Yale *Fellowships and Funding*
Center for International and Professional Experience

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Dear Mr. and Ms. Leondis,

I am very pleased to be back in touch with you regarding the Stacey Leondis '08 Memorial Fellowship. It is wonderful to be able to offer this fellowship to some of our most deserving undergraduates every year.

Now in my second year as Director of Fellowship Programs, my goal is to ensure the fellowships application experience is as accessible and efficient as possible for all. I believe the process of applying for fellowships is a key part of a Yale student's education. From start to finish, the application provides wonderful preparation for their remaining years at Yale and beyond through one-on-one advising, careful proposal revisions, and interview practice. We very much look forward to working with this year's applicants later in the semester.

I hope that you will enjoy reading the enclosed report(s) for the 2017 Stacey Leondis '08 Memorial Fellowship. It is inspiring and gratifying to hear such personal accounts of the transformative experiences made possible through your generosity. On behalf of the dean of Yale College, our colleagues and—most importantly—our students, I send you this heartfelt note of thanks.

With my very best wishes,

Rebekah Westphal
Director of Fellowship Programs
Center for International and Professional Experience

Enclosure(s)

Yale College
Center for International & Professional Experience

Sreeja Kodali
Saybrook, Class of 2018
Stacey Leondis SY'08 Summer Fellowship
United States

Exploring the cancer-causing potential of ALK ectodomain mutations

Anaplastic lymphoma kinase (ALK) is a cell membrane growth factor receptor, normally involved in regulating cell proliferation. Several cancers are caused by mutations in growth factor receptors like ALK that cause growth to be signaled inappropriately – leading to uncontrolled cell proliferation and thus cancer. Mutations in the ALK extracellular domain (ectodomain) have been found in cases of acute erythroid, B-cell acute lymphoblastic, and myeloid leukemia, as well as in neuroblastoma (NB). Currently, these mutations are not understood, and are not recognized as cancer-causing. My research aims to determine whether they are, and to ask whether ALK inhibitors should be used in cancer patients with these ALK mutations.

During the academic year I developed experimental systems to investigate whether these mutations are cancer-causing in many cellular contexts. My first studies used a classic oncogenesis assay in mouse fibroblasts – focus formation assays in which cancer-causing mutations cause cells to form visible spots called foci when grown in dishes. Unexpectedly, but interestingly, my results were negative. This result suggests that the mutated ALK is not a classic oncogene. In parallel with other work in the field, it now appears that these mutations may affect more complex aspects of ALK regulation.

My current hypothesis is that, rather than just activating ALK, the mutations cause cancer by altering the association of ALK with cell-surface heparin sulfate proteoglycans (HSPGs) – which bind to the ALK ectodomain and play an important role in ALK regulation. The fibroblasts I used contain large amounts of HSPGs while cells where activation was seen did not. I hypothesize that the cancer-causing effects of ALK ectodomain mutations may only be visible in cells that have lower HSPG levels.

Additionally, if ALK ectodomain mutations weaken inhibitory interactions with HSPGs, I hypothesize that they sensitize ALK to activation by ALK-activating ligands such as augmentor-alpha. It is possible that augmentor-alpha presence is required for the ectodomain mutations to be cancer-causing – and the cells used for my initial focus-formation assays do not express augmentors.

This summer I aimed to investigate whether ectodomain mutations sensitize ALK to its ligand augmentor-alpha by measuring ALK activation at different augmentor-alpha concentrations in a dose-response curve. I also aimed to study whether ALK ectodomain mutations found in neuroblastoma and several leukemias activate ALK by reducing the inhibitory effect of cell HSPGs by testing mutations in HSPG-free cells. Additionally, I hoped to hone my biochemistry lab technique, independently plan experiments, and critically think like a scientist while trouble-shooting my experimental design.

