PI: Tim Greenamyre

Grant Number: R01ES020718-03S1 (VICTER)

Grant Title: MtDNA Damage as a Biomarker for Environmental Mitochondrial Toxicity

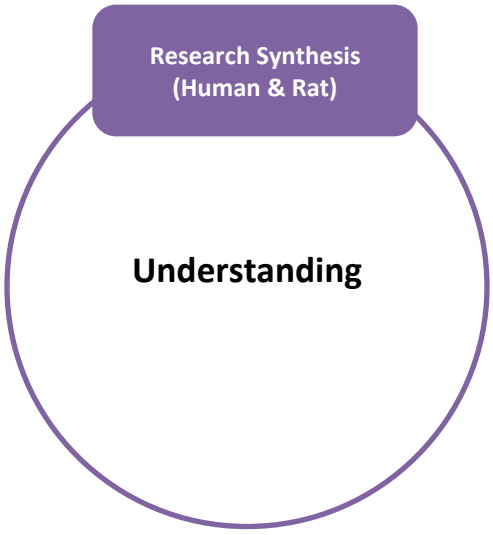
Background/Context: The Greenamyre laboratory (University of Pittsburgh) was funded to examine mitochondrial DNA (mtDNA) damage as a potential biomarker of exposure to environmental mitochondrial toxins. The PCR-based assay employed is extremely sensitive, quantitative and highly reproducible - and it detects any type of damage that slows polymerase progression. The basic premise is that mitochondrial toxins, by generating reactive oxygen species (ROS), will cause various forms of DNA lesions in the mitochondria. Parkinson's disease (PD), affecting about 1 million people in the United States, appears to involve both genetic and environmental factors - and systemic mitochondrial defects have been strongly implicated. The best characterized environmental risk factor for PD is occupational exposure to pesticides, many of which are mitochondrial inhibitors.

Key Translational Milestones

- Completed literature review of evidence for oxidative damage to nucleic acids, lipids, and proteins in both the brain and the peripheral tissues in human PD and in the rat rotenone model. (See Sanders and Greenamyre. 2013 for a full description of rat rotenone model, but basically, exposing rats to low doses of rotenone provides a model for Parkinson's Disease for both in vivo and in vitro studies.)
- Identified rotenone and paraquat as specific pesticides as bona fide risk factors for PD (Caroline Tanner and Sam Goldman; Parkinson's Institute, Sunnyvale, CA); both ROS-generating mitochondrial inhibitors. Exposure to the solvent, trichloroethylene (TCE), also increases the risk of PD. Importantly, existing genomic DNA samples are available from the epidemiological studies of Tanner and Goldman.
- mtDNA damage in blood and skeletal tissues in the rat rotenone model may provide a biomarker of past or ongoing mitochondrial toxin exposure
- LRRK2 mutations are associated with mtDNA damage
- Validated a new cellular phenotype that can be used for examining pathogenic mechanisms and screening therapeutic strategies.
- The first demonstration that abasic sites are readily detected in nigral dopaminergic, but not cortical neurons, in PD.
- In rats, TCE exposure is associated with inhibition of mitochondrial complex I.
- Will develop a biomarker for human environmental toxicants (ex. rotenone, paraquat, and TCE) and possibly, for Parkinson's disease using existing technology and staff, and banked DNA specimens
- Will test assay as a biomarker for PD in two independent epidemiological cohorts.

Starting Point Description:

- Literature review to understand evidence for oxidative damage to nucleic acids, lipids, and proteins in both the brain and the peripheral tissues in human PD and in the rat rotenone model.



Application and Synthesis Ring: Research Synthesis

Timeframe: 2011-2013

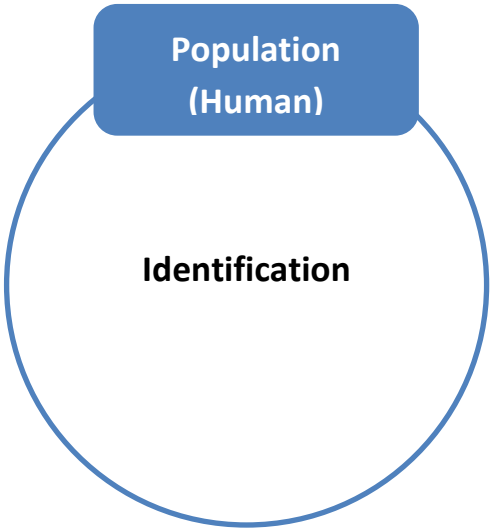
Collaborators:

- Review article by Greenamyre Lab

Citation:
Sanders and Greenamyre. 2013. Oxidative damage to macromolecules in human Parkinson disease and the rotenone model. Free Radic Biol Med. 2013 Sep;62:111-20. doi: 10.1016/j.freeradbiomed.2013.01.003.

