SENS Foundation
advancing rejuvenation biotechnologies

Research Report, November 2010

Proof-of-concept research is a critical component of SENS Foundation's mission, whether executed in our own Research Center, or in numerous affiliated universities, research organizations and other centers of excellence. Progress over the past year has established a scalable platform for our research activities, built on mature operational management and administrative controls.

Existing projects have been maintained or expanded, delivering advances toward specific milestones on the way to therapeutic deliverables. We have also been able to initiate several new projects, each with specific importance for critical-path research.

The relocation of our Research Center, and the upgraded facilities of the new laboratory space, promise to increase the speed of our progress in the year ahead. Our focus on providing the best environment possible for our researchers is, as ever, coupled with every effort to generate the funding necessary to deliver the results which underpin our mission.

We hope that you will find the following overview of our research activities interesting and informative. You can find updates and even-more detailed reports at our website, sens.org.

Tanya Jones
Research Operations Manager

Aubrey de Grey
Chief Science Officer

Michael Rae
CSO Team

Intramural Research Projects

SENS Foundation conducts intramural research in its Research Center in Mountain View, California. The primary focus of our intramural work is LysoSENS – investigating novel lysosomal hydrolases against intracellular aggregates that impair cell function – and we recently produced a detailed and comprehensive LysoSENS planning document in collaboration with our extramural project at Rice University.

We have also arranged for research in the MitoSENS strand – obviating mitochondrial DNA deletions – to be conducted at the Research Center, following the negotiation of a transfer agreement with Dr Corral-Debrinski covering materials produced, and used in, previous successful work by her group. Dr Matthew "Oki" O'Connor joined us in September to initiate this project.

LysoSENS

2010 milestones. The creation of an internal A2E synthesis capability enabled efficient target enzyme evaluation. Our successful development of a protein purification protocol resulted in the isolation of an active, A2E-degrading enzyme.

The year ahead. We will express and purify additional candidate enzymes for both macular degeneration and atherosclerosis projects; establish activity of these additional enzymes *in vitro*; perform cell-uptake studies, in RPE cells for A2E and in macrophages for 7-ketocholesterol (7KC); and perform initial toxicity studies.

One of our priorities has been to overcome the limitations on our ability to test potential A2E-degrading enzymes, caused by a restricted supply of the target molecule. We have now successfully synthesized A2E, purifying it by flash chromatography and HPLC, and confirming it to be spectrophotometrically identical to that produced in Janet Sparrow's laboratory. Our ability to produce this material 'in house' is a key element in the expansion of our screening and evaluation of existing and newly-purified A2E-degrading enzymes.

In collaboration with students at the State University of New York at Plattsburgh, we have also made significant progress in improving the A2E-degrading capacity of versatile peroxidase (an extant enzyme previously identified by screening a commercial library) through chimeric addition of an additional moiety. In addition, we recently observed enzymatic activity in one of these enzymes during an expression optimization project, and we are now planning to conduct further tests of this particular enzyme against A2E.

Our protein purification process has been improved by acquisition of new equipment and modifications to our protocols. SDS-PAGE gel confirmed the superior purity of proteins produced under this new regime. We have also subcloned cholesterol ester hydrolase (a putative microbial 7KC hydrolase) into an expression vector, so that we can optimize this enzyme's expression and purification, for further characterization of its 7KC-degrading properties.

Researchers. Lorenzo Albanello, Daniel Kimbel, Max Peto
Budget. $200,000 in 2010; $300,000 in 2011

MitoSENS

2010 milestones. We have established an in-house MitoSENS program, based on previous, funded work in the INSERM laboratory of Dr Corral-Debrinski.

The year ahead. We will transiently express mitochondrially-encoded proteins (ND1, ND4, ATP6) from provided expression plasmids; confirm localization to mitochondria by immunofluorescence; design and synthesize "nuclear" cytochrome B followed by similar expression and localization; demonstrate Complex III functional rescue with nuclear expression of cytochrome B.

Dr Corral-Debrinski's laboratory is providing SENS Foundation with the materials produced and used in previous successful work by her group. These materials – all of which we expect to have received by the end of November 2010 - include the plasmid pCMV in which nuclear versions of the human mitochondrial ND1, ATP6, and ND4 genes were synthesized. They will allow us to independently test the ability of allotopic expression of mitochondrial genes from the nucleus to replace the function of mitochondrially-expressed genes, restoring the activity of respiratory chains deficient in these proteins due to mitochondrial mutations.