At the end of the summer, I developed several neuroblastoma cell lines that stably expressed ALK ectodomain variants, which can be used to reliably investigate whether ectodomain mutations sensitize ALK to activation by augmentor-alpha. I also developed several HSPG-free cell lines stably expressed ALK ectodomain variants, which can be used to reliably investigate the effect of HSPGs. I honed a protocol to treat cells with augmentor-alpha and subsequently harvest and lyse them to visualize activation induced by augmentor-alpha. Creating stable cell lines and visualizing ALK expression and activation in the cell lines posed more challenges that expected, including cell contamination, low cell viability, and low cell transfection

efficiency. This summer I created the systems and protocols that are needed to produce data, and I will be able to complete the experiments this fall.

Through this summer experience I gained deeper insight into biochemical research, which is important to me as a molecular biophysics and biochemistry major and aspiring physician. I learned that communicating with mentors, planning lab work carefully, and recording meticulous records help immensely. I developed an expertise in mammalian cell culture work, harvesting mammalian cells, and western blots. I designed experiments to minimize room for technical errors and to include positive and negative controls that ensure assays worked as expected. I critically reexamined experimental design to identify issues and optimize design. I learned that producing high-quality data takes time and that resilience is an important trait in scientists.

In the future I hope to build on this experience academically by completing my experiments this fall, starting a new project that exposes me to new biochemical techniques, and taking more biochemistry courses. This experience confirmed my interest in biomedical science and my desire to go to medical school.

I am incredibly grateful to Steve and Lynn Leondis and John and Irene Gruska without whose help this experience would not have been possible. Thank you very much.

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Christine Xu
Saybrook College, Class of 2018
Stacey Leondis '08 Fellowship
Stanford, CA, USA

Stem Cell Research at the Stanford Institute for Stem Cell Biology and Regenerative Medicine

With the support of the Stacey Leondis '08 Fellowship, I was a research intern this summer at the Stanford Institute for Stem Cell Biology and Regenerative Medicine. Continuing from my research last summer, I worked on a project to differentiate human embryonic stem cells (hESCs) into neural progenitors of the midbrain. The developing midbrain gives rise to several key regions of the brain, including the dopaminergic neurons of the substantia nigra that are lost in Parkinson's disease. We hope that our work will accelerate possibilities of transplantable stem cell-derived progenitors for treating neurodegenerative disorders.

As an incoming senior, I hoped that this summer experience would be formative in shaping my research ideas and future career goals. I am applying to medical schools and plan to become a physician-scientist in the future. This summer, my goals were to challenge myself to develop my independent research skills, gain a clearer idea of my research and clinical goals in the future, and to aid my mentor in the establishment of his new lab group by working closely in our team.

My mentor, Dr. Kyle Loh, graduated last year after working as a PhD student in the lab of Dr. Irving Weissman. With a recent appointment to the Stanford faculty, he has been leading our team in the Weissman lab to form an independent lab group. I took part this summer—an exciting time for us—as our team began to define our goals and establish the foundation for our new lab. Working in this close-knit group, I learned about collaboration and leadership. I learned about the career path of a researcher, and what it takes to shape one's research interests into concrete goals and plans.

As for my personal development, I wanted to challenge myself to conduct research with a new level of independence and vision. Having worked in this lab the previous summer and collaborated remotely throughout the academic year, I was already comfortable with the techniques used in our group and the science behind our work. However, I wanted to not just understand but to also lead a project with my own insight. Previously, our lab had characterized the differentiation of hESCs into progenitors of different regions of the brain. This summer, I proposed a project to focus on the midbrain, given my interest in this region and especially in producing dopaminergic neurons, which could someday be useful in stem cell therapies. I characterized the function of signaling pathways, such as Hedgehog and Wnt, that pattern the cells of the developing midbrain. I generated progenitor cells of the ventral midbrain, which later in development gives rise to dopaminergic neurons.