Translational Narrative:
What led to the next step?
How did the idea evolve?
Who was involved?
What needed to happen (collaborations, tools, technologies, serendipity) to cross the translational bridge?
How did you know what to do next?

TRANSLATIONAL POINT 2



Translational Research Description:

- Identified rotenone and paraquat and TCE as risk factors for PD

Science and Setting Translational Ring

Driver: Identification
Experimental Setting: Population
Organism: Human

Timeframe: xxxx? - 2011

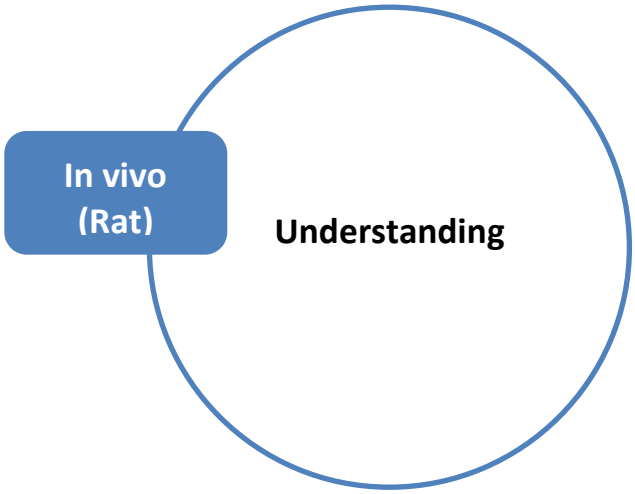
Collaborators:

- The Parkinson's Institute

Citation:

Tanner CM, Kamel F, Ross GW, Hoppin JA, Goldman SM, Korell M, et al. Rotenone, Paraquat and Parkinson's Disease. Environ Health Perspect. 2011 Jun;119(6):866-72. doi: 10.1289/ehp.1002839.

TRANSLATIONAL POINT 3



Translational Research Description:

- mtDNA damage in blood and skeletal tissues in the rat rotenone model may provide a biomarker of past or ongoing mitochondrial toxin exposure

Science and Setting Translational Ring:

Driver: Mechanistic Understanding
Experimental Setting: In vivo (Subcellular)
Organism: Rat

Timeframe: 2013-2014

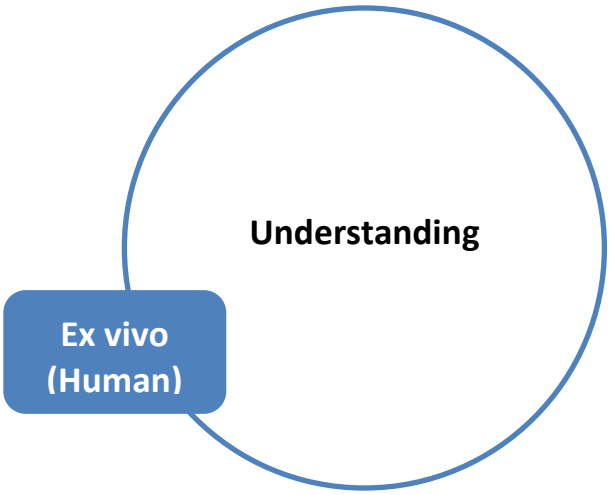
Collaborators:

- Greenamyre Lab

Citation:

Sanders, Howlett, McCoy, Greenamyre. 2014. Mitochondrial DNA damage as a peripheral biomarker for mitochondrial toxin exposure in rats. Toxicol Sci. 2014 Dec;142(2):395-402. doi: 10.1093/toxsci/kfu185.

