TRC STAR PROJECT PROPOSAL (OHSU-MIT-FHCRC-NTP-PARADIGM)
DNA ALKYLATION IN NEURODEGENERATIVE DISEASE AND CANCER

PROTOCOL (7/12/04)

PARTICIPANTS (Cooperative Research Members: CRMs)

1. FHCRC/UW: Helmut Zarbl (PI), Terry Kavanagh (UW)
2. MIT: Leona Samson (PI); Rebecca Fry, Lisienne Meira
3. NIEHS/NTP: Mike Cunningham (long-term studies only – may not be performed)
4. OHSU: Peter Spencer (PI), Glen Kisby, Mohammad Sabri, Valerie Palmer, Dean Sproles, Alex Cranson, Safia Baggia
5. PARADIGM: Pat Hurban, Ed Lobenhofer

GENERAL NOTES:

1. The experimental design (below) is based on the approved and funded Star project proposal and the modifications arising from our mutual discussions.

2. As the first step, wild-type mice will be used to check gene expression consistency among members of the Star project group.

3. All of the labeling and hybridization work will be performed by Paradigm using the NIEHS TRCP accounts. A single commercial laboratory will therefore receive purified RNA samples from all three cooperating laboratories. The Agilent platform will be used unless excessive delays demand substitution of another platform (Affymetrix).

4. Studies will commence with wild-type mice as follows:

TEST ARTICLES AND DOSAGES:

1. Methylazoxymethanol actetate (MAM). 20 mg/kg
2. Methylnitrosurea (MNU). 10mg/kg
3. Methylmethanesulfonate (MMS). 100 mg/kg (provisional)

WORKLOAD DISTRIBUTION AND ORDER OF EXPERIMENTATION:
Begin with MAM (OHSU, FHCRC/UW) and MNU (MIT/OHSU)
Later conduct MMS studies (MIT, FRCRC/UW).

QUALITY-CONTROL CHECK:
Hold for one week with food and water ad libitum.
Vehicle-injected controls (one group)
Termination at 24 hours following injection
Harvest tissues, extract RNA
Hybridize at Paradigm
Compare three institution-specific data sets

Proceed with experimental design if correlation is high (>90%)
Problem solve and repeat if correlation is <90%.

EXPERIMENTAL DESIGN:
MAM in common vehicle vs. common vehicle (i.e. 2 groups).
MNU in common vehicle vs common vehicle (i.e. 2 groups).
MMS in common vehicle vs. common vehicle (i.e. 2 groups).

TIMEPOINTS:
6h, 24h, 48h, 7days following single injection
Commence with 24-hour timepoint

INSTITUTIONAL TABLES TO SHOW ANIMALS TO BE TREATED (AND TERMINATED) BY AGENT, TIME AFTER TREATMENT, AND GENOTYPE:

FHCRC/UW	**Vehicle**	**MAM**	**MNU**	**MMS**	**Totals**
0	-	-	-	-	
6 hours	4 (3)	4 (3)	-	4 (3)	12 (9)
24 hours	**4 (3)**	**4 (3)**	**-**	**4 (3)**	**12 (9)**
48 hours	4 (3)	4 (3)	-	4 (3)	12 (9)
7 days	4 (3)	4 (3)	-	4 (3)	12 (9)
WT total	16 (12)	16 (12)	-	16 (12)	48 (36)
AAG total	16 (12)	16 (12)	-	16 (12)	48 (36)
MGMT total	16 (12)	16 (12)	-	-	32 (24)
All mice total	48 (36)	48 (36)	-	32 (24)	128 (96)

MIT	**Vehicle**	**MAM**	**MNU**	**MMS**	**Totals**
0	-	-	-	-	-
6 hours	4 (3)	-	4 (3)	4 (3)	12 (9)
24 hours	**4 (3)**	**-**	**4 (3)**	**4 (3)**	**12 (9)**
48 hours	4 (3)	-	4 (3)	4 (3)	12 (9)
7 days	4 (3)	-	4 (3)	4 (3)	12 (9)
WT total	16 (12)	-	16 (12)	16 (12)	48 (36)
AAG total	16 (12)	-	-	16 (12)	32 (24)
MGMT total	16 (12)	-	16 (12)	-	32 (24)
All mice total	48 (36)	-	32 (24)	32 (24)	112 (84)