This lab experience has informed my ideas of what my future work may involve. Working on this project as Stanford has been hugely inspiring to me, and I want to continue stem cell during medical school and perhaps after that during my career. During my undergraduate years at Yale, I have been volunteering with a program at Yale New-Haven Hospital called Elder Horizons, which aims to promote mental health and recovery

among patients over 70. I worked with many patients who suffered from neurocognitive disorders such as Alzheimer's disease. I saw the immense toll that these disorders could take on patients. Through my research, I am beginning to understand how research could lead to better treatments for these patients. Someday, I would like to work directly with patients, perhaps in neurology, as well as pursue research that complements my clinical work. With a greater understanding of how my clinical and research goals overlap, I am ready to pursue the next step of my career.

I would like to thank my mentors at Stanford, especially Dr. Weissman for welcoming me into his lab last year, and Dr. Loh for his continued mentorship and guidance. In addition, I am grateful for the generous support of Yale fellowships that have made this experience possible for me. I truly appreciate having had these incredible opportunities during the past two summers. Having learned through my experiences, I feel prepared and excited to pursue my future after Yale.

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Aviva Rabin-Court
Saybrook College, Class of 2019
Stacey Leondis SY'08 Summer Fellowship
USA

Research Fellowship in Dr. Shulman's Metabolism Lab

One of the first things that I learned about research is that it never moves at the pace that you expect it to. When you think your experiments will be quick, boring, and confirm your hypotheses, they yield only bewildering data points, unexpected trends, and new paths of investigation. When you expect a project to stretch from the time that you apply for a fellowship to the time that you receive it, it invariably wraps up in a couple of weeks. By the summer, the caloric restriction project that I had initially submitted to the fellowship office was in its final stages, with only a few experiments remaining to appease the reviewers. The project investigated how caloric restriction reverses type two diabetes, and ultimately found that caloric restriction, and the resulting decrease in fat intake, lowers acetyl co-A and ectopic lipid content. This decrease in acetyl coA in turn suppressed PKCE translocation, improving insulin sensitivity, while upstream effects reduced the rate of gluconeogenesis. By the start of my fellowship, this story was old news; we had moved on to adjacent questions. How does starvation shift metabolism? What happens when caloric restriction is pushed further? How does the body limit proteolysis and ensure that fuel remains in circulation for the brain?

The manuscript for the starvation project was already in progress when I arrived—it was a behemoth. To discuss it in full here would be a fitting description of my summer, as I spent a lot of time staring at the figures in bemusement and, finally, comprehension. In it, Dr. Shulman and Dr. Perry take on many Great Men of Science--there's some small spat with Krebs, which is all in a day's work in the Shulman Lab. The central question of the project was how the body transitions from glucose to fat metabolism during starvation. The reigning theory was that this transition was mostly controlled by insulinopenia; however, we found that hypoleptinemia was necessary in addition to insulinopenia. Further investigation revealed that it was through activation of the hypothalamic-pituitary-adrenal axis that hypoleptinemia resulted in increased rates of lipolysis and hepatic acetyl co-A content.

Working on this project, I tried to review a diagram of the pathway we had uncovered fairly often, in order to recall the larger picture. I spent most of my days focused on a very small piece of a figure: I spent one week on a single paragraph of the behemoth, preparing samples of white adipose, brown adipose, muscle, brain, heart, liver, and kidney from various rats for alanine and glutamate extractions, so that we could analyze the samples to find out the pyruvate dehydrogenase to citrate synthase flux in each tissue. This ratio is a readout of how much the tissue is relying on glucose or fat; in the starved animals, reduction in glycogenolysis was associated with an increasing reliance on fat oxidation. Essentially, starved animals ran out of glycogen and shifted to fat. Leptin replacement studies suppressed corticosterone, thereby suppressing lipolysis and reversing this physiological response.