Dr Matthew "Oki" O'Connor, who has recently joined our staff, has been familiarizing himself with Dr Corral-Debrinski's allotopic expression protocols, with her input and advisement. This skills transfer, coupled with improvements to the protocols themselves and Dr O'Connor's existing laboratory experience, are all designed to avoid the recurrence of previous issues in the replication of Dr Corral-Debrinski's results. Our next steps will be to replicate and extend Dr Corral-Debrinski's success in overcoming translocation issues with hydrophobic proteins expressed in the nucleus.

We will then continue by designing and synthesising ATP8 (suitably modified for nuclear expression) followed by expression and localization. Co-expression and co-localization of ATP6 and ATP8 in mitochondria will be the next step, followed by the development of a functional assay to show that Complex V (ATP Synthase) activity is rescued by nuclear expression of these proteins. The achievement of this milestone would constitute the first complete rescue of a respiratory chain complex from multiple defective mitochondrial-encoded components.

Researchers. Matthew "Oki" O'Connor
Budget. $30,000 in 2010; $120,000 in 2011

Extramural Research Projects

In addition to its intramural projects, SENS Foundation provides funding to a number of centers of excellence in the USA and globally. Our collaborations continue to increase in number and breadth, with a new project – based in Houson, Texas and Dubin, Ireland – starting in late 2010.

LysoSENS

Location. Rice University, Houston, Texas

2010 milestones. We have designed and synthesised modified versions of eight microbial hydrolases, the expressions of which are upregulated by 7KC; developed an HPLC assay; and expressed proteins for target candidates.

The year ahead. We will develop further expression testing for cloned proteins and conduct *in vitro* activation testing.

Jacques Mathieu has designed and synthesised modified versions of the genes for 13 enzymes that are upregulated in microbes during breakdown of 7-ketocholesterol (7KC), a cholesterol oxidation product believed to be a key mediator of atherogenesis and several other degenerative diseases. Mathieu cloned the underlying genes for these 13 enzymes, and then modified them for transgenic expression in *E. coli*, reducing their content of problematic base pairs and eliminating several common restriction sites that would have lead to their destruction in the expressing bacteria. He is currently isolating 3 additional proteins that also appear to be involved in metabolizing 7KC, and has initial results suggesting the ability of at least one enzyme to transform it, albeit slowly.

Mathieu has also designed in principle a vector to deliver these genes directly to the lysosome, to enable enzyme expression where required to break down aggregates accumulating in the cell. He is now in the process of putting such a vector together with "payload" genes for the enzymes.

Mathieu is being assisted by Rob O'Callahan, whose other project is to identify bacterial enzymes that attack protein crosslinks in lipofuscin, the less well-defined recalcitrant waste common to aged lysosomes. O'Callahan is now focused on a protein-degrading enzyme from the brewer's yeast, *Saccharomyces cerevisiae*, that attacks a broad range of proteins. He is working to load it into a vector for expression in mammalian cells. Once they have confirmed that it can work in such cells, the team intend to introduce mutations into the gene, to broaden its possible range of protein targets, allowing it to attack the multifarious bonds in lipofuscin.

Researchers.	Pedro Alvarez, Jacques Mathieu, Robert O'Callahan
Budget.	$70,000 in 2010; $85,000 in 2011

ApoptoSENS and RepleniSENS

Location. University of Arizona, Tucson, Arizona

2010 milestones. We have constructed a prototype T-cell "scrubber" and confirmed that it reduces the abundance of dysfunctional immune cells; prepared suitably infected mice and verified their immune status; and applied permutations of immunorejuvenation therapies.

The year ahead. We will analyse the data collected and publish results.

The project seeks to test whether removal of accumulated age- and virus-related T-cell clonal expansions and/or T-cell repertoire rebalancing (i) restores functionality of T-cell compartment in the aged organism, and (ii) improves immune defense against new infection. This series of longitudinal experiments was designed using 2 mouse models of persistent viral infection known to result in the development of virus-specific CD8+ T-cell expansions (TCE). Such expansions are thought to "crowd out" the naïve T-cells needed to target novel pathogens, limiting the ability of the immune system. These experiments will directly test methods for the removal and/or neutralization of virally-induced expansions in aging mice, and the ability of these methods to rebalance and rejuvenate the aging immune system to restoring youthful, robust immune defense.

