CAMB STUDENT NEWSLETTER

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IN THIS ISSUE

LETTER FROM THE EDITORS

Dear CAMB students, faculty, and alumni:

Welcome to the first issue of the CAMB Student Newsletter. Our mission is to bring together the CAMB community by celebrating the accomplishments and interests of current and former CAMB students. Every issue of the newsletter, released quarterly, will include commentaries on recent publications by CAMB students as well as discussions with recent CAMB alumni about their transition from the program. Each issue will also include special interest articles that will cover a range of topics relevant to the CAMB community, such as interviews with new faculty members and student involvement in community outreach efforts. We have also started a blog that will include even more articles and will be updated on a regular basis. We encourage you to visit the blog at http://cambnewsletter.wix.com/blog.

We are an independent, student-run organization, and this newsletter would not have come together without our wonderful team. We thank the entire team for responding to our initial call for participants and for their ideas and hard work in putting together this inaugural issue. In particular, we would like to recognize Arwa Abbas for design of the logo, Lindsey Weed and Inny Feltzin for formatting the newsletter, and Hayley Hanby and Iryna Shakhmantsir for launching the blog. It is clear that the talents of CAMB students reach far beyond the bench. We extend our thanks to Dr. Dan Kessler and Meagan Schofer for their encouragement and enthusiasm for this project.

Current students who are interested in writing for the newsletter or the blog should contact us at cambstudentnews@gmail.com.

We hope the CAMB Student Newsletter becomes a valuable forum to recognize student accomplishments and strengthens the CAMB community for years to come.

Sincerely,

Kate Palozola and Neha Pancholi

Editors-in-Chief

RESEARCH SPOTLIGHT

Holy shift! Changes in poop mark disease progression

Neha Pancholi

Animal models of disease are crucial to understanding disease progression in humans. In a recent article published in the *American Journal of Primatology*, Hannah Barbian, a fifth-year MVP student in Beatrice Hahn's lab, exemplified this concept. The Hahn lab researches the disease progression and evolution of simian immunodeficiency virus (SIV) and human immunodeficiency virus (HIV). A long-standing collaboration with the Jane Goodall Institute and Gombe National Park in Tanzania has supplied the lab with a breadth of chimpanzee samples. The Hahn lab previously used chimpanzee samples to demonstrate that HIV-1, the etiologic agent of the human AIDS epidemic, originated from a cross-species transmission from chimpanzees into humans. Samples from Gombe National Park are especially useful because despite being wild, the chimpanzees are closely monitored, so information about their health, age, and behavior is readily available.

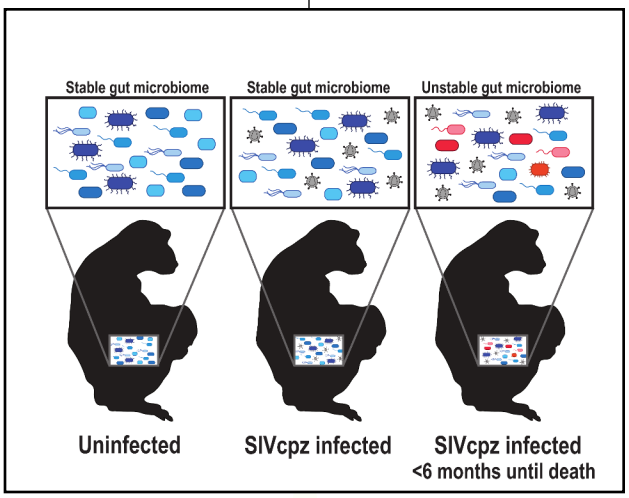
Hannah used fecal samples from Gombe National Park to investigate the relationship between SIVcpz infection and the chimpanzee gut microbiome. Understanding the effect of SIVcpz infection on wild chimpanzees is challenging because routine blood collection of wild chimpanzees is invasive and therefore unethical. This prevents monitoring of viral load or CD4 T cell numbers in infected individuals. "This project was exciting because we hoped to use the microbiome to get more clues into what is going on in SIVcpz infected animals," said Hannah. "Most markers of AIDS progression in humans are found in blood. So when studies were published showing that the human gut microbiome becomes dysbiotic with HIV-1

infection, and may even be linked to disease progression, we were really excited to apply this technique to our chimpanzee fecal samples."

Hannah Barbian, MVP

Hannah and colleagues isolated nucleic acids from chimpanzee fecal samples and used multiple approaches to identify the constituents of the gut microbiome. Metagenomic sequencing identifies all organisms within a sample, including bacteria and viruses, but it requires large quantities of fecal material. Since fecal samples are valuable and limited, only a few samples were analyzed by metagenomic sequencing. There were no statistically significant differences between the bacteria identified in samples from SIVcpz-infected and uninfected chimpanzees. However, samples from all infected chimpanzees had greater amounts of bacteria from the *Prevotellaceae* family.

