Graduate Internship placements:

Students who are selected for interviews, based on their application, will be contacted to rank their interest in the host lab placements. At this time, they will be able to express locational restrictions.

PI	Affiliation	City	State	Associated SENS strand
Amutha Boominathan	SENS Research Foundation	Mountain View	California	MitoSENS
Hadi Rebbaa	SENS Research Foundation	Mountain View	California	RepleniSENS
Amit Sharma	SENS Research Foundation	Mountain View	California	ApoptoSENS and RepleniSENS

Amutha Boominathan (SENS Research Foundation, Mountain View, CA):

Mitochondrial are vital organelles in eukaryotes that convert chemical energy from substrates to potential energy in the form of ATP. They also play important roles in various cellular pathways such as calcium and iron homeostasis, lipid biosynthesis, and innate immunity. Mitochondrial dysfunction with age has been observed in several diseases such as sarcopenia, Parkinson's, and Alzheimer's diseases. Furthermore, inherited and acquired mutations in the mtDNA are the cause of several musculoskeletal and neurodegenerative diseases. Using a gene therapy approach, our goal is to delineate the optimal parameters required to express the 13 protein-coding genes in the mitochondrial DNA from the nucleus and restore function. We are also exploring small molecules that can modulate the turnover and removal of dysfunctional mitochondria. Our recent progress can be seen https://pubmed.ncbi.nlm.nih.gov/27596602/ and https://pubmed.ncbi.nlm.nih.gov/31981894/. Students will get the opportunity to explore either the 1) gene therapy approach or 2) the small molecule approach to restore mitochondrial function. The lab utilizes human cybrid cell lines and rodent models with specific mutations in the mitochondrial DNA to evaluate these strategies using computational techniques and molecular and cellular biology.

https://www.sens.org/engineering-new-mitochondrial-genes-to-restore-mitochondrial-function-mitosens/

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1 Cite Share
The Mitochondrial Genome in Aging and Disease and the Future of Mitochondrial Therapeutics.
Saravanan S, Lewis CJ, Dixit B, O'Connor MS, Stolzing A, **Boominathan A.**
Biomedicines. 2022 Feb 18;10(2):490. doi: 10.3390/biomedicines10020490.
PMID: 35203698 Free PMC article. Review.

2 Cite Share
Rapid enrichment of mitochondria from mammalian cell cultures using digitonin.
Dixit B, Vanhoozer S, Anti NA, O'Connor MS, **Boominathan A.**
MethodsX. 2020 Dec 23;8:101197. doi: 10.1016/j.mex.2020.101197. eCollection 2021.
PMID: 34434723 Free PMC article.

3 Cite Share
Stable nuclear expression of ATP8 and ATP6 genes rescues a mtDNA Complex V null mutant.
Boominathan A, Vanhoozer S, Basisty N, Powers K, Crampton AL, Wang X, Friedricks N, Schilling B, Brand MD, O'Connor MS.
Nucleic Acids Res. 2016 Nov 2;44(19):9342-9357. doi: 10.1093/nar/gkw756. Epub 2016 Sep 4.
PMID: 27596602 Free PMC article.

4 Cite Share
Codon optimization is an essential parameter for the efficient allotopic expression of mtDNA genes.
Lewis CJ, Dixit B, Batiuk E, Hall CJ, O'Connor MS, **Boominathan A.**
Redox Biol. 2020 Feb;30:101429. doi: 10.1016/j.redox.2020.101429. Epub 2020 Jan 11.
PMID: 31981894 Free PMC article.

Amit Sharma (SENS Research Foundation, Mountain View, CA): ApoptoSENS team investigates the interplay between the innate immune system and senescent cells. The innate immune system plays a critical role in the maintenance of tissue homeostasis and the response to cellular damage and stress. Cellular senescence, a state of irreversible growth arrest that occurs in response to cellular stress, the innate immune system has been shown to play a critical role in promoting and suppressing senescence. One of the primary mechanisms by which the innate immune system promotes senescence is activating the senescence-associated secretory phenotype (SASP), which involves the secretion of pro-inflammatory cytokines, chemokines, and growth factors. These SASP factors can act in an autocrine and paracrine manner to reinforce senescence and contribute to developing age-related diseases.

On the other hand, the innate immune system (natural killer cells, macrophages, and other immune cells) can also play a role in suppressing senescence by promoting the clearance of senescent cells through immune surveillance mechanisms. Understanding the role of the innate immune system in pathologies caused by increasing senescence burden is crucial for developing new strategies to prevent or treat age-related diseases. By manipulating the immune response to senescent cells, it may be possible to promote the clearance of these cells and prevent the development of chronic inflammation and tissue damage associated with aging. Additionally, targeting the SASP and other immune-mediated mechanisms of senescence may provide new therapeutic avenues for treating age-related diseases. We use various molecular and cellular biology assays in the cell culture and mouse model.

The major questions we are interested in investigating are the following.

1. How senescent cells contribute to systemic inflammation and how they affect NK cells and other innate immune regulators, especially in the context of cellular senescence. Others have reported an age-related decline in NK cell numbers. We have observed that NK cell cytotoxicity (NKCC) may decline with age as well, partly due to intrinsic changes in NK cells with age and partly because of extrinsic factors contributed by inflammation (inhibitory cytokines such as interleukin-10 (IL-10) and transforming growth factor-beta (TGF-β)). SASP factors can induce the expression of inhibitory receptors on NK cells, such as NKG2A and CD94/NKG2A, which can inhibit NK cell activity. Additionally, SASP factors can upregulate the expression of ligands for inhibitory receptors, such as HLA-E, on the surface of senescent cells, which can further inhibit NK cell function. We have observed that a sub-set of senescent cells can escape NKCC. A study of senescent human dermal fibroblasts found that senescent cells exhibited significant heterogeneity as subpopulations of senescent cells with distinct gene expression profiles. We are investigating if subpopulations of senescent cells are resistant to immune clearance.