Although I remain in awe of much of what I learned in the lab (including some truly hair-raising gossip about Banting & Best), I am possibly most grateful for the chance to see how one question spirals into the next. In the last couple of weeks of the starvation project, we began to look into a related problem: SGLT2 inhibitors. SGLT2 inhibitors, or Dapagliflozin, Canagliflozin, and Empagliflozin, as they are commercially known, are used to treat both type 1 and type 2 diabetes. They lower glucose levels by inhibiting sodium-glucose

transporter 2, causing more glucose to be excreted in urine. They have a very dangerous (and deeply weird) side effect—they can cause euglycemic ketosis. Diabetic ketoacidosis is a serious complication of diabetes, in which the buildup of ketones in the bloodstream cause vomiting, dizziness, confusion—if left untreated, it can be fatal. In patients who are using SGLT2 inhibitors, DKA can go unnoticed because it occurs with normal blood sugar. In addition to making SGLT2 inhibitors a clinical conundrum, euglycemic DKA makes SGLT2 inhibitors a source of considerable bewilderment to metabolic researchers. Ketosis is generally caused by high blood sugars; what would cause it to occur with normal blood sugars?

Our first hypothesis was that it was the old starvation pathway causing havoc. Dapagliflozin was clearly lowering blood sugar too much, causing regulation through the HPA axis to kick in, increase lipolysis and thereby drive dangerous amounts of ketogenesis. This hypothesis was a little rickety, because it relied on variation within a normal blood glucose range to be sufficient to interfere with the HPA axis. Sure enough, our initial infusion studies showed insufficient drops in blood sugar to drive our theoretical pathway, even with suprapharmacological doses of Dapagliflozin. Upon processing the samples for ketone turnover—grasping at straws with little hope of an answer—we found a huge increase in ketone turnover in the experimental group, far too wild to be explained by the miniscule differences in blood sugar. Some other thing must be driving this increase in ketogenesis—could it be hypovolemia, increasing norepinephrine and thereby interfering in this same HPA axis pathway?

This question was approximately where we left off—I look forward to returning to the school year and finding out the answer. I'm very grateful, as a (hopeful!) future clinician, to have the chance to work in the behind the scenes of pharmacology, and see how the problems that will confront me later in clinical settings are farmed out and researched. I would like to thank Yale College and the selection committee for granting me the Stacey Leondis SY '08 Summer Fellowship—Saybrook once again helping me live my dream!--and for giving me the opportunity to participate in truly incredible research with some inspiring people this summer. I learned a lot—both about the Krebs cycle and about how to ask questions—and I hope to build upon my work this summer during the school year.

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Elizabeth Scheibe
Saybrook, Class of 2019
Stacey Leondis SY'08 Summer Fellowship
Paris, France

Mammary Gland Research in Paris, France

Before setting foot in the Fre lab at the Curie Institute for Cancer Research in Paris, France, I had two broad goals. The first had a purely scientific focus: I planned to participate in the research of mammary gland stem cells, and more specifically, to conduct experiments to elucidate the role of the NOTCH signaling pathway in mammary stem cell differentiation, proliferation, and tumorigenesis. My second goal was less concrete: to envelop myself in a lab environment that was not only culturally distinct from the labs I have worked in in the United States due to location, but also was unique in that it was overwhelmingly female-driven. In order to accomplish these goals, I lived in an apartment in central Paris for 9 weeks, within walking distance of the Curie Institute, and worked 8 weeks in the lab, from May 15th to July 15th, for an average of 35 hours a week.

The Stacey Leondis SY'08 Summer Fellowship supports summer internships and research projects for Saybrook students who train to undertake medical or biomedical research, and seeks to provide the opportunity to participate in original research, clinical experiences or other forms of hands-on experiential learning in science, and particularly medicine. Thanks to the generosity of this fellowship, I was able to undertake biomedical research in a hands-on manner. Specifically, during my time in the lab, I worked closely with Bethan Lloyd-Lewis, a lab member whose work is focused on using genetically modified mouse models and novel intra-vital imagining techniques to visualize the role of the NOTCH protein in basal and luminal mammary stem cells. Specifically, we imaged live mice over the course of pubertal development. Our goal was to track the differentiation and proliferation of NOTCH1-positive cells in mammary ductal structures and determine the role that different cell types play in the formation of terminal end buds. NOTCH1 guides the cell fate of mammary stem cells, which differentiate into either basal or luminal lineages. Previous work done by the Fre lab and others has shown that NOTCH1 appears to drive the differentiation of the luminal lineages, but until now, this has not been visualized in real-time. The intra-vital confirmation of the previous findings of the Fre lab has important implications in the identification of breast cancer subtypes based on cellular origin. Additionally, the intra-vital tracking of mutant mouse lines will allow for a deeper understanding of pathogenic and tumorigenic mammary stem cell development.