The authors also identified bacteria from fecal samples with 16S ribosomal RNA sequencing. Unlike metagenomic sequencing, this approach requires little starting material, which allowed the use of a much larger pool of samples from the past fifteen years. Results from 16S rRNA sequencing were similar to those obtained from metagenomics sequencing: the gut composition of infected chimpanzees was not significantly different than that of uninfected chimpanzees, but *Prevotellaceae* was again enriched in infected samples. Analysis of the virus composition showed an abundance of Chimpanzee Stool Associated Circular Virus and Chimpanzee Adenovirus in both uninfected and infected chimpanzees.



Changes to the gut microbiome of SIVcpz-infected chimpanzees are observed shortly before AIDS-related death. SIVcpz infection does not dramatically affect the composition of the gut microbiome during earlier stages of disease. (Illustration by Hannah Barbian)

Interestingly, there was a dramatic difference observed in samples from infected chimpanzees that were collected shortly before their AIDS-related deaths. The bacterial composition of these samples was significantly different from uninfected chimpanzees and from infected chimpanzees that died of other causes. The samples collected close to death were also significantly different from samples collected from the same individuals at earlier time points.

These results demonstrate that there is a drastic shift in the composition of the chimpanzee gut microbiome shortly before an AIDS-related death. The authors comment that this is likely due to the decline of effective immune responses.

The microbiome changes observed at the end stages of SIVcpz infection have implications for future SIVcpz studies and for chimpanzee health. Monitoring infected chimpanzees for a shift in the gut microbiome could serve as an indicator of upcoming rapid disease progression that could prompt close observation of the chimpanzees to learn more about the final stages of AIDS. Identifying chimpanzees that are close to death could even prompt medical intervention if necessary. Beyond informing SIVcpz pathology, Hannah's studies also have implications for HIV-1. Previous studies have shown that HIV-1 infection, like several other infections, is correlated with changes to the gut microbiome. Since antibiotic and antiretroviral use is common in HIV-1-infected patients, it is unclear whether the observed alterations are directly due to HIV-1 infection. Wild-living chimpanzees, therefore, provide a suitable model system to study the impact of retroviral infection on the primate gut microbiome in the absence of antibiotic and antiretroviral use. Since Hannah and colleagues found that infection of wild chimpanzees did not significantly affect the gut microbiome until close to death, the previously reported effects of HIV-1 infection on the human gut microbiome are likely indirect effects of antiretroviral and antibiotic use.

Hannah continues to use fecal samples from Gombe National Park in her thesis work to study SIVcpz progression. She is currently analyzing recently collected fecal samples for new and existing SIVcpz infection and is developing more sensitive assays to sequence and quantify RNA from fecal samples.

Barbian, H., Li, Y., Ramirez, M., Klase, Z., Lipende, I., Mjungu, D., Moeller, A.H., Wilson, M.L., Pusey, A.E., Lonsdorf, E.V., Bushman, F.D., and Hahn, B.H. Destabilization of the Gut Microbiome Marks the End-Stage of Simian Immunodeficiency Virus Infection in Wild Chimpanzees. ***American Journal of Primatology.*** **2016.**

The fluidity of roles of epigenetic regulators

Gleb Bazilevsky

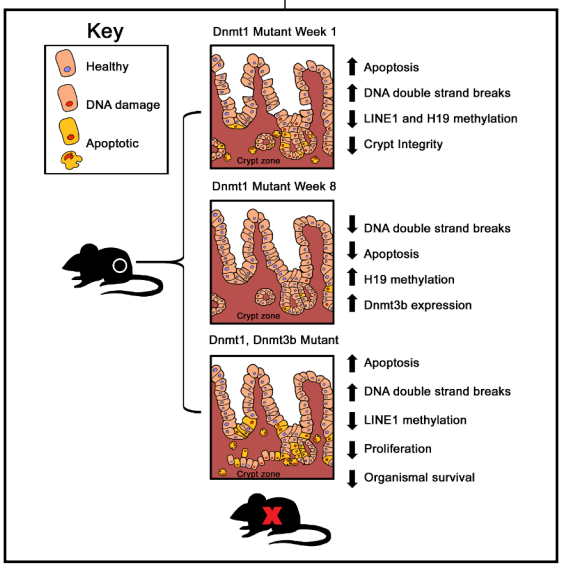
The Cell and Molecular Biology (CAMB) program spans a daunting breadth of biological inquiry, with students and investigators participating across six dynamic and complementary programs. This diversity is exemplified by the research of Ellen Elliott, recent alumna of the Developmental, Stem Cell, and Regenerative Biology (DSRB) program and the Kaestner laboratory. Her work integrates the methods and concepts of regenerative biology, organismal development, physiology, and epigenetic regulation to investigate the role of DNA methylation in mammalian intestinal epithelia. In the January 2016 issue of *eLife*, Ellen and colleagues present that the DNA methyltransferase Dnmt3b can rescue detrimental DNA hypomethylation and stalled stem cell maturation caused by the loss of the methyltransferase Dnmt1 in adult mouse intestinal epithelia.

