but no ink.

If a puncture does occur, the area will be immediately wiped with a paper towel soaked in 1:5:1 CLIDOX.

As soon as possible, the person will exit the room following standard procedures without touching the punctured section of skin. The area of skin should then be disinfected and washed with soap and water.

The person will then contact staff health services and follow the accident procedure set up with staff health.

**Workflow for mouse injections:
From protocol submission:**

All surgical procedures are done according to SOP #333-03. The entire procedure takes approximately 15 - 30 min per animal. Animal will be allowed to recover and monitored according to SOP #303-03 and 330-03.

- Mice brought into the room
- All procedures involving vectors carried out inside safety hood
- Mouse anaesthetised .
- Skin preparations done
- Placed on Kopf stereotaxic apparatus
- Towed
- A 1 cm midline on the scalp
- Skin retracted
- a burr hole drilled (according to pre-determined coordinates)
- Drill assembly is removed from stereotaxic apparatus and the mouse on the stereotaxic apparatus is moved into the hood.

Inside the bio-safety cabinet, we will have the vector to be used in an eppendorf tube on ice. There will be a small covered container with 1:5:1 CLIDOX solution inside the hood to immerse syringe and needle assembly following injections.

- The eppendorf tube will be carefully opened and 1 – 5 microliters of vector will be taken into a Hamilton syringe and needle assembly
- The eppendorf will be closed and placed back on ice
- Syringe will be placed into injection holder on stereotaxic apparatus
- The vector will be slowly injected at ~ 0.5 microliters /min
- Needle will be left in place for 3 min and slowly withdrawn
- Syringe and needle assembly will be removed and placed in 1:5:1 CLIDOX solution
- Wound will be swabbed with cotton tips and they will be disposed of into bags for incineration
- Wound will be closed in single layer with skin clips
- Animal will be recovered under heat lamp
- Eppendorf tube containing any remaining vector will be submerged open in 1:5:1 CLIDOX solution

For both types of procedures:

Post-operative care and monitoring will be done by Dr. Jiahui Liu

- After pre-determined length post-injection (3 days to 12 months)
- Animal will be anaesthetized and placed supine on a tray in a bio-safety cabinet
- Abdomen and thorax will be opened by a midline incision
- Saline (20mL) followed by 2% paraformaldehyde solution (60mL) will be passed through the heart into the aorta
- Effluent is collected in a container for proper disposal
- At the end of the perfusion, animal's head will be opened and brain and other organs will be removed and placed into a container with phosphate buffered saline
- All instruments will be placed in 1:5:1 CLIDOX solution
- Animal carcass will be incinerated.

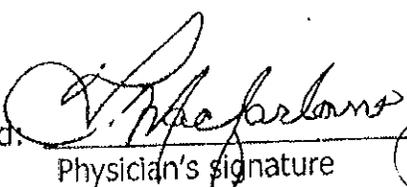


Workplace Health

SUBJECT: Animal Bites

OBJECTIVE: To provide education, First Aid and, when necessary, ongoing medical management of animal bites

- PROCEDURE:**
1. Educate persons working with animals that **all** animal bites are potentially serious and require immediate First Aid.
 2. The bite wound should be washed immediately in warm soapy water.
 3. If the wound is bleeding, attempt to encourage bleeding by gently expressing the wounded area.
 4. After washing, cover the wound with a dry sterile dressing
 5. Go to Workplace Health, Room 25, University Community Centre (UCC) for further assessment and treatment. After hours go to University Hospital Emergency Department.

Approved:  Physician's signature  Nurse's signature Aug 3/2005 Date