The study began at the end of 2008 and will conclude in June 2011. At the beginning of the study, 180 mice were received and sorted into five groups for treatment and control. Mice were infected with HSV-1 or MCMV, or were not infected. Evaluation of the virus-specific CD8 T-cell populations in all infected mice showed evidence of the initial inflation of those populations within aging mice, particularly within the MCMV-infected groups. At 55 weeks, the dual expression of the cell-surface markers KLRG1 and PD-1 by virus-specific CD8 T-cells (an indication of functionally "exhausted" cells) was beginning to emerge, particularly within MCMV-infected groups.

In late May and June 2010, additional therapeutic interventions were performed. In August, all groups were challenged with the bacterium *Listeria monocytogenes*. Young adult control animals (not infected with HSV or MCMV) were included as an additional challenge group. (This will allow a study of the differences between adult and old responses, of the ways in which life-long infection may further compromise the response of old mice, and of how therapeutic intervention may improve that.) Seven days following challenge animals were euthanized and tissues collected for analysis. Samples were evaluated with over 30 markers to determine: (1) the functional capacity of *Listeria*-specific CD4 and CD8 T-cells to produce 5 different effector cytokines needed to mount an attack in response to specific antigen; (2) the transcriptional upregulation of key molecules required for various T-cell populations to achieve optimum effector function; and (3) the size of the virus-specific TCE population, as well as the phenotypic markers expressed by these cells.

The experiment generated over 10 GB of flow cytometry data that are currently being analyzed. A comprehensive analysis report is due for delivery at the beginning of December 2010.

Researchers.	Janko Nikolich-Zugich, Megan Smithey
Budget.	$120,000 in 2010; $60,000 in 2011

OncoSENS

Location. Albert Einstein College of Medicine, Bronx, New York

2010 milestones. We developed a method to determine gene-specific DNA methylation patterns in single cells; tested this protocol for gene-specific DNA methylation patterns in different types of single cell; and optimized the protocol to isolate the nuclei of single neurons.

The year ahead. We will develop a novel method for single-cell global gene methylation analysis and study cell-to-cell variation in DNA methylation patterns among neurons during mouse aging.

Dr Vijg is studying the accumulations and effects of mutations and/or epimutations in aging mice. The variability in gene expression among single cells in tissue may arise from heterogeneous responses to both intrinsic and extrinsic perturbations, and/or to cell-to-cell differences in the nature and magnitude of the perturbations to which each is subjected. Dr Vigj's group, and others, have shown that aging is associated with increased stochastic deregulation of cellular gene expression, giving rise to cellular mosaics of diverging transcriptomes. These may be due in part to the differential loss of normal epigenetic patterns in different cells. If so, it would greatly increase the difficulty of achieving an OncoSENS-based solution to repairing or obviating age-related nuclear DNA mutations. Testing this requires epigenetic patterns to be analyzed at the single-cell or single-molecule level. Dr Vijg is now making progress toward resolving this issue.

Dr Vijg's group have now developed and finalized a novel protocol to assess stochastic epigenetic changes at the level of a single cell. They have been able to show that the protocol works with different cell types such as single hepatocytes, single fibroblasts and single neurons. In the process, they have also developed techniques to isolate single neuronal nuclei for testing. To test the protocol, they have chosen promoter regions of genes with tissue-specific epigenetic signatures (hypomethylation in the liver vs. hypermethylation in the brain, for example) and have proven that the protocol can detect the correct DNA methylation patterns at the single-cell level.

The group has been able to analyze promoter regions of a number of individual genes using technology routinely run in Einstein's Genomics Core. However, full investigation requires being able to assess gene methylation patterns in all, or at least all organ-specific, genes in a cell, and, ideally, throughout the entire genome (or a representation of it). Therefore, Dr Vijg's group are currently working on a novel method that combines the single-cell DNA methylation pattern method with strategies for library preparation, for next-generation sequencing. They anticipate that this genome-wide method approach will allow them to interrogate approximately 1.2 million single-cell cytosines (in CG dinucleotides) in a single experiment.

Depending on the results of this work, they will proceed with assessing stochasticity using either gene-specific DNA methylation patterns or genome-wide ones. They are currently working to publish on the novel method to determine gene-specific DNA methylation patterns in single cells.