OHSU	**Vehicle**	**MAM**	**MNU**	**MMS**	**Totals**
0	-	-	-	-	
6 hours	4 (3)	4 (3)	4 (3)	-	12 (9)
24 hours	**4 (3)**	**4 (3)**	**4 (3)**	-	**12 (9)**
48 hours	4 (3)	4 (3)	4 (3)	-	12 (9)
7 days	4 (3)	4 (3)	4 (3)	-	12 (9)
WT total	16 (12)	16 (12)	16 (12)	-	48 (36)
AAG total	16 (12)	16 (12)	-	-	32 (24)

MGMT total	16 (12)	16 (12)	16 (12)	-	48 (36)
All mice total	48 (36)	48 (36)	32 (24)	-	128 (96)

COMMON VEHICLE:
Freshly prepared 100 microliters 0.05% Sigma HPLC-grade acetic acid in saline.

ANIMAL NUMBER
N=4/group/timepoint.
Harvest tissue from four animals. Select three best-quality RNA samples.

ANIMAL SUPPLIER (wildtype only):
Charles River. Each CRM will order their own animals according to the following protocol. Jackson Laboratory (or Charles River) will be approached to determine whether they could serve as the sole supplier of aag nulls and mgmt nulls.

ANIMALS:
12-week-old C57 black males (females will be done later, time-permitting).

ANIMAL HOUSING:
Sterilize cage before use.
Do not ear-tag the animal.
House one animal per cage.
House on corn cob bedding (alternative: non-cedar wood chips)

ANIMAL ACCLIMATION:
Arrive at CRMs at 11 weeks of age.
Place individually in cages to be used for study.
Hold for one week with food and water ad libitum.

ANIMAL FEED
Purina standard mouse chow.
Purchase by OHSU (3 x 50 lb. Bags) for delivery to three study sites prior to 2/20/04.
Provide ad libitum from time of delivery to termination.

ANIMAL DRINK
Tap water in bottle or automatic water supply: Take sample of water in falcon tube, freeze at –20 degrees C, for subsequent analysis in the event one of the three collaborating institutions show marked data variance).

ANIMAL BODY WEIGHT:
Weigh on arrival (try to ensure animals reach ~25g target for treatment commencement)
Weigh prior to treatment at 11:00 a.m.
Weigh daily at 11:00 a.m. throughout experiment (24-h and 7-day timepoints only)

TREATMENT GROUPS:
Single i.p. injection

Target volume: 100 microliter
Final volume: Adjusted to body weight.

INJECTION METHOD:
Hamilton glass syringe with ~26-guage metal needle

INJECTION SITE:
Lower left quadrant of abdomen

TERMINATION:
Termination by decapitation at neck (sharp scissors or guillotine)
(Initial studies may vary: e.g. FHCRC used carbon dioxide).

TIMEPOINTS:
6h, 24h, 48h, 7days following single injection

TISSUE PREPARATION (WHOLE TISSUES)
First remove whole brain (cut at medulla oblongata) as fast as possible.
Then remove principal (largest) lobe of the liver.
Place tissues in ice-cold RNALater
Cut brain and liver into right and left halves.

TISSUE PREPARATION (HALF TISSUES)
1. Right side tissues for RNA extraction and hybridization.
- Immerse in ice-cold RNALater
- Store at 4oC until extraction (within one week of tissue harvesting/freezing)
- At time of extraction, rince in nuclease-free water
- Immerse in cold homogenization Buffer RLT
- Employ Qiagen kits RNeasy
- Check purity with Bioanalyzer
- Send to Paradigm
Mouse Custom Chip by Agilent, Cy3, Cy5 labeling, and single dye flips.
Change to alternative platform (Affymetrix) if delays are excessive.

2. Left side tissues for DNA extraction and DNA damage determination.
- Snap freeze in liquid nitrogen.
- Work on dry ice.
    - Store tissue at -80C.

HYBRIDIZATION
Use random number scheme to select hybridization sequence (to avoid batch-related artifacts affecting experimental groups).

TISSUE EXCHANGE:

FHCRC and MIT to send their half brains on dry ice. Address to Valerie Palmer (palmerv@ohsu.edu) and Glen Kisby (Kisby@ohsu.edu) at OHSU for assessment of DNA adducts

TISSUE RETENTION:
Retain RNA samples at –80oC in each of the three CRMs.

END