Ellen's work is a product of the Kaestner lab's efforts to understand the pathways of liver, pancreatic, and intestinal organogenesis using cell culture and mouse models. The lab uses these methods in part to study the contribution of DNA methylation to gene regulation. DNA methylation is a heritable covalent modification of eukaryotic DNA that some organisms use to differentially regulate maternally and paternally derived chromosomes. This modification is essential for the timely, dosage-controlled expression of many genes, especially those genes involved in embryogenesis and development. These genes are methylated by two classes of methyltransferases, each with distinct targets and mechanisms of action. One class consists of the 'maintenance' methyltransferases Dnmt3a and Dnmt3b that faithfully copy the methylation patterns of parent DNA strands onto daughter DNA strands during semi-conservative DNA replication. The other class consists of the 'de novo' methyltransferase Dnmt1 that can lay down new methyl marks on DNA without a pre-existing template. The loss of either class of

Ellen Elliott, DSRB

methyltransferases can lead to the loss of epigenetic information and can be fatal to actively-dividing cells, including embryonic stem cells.

Over the course of three publications, Ellen delves into the role of the Dnmt1 methyltransferase in the development and operation of the 'crypt' stem cells that replenish the mature cells constituting the intestinal villi. She proposes a novel relationship between the two methyltransferase classes by elegantly demonstrating that adult mouse crypt cells can overcome the loss of Dnmt1 by adapting and repurposing Dnmt3b. This compensation explains the foundational observations that Dnmt1 ablation only transiently affects the mature intestine, while being absolutely necessary for perinatal crypt maturation. Conditional Dnmt1-null adult intestines experience expansive hypomethylation at LINE1 and H19 elements and a global increase in DNA damage. However, these intestinal Dnmt1-null cells can survive and proliferate outwards, unlike in other actively-replicating tissues, where the Dnmt1 deletion is lethal. Contrary to expectations that Dnmt1 loss would arrest cell growth, the conditional knockout crypts even appear to proliferate outwards at an accelerated rate. The result is striking. The crypt cells amass without being able to differentiate into mature epithelial cells. Yet, the mice can survive the Dnmt1deletion and recover wild-type crypt cell functionality. Within a week, Dnmt3b (though not Dnmt3a) mRNA and protein levels increase to compensate for the loss of Dnmt1, with a return to control levels of DNA methylation. Double mutants for Dnmt1 and Dnmt3b are not so fortunate. The double conditional knockout mice are severely morbid in 60% of cases, with decimated epithelial integrity, demethylated LINE1 and H19 loci, and significant DNA damage. The remaining 40% are only able to survive through the sporadic escape of crypt cells from Cre-recombination, repopulating the crypt zone with Dnmt3b-positive cells. Dnmt3b acquires added import and functionality as it compensates for Dnmt1. Control experiments show that Dnmt3b depletion alone does not affect crypt functionality, and that Dnmt3a cannot compensate for the Dnmt1/Dnmt3b null mice. Moreover, constitutive or inducible Dnmt3a or 3b loss in the intestinal epithelium by itself has no discernable phenotype or effect on viability.



The 'de novo' DNA methyltransferase Dnmt3b compensates Dnmt1-deficient intestinal epithelium.
Top Panel: Intestinal epithelium-specific ablation of the maintenance DNA methyltransferase Dnmt1 in adult mice leads to abnormalities in tissue morphology. Loss of Dnmt1 increases DNA double stranded breaks and cell apoptosis as determined by increases in γH2AX foci and TUNEL staining. Mutant crypt epithelial cells show decreased methylation at LINE1 retrotransposons and the H19 imprinting control. **Middle Panel**: The intestinal epithelium can recover from Dnmt1 ablation. Within 8 weeks Dnmt1 mutant cells resemble healthy controls in morphology, levels of DNA double stranded breaks and apoptosis. Methylation at H19 but not LINE1 is also restored. Mutants have specifically increased expression of the DNA methyltransferase Dnmt3b. **Bottom Panel**: Tissue-specific deletion of both Dnmt1 and Dnmt3b is severely deleterious to the intestinal epithelium. Double mutants have grossly abnormal epithelium including increased crypt cell apoptosis, decreased DNA methylation at H19 and LINE1 loci and extensive DNA damage. The double mutant cells do not recover the control phenotype over time. (Illustration by Arwa Abbas)