Researchers.	Jan Vijg, Silvia Gravina
Budget.	$126,000 in 2010; $126,000 in 2011

ApoptoSENS

Location. Buck Institute for Age Research, Novato, California

2010 milestones. Work at the Foundation's Research Center identified senescence markers and toxins. We have engineered transiently active suicide genes.

The year ahead. We will engineer permanently active suicide genes; determine their effect on the abundance of senescent cells; and determine further, downstream effects.

Kevin Perrott, working under the guidance of Dr Judith Campisi, is screening compounds for their effectiveness in eliminating cells exhibiting the "Senescence Associate Secretory Phenotype" (SASP), which produces pro-inflammatory molecules implicated in aging. The isolation, identification, and analysis of potential SASP-moderating compounds will comprise the bulk of this three year project.

Researchers.	Judith Campisi, Kevin Perrott
Budget.	$30,000 in 2010; $60,000 in 2011

AmyloSENS

Location. University College Dublin, Dublin, Ireland
University of Texas, Houston, Texas

2010 milestones. This is a new project for the Foundation.

The year ahead. We will identify antibodies and fragments that bind to, and catalytically cleave, transthyretin fibrils and high-copy-number oligomers; and identify the structure and composition of other amyloids and their role in age-related diseases.

In previous work, these researchers have developed antibodies and catalytic antibody fragments which target Alzheimer's and TTR amyloids, and which are now in various phases of clinical development, up to phase III clinical trials.

The current project is a four-phase collaboration to develop (a) an antibody for diagnosing transthyretin (TTR) amyloidosis, which is implicated in age-related cardiac structural and functional decay, and (b) novel antibody fragments capable of catalytically cleaving TTR aggregates.

Researchers.	Brian O'Nuallain (Dublin), Sudhir Paul (Texas)	
Budget.	Dublin:	$40,000 in 2010; $40,000 in 2011
	Texas:	$50,000 in 2010; $50,000 in 2011

Research Operations

Research Center Relocation and Expansion

In late 2009 and early 2010, we found that the location of the Research Center – in an incubator facility – was limiting the progress and expansion of our research. In addition to requiring more space in which to house expanded laboratories and staff, our facility had been beset by microbial contamination problems. An internal investigation of the incubator space's biosafety cabinet, fume hood, and countertops – which did not meet Good Laboratory Practice (GLP) standards – revealed that we could not maintain a sterile environment for the microbiological experiments at the core of LysoSENS and MitoSENS research. All cloning, culturing, and enzymatic expression work was subject to contamination, resulting in substantial loss of time and materials.

The identification of an appropriate facility took several months, but our efforts have been repaid, with our new location delivering greater functionality and nearly three times the usable space of our original facility. The new space required the replication of incubator facilities, including an autoclave, water filtration system, a -80°C freezer and laboratory benches. (Our previously-inoperable HPLC was recently repaired by regular volunteer Anne Corwin.) Contamination issues have been specifically addressed, including the purchase and refurbishment of a used biosafety cabinet with new HEPA filters and UV light, and the establishment of improved engineering controls and protocols. The new floorplans are also configured to separate cell culture activities from those involving yeast or bacteria, further reducing the risk of experimental cross-contamination. We anticipate completion of renovations in mid-November.

The new facility has increased office space for Research Center staff and visitors, including a conference room and offices for our CEO, CSO and Academic Coordinator.

Budget. $30,000 in 2010; $100,000 in 2011

Other Operational Matters

Research Development Committee: The Foundation's Research Development Committee (RDC) was formalized in 2010, with membership comprising Aubrey de Grey, Tyler James, Tanya Jones, Michael Kope, Sarah Marr and Michael Rae. The RDC prioritizes existing and proposed projects in the light of research priorities and available funding.

Staffing and Related Issues: Lorenzo Albanello has been joined at the Research Center by Daniel Kimbel, Max Peto, and Matthew "Oki" O'Connor. These 2010 hires provided an opportunity for us to refine the overall Foundation hiring policies, which are now in place. We are in the process of acquiring a Mountain View business licence, property and liability insurance, and Director's and Officer's insurance.

Finances: The Research Center has a separate bank account – which only receives funds from the Foundation's main account – to clarify and simplify financial management. As part of the wider processing of the Foundation's accounts, fixed assets have been inventoried, and a consumables valuation prepared.