2. Even though like SA b Galactosidase, $p16^{INK4A}$, $p21^{cip1}$, etc., are routinely used, these are non-specific. We have recently identified several surface biomarkers of senescence by proteomics and phage display analysis and are validating them. We are also developing chimeric antigen receptors-NK cells based on our makers to target senescent cells in aging and age-associated disease models.

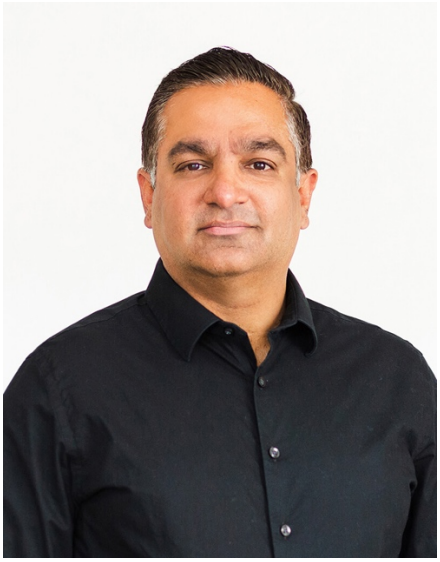
3. Understanding the mechanisms underlying senescence propagation is essential for developing strategies to target senescent cells and prevent or treat age-related diseases. In

addition, to declining immune surveillance with age, the SASP secreted by senescent cells is known to propagate senescence in an autocrine and paracrine manner. Our unpublished data indicated that the increased secretion of protein aggregates might be an important puzzle piece. We are testing whether engineered catalytic antibodies could target protein aggregates like tau oligomers in this context. With our collaborators, we are engineering an oligomeric tau antibody by replacing its light chain with a catalytic antibody to test if this recombinant tau catalytic antibody can hydrolyze tau oligomers. I am also interested in understanding how these proteins may drive systemic inflammation and a declining innate immune system.

4. Mechanism of death resistance of senescent cells can arise through various mechanisms, including upregulation of anti-apoptotic proteins, activation of survival pathways, and decreased expression of senolytic targets. For example, we have recently identified that a sub-set of SASP-secreting senescent cells can engage resistance to ferroptosis (iron-dependent apoptosis) and several senolytic drugs that target anti-apoptotic BCL family proteins. Furthermore, we demonstrated a novel senolysis approach based on the propensity of senescent cells to accumulate labile ferrous iron accumulation. In addition, we have unpublished data showing another vulnerability of senescent cells that can lead to a therapeutic intervention that does not depend on inhibiting anti-apoptotic drugs that could be much safer alternatives.

These research projects will help develop a better understanding of aging and inform novel therapeutic interventions for aging and related diseases.

https://www.sens.org/catalyzing-degradation-of-tau-aggregates/
https://www.sens.org/enhancing-innate-immune-surveillance-of-senescent-cells/



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Brauning A, Rae M, Zhu G, Fulton E, Admasu TD, Stolzing A, **Sharma A.**
Cells. 2022 Mar 17;11(6):1017. doi: 10.3390/cells11061017.
PMID: 35326467 **Free PMC article.** Review.

2 Cite Share
Role of immune cells in the removal of deleterious senescent cells.
Kale A, **Sharma A**, Stolzing A, Desprez PY, Campisi J.
Immun Ageing. 2020 Jun 3;17:16. doi: 10.1186/s12979-020-00187-9. eCollection 2020.
PMID: 32518575 **Free PMC article.** Review.

3 Cite Share
Enhanced co-culture and enrichment of human natural killer cells for the selective clearance of senescent cells.
Kim K, Admasu TD, Stolzing A, **Sharma A.**
Aging (Albany NY). 2022 Mar 4;14(5):2131-2147. doi: 10.18632/aging.203931. Epub 2022 Mar 4.
PMID: 35245208 **Free PMC article.**

4 Cite Share
Musashi expression in intestinal stem cells attenuates radiation-induced decline in intestinal permeability and survival in Drosophila.
Sharma A, Akagi K, Pattavina B, Wilson KA, Nelson C, Watson M, Maksoud E, Harata A, Ortega M, Brem RB, Kapahi P.
Sci Rep. 2020 Nov 5;10(1):19080. doi: 10.1038/s41598-020-75867-z.
PMID: 33154387 **Free PMC article.**

Hadi Rebbaa (SENS Research Foundation, Mountain View, CA): Aging is a major risk factor for chronic diseases and the ensuing functional decline of the body. The accumulation of damaged/senescent cells in in various tissues overtime is a hallmark of aging and a contributing factor to the associated diseases.One of the ressearch projects at the RepleniSENS program focuses on the discovery of novel approaches to reduce the rate of senescent cell accumulation through selective targeting for elimimiantion. We are investigating key vulnerabilities in senescent cells and leveraging them to develop a unique approach for selective elimination of these cells. The student will be participating in the validation of drug candidate selectivity toward senescent cells in vitro and in tissue specimens obtained from drug-treated and control mice. Methods such as cell culture, immunohistochemistry, qPCR, flow cytometry and western blot will be used for these validation studies.

https://www.sens.org/exploring-synergies-between-senolysis-and-stem-cell-therapy/

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Identification, mechanism of action, and antitumor activity of a small molecule inhibitor of hippo, TGF-β, and Wnt signaling pathways.
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Chu F, Chou P, Mirkin BL, Mousa SA, Rebbaa A.Aging Cell. 2008 Aug;7(4):516-25. doi: 10.1111/j.1474-9726.2008.00403.x. Epub 2008 Jul 8.PMID: 18489729 **Free PMC article.**