In order to view the cells develop in the same mouse over the course of several days, we surgically placed windows over the right 4th and 5th mammary glands of pubertal mice. To optimize image quality, we developed a regimen of anti-inflammatory drug doses. Because intra-vital imaging of the mammary gland in pubertal mice is a new technique, I was able to experience firsthand the challenges, setbacks, and troubleshooting improvisations that form a critical, but often overlooked part of the scientific process. Rather than becoming frustrated or discouraged, I witnessed Bethan's flexibility and creativity; this gave me confidence to try new strategies while working on my own project.

Independently, I sought to identify the pathological profile of a mutant mouse with constituently active Wnt pathway signaling, which leads to the buildup of excess beta-catenin, and has been associated with a wide variety of conditions including skin cancers and epidermal hyperplasia. In order to achieve this, I was trained in cryosectioning, immunostaining, and confocal microscopy, which were all new techniques for me. Because the

lab had not previously stained this tissue, there was no roadmap for success—I had to learn through trial and error which antibodies worked under which procedural modifications. This process allowed me to understand the principles behind immunostaining, microscopy, and mammary stem cell development on a level that would not have been possible through classroom instruction alone, and deepened my hunger and fascination for not only these specific topics, but also scientific inquiry as a whole. This fellowship provided me with the unique opportunity to experience the thrill of biomedical research outside of the traditional bounds of a classroom, and without the associated pressures of school life.

As I alluded to previously, the lab culture provided an invaluable learning experience all its own. In contrast with certain labs I have observed in the United States, the lab ethos was one of support and encouragement, and I was able to witness others around me going through the process of setback and reassessment free from admonition or punishment; it became clear that this was an environment where failure is not only acceptable, but in fact forms as key a component of the scientific process as success does. This realization served to reassure me that research is not a brutal, thankless and lonely field, as popular opinion suggests, but rather that the research community can be tight-knit and collaborative, and is in fact the kind of community in which I can see myself in my future career. Without the generous support of this fellowship, I would not have been able to have such an eye-opening experience, and I am therefore incredibly thankful for the opportunity and support.

In addition to the supportive lab ethos, the Fre lab differs from other labs I have observed because it is overwhelmingly female-driven. Not only is the Principal Investigator a woman, but 4 out of 5 of the other lab members were women. As a young woman in science, I have frequently experienced and observed stereotypes about women in STEM at play—I have been told firsthand that I am "too fragile" to be a doctor. What I witnessed in Paris was the antithesis of this—in lab meetings and at the bench, these women fearlessly expressed their opinions and acted on their instincts without a second thought. They were inspirational to me because they were utterly themselves in the lab, unencumbered by the stereotype-driven need to appear "agreeable" rather than "bossy." Therefore, though the focus of this fellowship is biomedical research, it also allowed me to see models for my potential as a woman in science, not only today, but also in my future career.

In conclusion, I would like to extend my sincere and deep gratitude to Steve and Lynn Leondis, John and Irene Gruska, and the Saybrook College selection committee for giving me the opportunity to conduct research in Paris this summer in Stacey's memory. Without the support of this generous fellowship, I would not have had the opportunity to experience the scientific thrill and personal discovery that I was able to at the Curie. Additionally, I would not have been able to participate in cancer research that is critical not only because it has the potential to save the lives of millions of people affected by the disease, but also because it also has the power to touch the lives of the family members of these individuals. As someone who has lost three family members to cancer, I am particularly passionate about finding new treatments, and eventually a cure, for this disease.