TRANSLATIONAL POINT 4



Translational Research Description:

- LRRK2 mutations are associated with mtDNA damage

Science and Setting Translational Ring

Driver: Mechanistic Understanding
Experimental Setting: Ex vivo/Subcellular
Organism: Human

Timeframe: 2011-2014

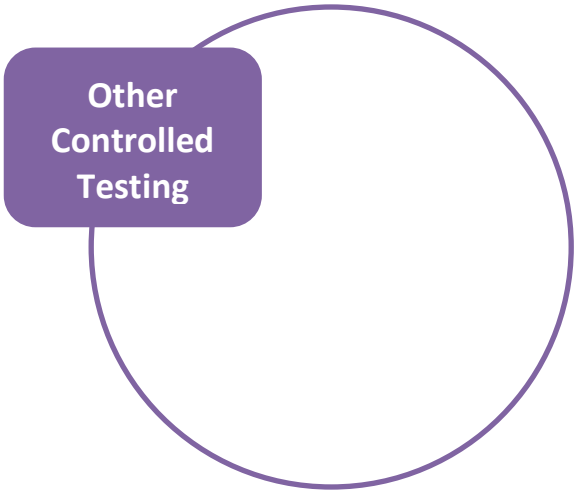
Collaborators:

- Greenamyre Lab
- Sangamo BioSciences, Inc.
- McLean Hospital/Harvard Medical School
- The Parkinson's Institute

Citation:

Sanders at al. 2014. LRRK2 mutations cause mitochondrial DNA damage in iPSC-derived neural cells from Parkinson's disease patients: reversal by gene correction. Neurobiol Dis. 2014 Feb;62:381-6. doi: 10.1016/j.nbd.2013.10.013.

TRANSLATIONAL POINT 5



Translational Research Description:

- Validated a new cellular phenotype that can be used for examining pathogenic mechanisms and screening therapeutic strategies.

Controlled Testing Ring: Other Controlled Testing, Initial Phenotype Validation

Timeframe: 2011-2014

Collaborators:

- Greenamyre Lab
- Sangamo BioSciences, Inc.
- McLean Hospital/Harvard Medical School
- The Parkinson's Institute

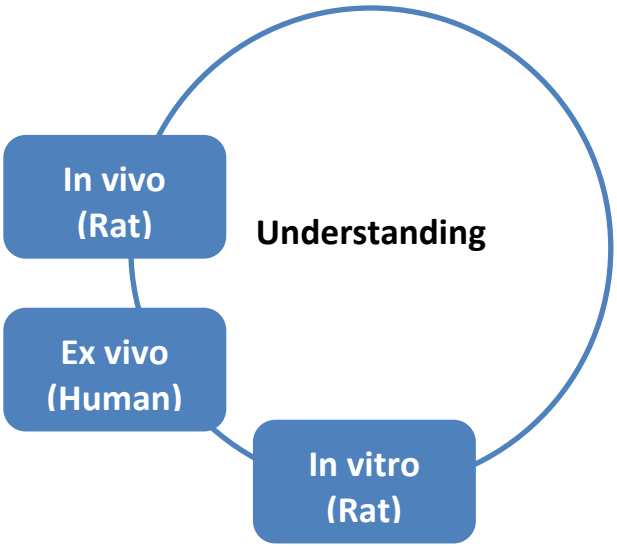
Citation:

Sanders at al. 2014. LRRK2 mutations cause mitochondrial DNA damage in iPSC-derived neural cells from Parkinson's disease patients: reversal by gene correction. Neurobiol Dis. 2014 Feb;62:381-6. doi: 10.1016/j.nbd.2013.10.013.

TRANSLATIONAL POINT 6

Translational Research Description:

- The first demonstration that abasic sites are readily detected in nigral dopaminergic, but not cortical neurons, in PD.



Science and Setting Translational Ring:

Driver: Mechanistic Understanding

Experimental Setting: in human postmortem brain tissue and in in vivo and in vitro models of PD

Organism: Human, Rat

Timeframe: 2011-2014

Collaborators:

- Greenamyre Lab
- UC Berekely Dept of Chemistry
- Pittsburgh Institute for Neurodegenerative Diseases and Department of Neurology, University of Pittsburgh
- Department of Pathology
- Department of Pharmacology and Chemical Biology
- Pittsburgh Institute for Neurodegenerative Diseases and Department of Neurology

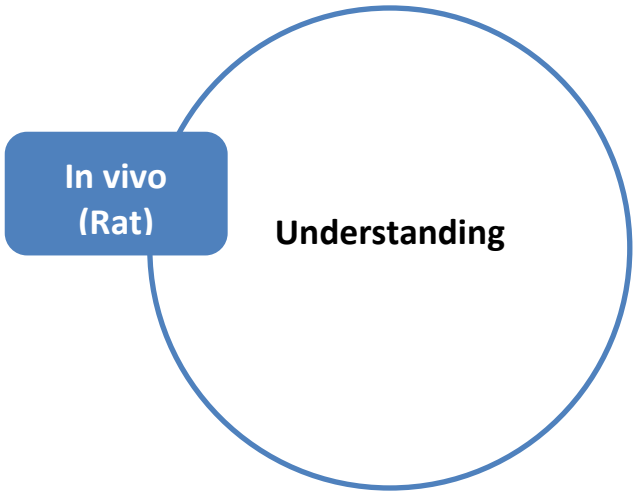
Citation:

Sanders et al. Mitochondrial DNA damage: molecular marker of vulnerable nigral neurons in Parkinson's disease. Neurobiol Dis. 2014 Oct;70:214-23. doi: 10.1016/j.nbd.2014.06.014.

TRANSLATIONAL POINT 7

Translational Research Description:

- In rats, TCE exposure is associated with inhibition of mitochondrial complex I (Question for PI or PO - Was this part of research? Or a previous finding?)



Science and Setting Translational Ring:

Driver: Mechanistic Understanding
Experimental Setting: In vivo
Organism: Rat

Timeframe: 2011-2014

Collaborators:

- Greenamyre Lab

Citations:

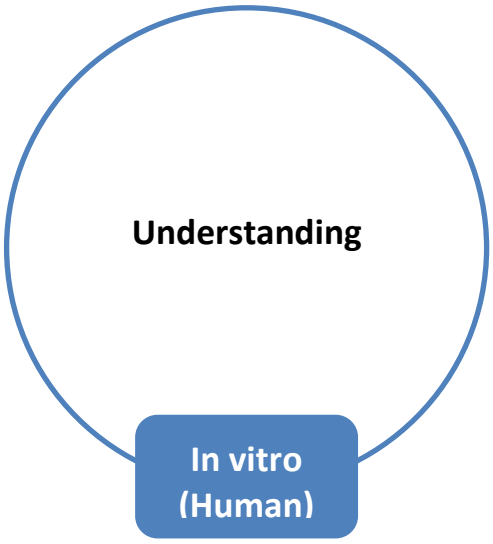
Zharikov et al. 2015. shRNA targeting α-synuclein prevents neurodegeneration in a Parkinson's disease model. J Clin Invest. 2015 Jul 1;125(7):2721-35. doi: 10.1172/JCI64502.

Tyurina et al. 2015. LC/MS analysis of cardiolipins in substantia nigra and plasma of rotenone-treated rats: Implication for mitochondrial dysfunction in Parkinson's disease. Free Radic Res. 2015 May;49(5):681-91. doi: 10.3109/10715762.2015.1005085.

TRANSLATIONAL POINT 8

Planned Translational Research Description:

- Develop a biomarker for rotenone, paraquat, and TCE and possibly, for Parkinson's disease, using banked DNA specimens



Science and Setting Translational Ring:

Driver: Identification
Experimental Setting: In vitro
Organism: Human

Timeframe: Still to do

Collaborators:

- Not known yet

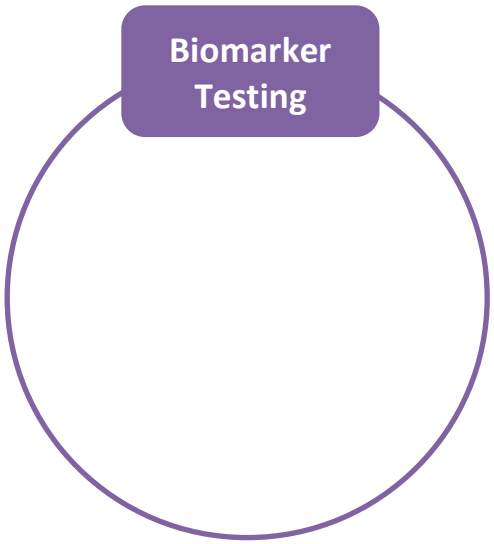
Source:
Grant Application.

TRANSLATIONAL POINT 9

Planned Translational Research Description:

- Test assay as a biomarker for PD in two independent epidemiological cohorts

Controlled Testing Ring: Biomarker Testing



Timeframe: Still to do

Collaborators:

- Not known yet

Source:
Grant Application.