Of course, Ellen's work is not without unanswered questions. She acknowledges that her group does not understand the mechanism by which Dnmt3b can properly methylate the Dnmt1 targets – if Dnmt3b manages to remethylate hemimethylated DNA or if it waits until the DNA is entirely demethylated before restoring the wild-type methylation pattern through another mechanism. "It would be interesting to see where the methyltransferases are binding," says Ellen. She hypothesizes that Dnmt3b might assume Dnmt1 binding sites and protein interactions as it compensates for Dnmt1 loss. Ellen also hopes that other specialists of intestinal cell biology will investigate the source and specific loci of the DNA damage occuring after Dnmt1 ablation. The answers to these questions might complement one another, as the damaged loci might be reconfigured targets for Dnmt3b.

This work by Ellen and colleagues demonstrates the fluid roles and relationships of epigenetic factors in developing and adult cells, clearing further ground for the research of intestinal cell biology and organogenesis on the whole. A better understanding of these processes and mechanisms has the potential to redefine current dogmas about DNA methylation and present novel strategies for the treatment of developmental and proliferative disorders in the intestine and other organs.

Ellen Elliott is currently studying the genetics of immune-cell lncRNAs as a post-doc at the Jackson Laboratory in Farmington, Connecticut.

Elliot, E.N., Sheaffer, K.L., and Kaestner, K.H. The 'de novo' DNA methyltransferase Dnmt3b compensates the Dnmt1-deficient intestinal epithelium. *eLife*. 2016;5.

SPECIAL INTEREST: BOOK REVIEW

A Brief History of Creation: Science and the Search for the Origin of Life

By Bill Mesler and H. James Cleaves II

Virzhiniya Feltzin

How did life first appear on Earth? What can the origin of life tell us about life's meaning? In their new book "A Brief History of Creation", journalist Bill Mesler and organic chemist H. James Cleaves II offer a thorough and compelling narrative of the history of man's search for answers to questions as old as mankind itself.

The book begins with an interesting – though perhaps overly detailed – account of early religious and philosophical beliefs on life's creation, and then proceeds to examine contemporary scientific theories on the origin of life, making a case for why the scientific method is the best approach to tackle this question. It ends with a discussion of how the quest to understand the origin of life portrays the nature of science itself, the character of a scientist, and the challenges major theories and discoveries face before they are adopted by the general public. The authors include an appendix with several protocols for spontaneous generation of life, and all CAMB students are encouraged to attempt to replicate these in their lab.

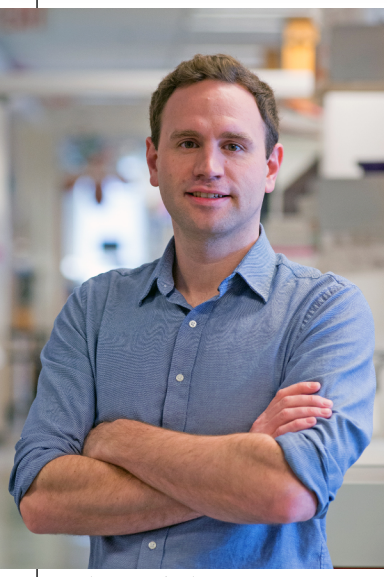
Researchers of all disciplines who are interested in the history of scientific discovery will enjoy the book's analytical depth and the abundance of entertaining historical detail, such as the fact that Aristotle ran a zoo as part of his Lyceum, or that Stanley Miller was an informant to the CIA on origin-of-life discoveries in Moscow during the Cold War. At the same time, the lively writing style and the way in which the authors humanize the scientists involved will appeal to a general audience.

"A Brief History of Creation" is available in hardcover, electronic, and audiobook formats from all major online and local retailers. The book will be released in paperback format on December 6th, 2016.

Welcoming New-CAMB-ers:

A Faculty Profile on Matthew Good, Ph.D

Francesca Tuazon



Matthew Good, Ph.D,
Assistant Professor of Cell and Developmental Biology

Matthew Good, Ph.D., is a recently appointed Assistant Professor in the Department of Cell and Developmental Biology at the Perelman School of Medicine with a secondary faculty appointment in the Department of Bioengineering at the School of Engineering and Applied Science. In addition to being part of the CAMB graduate group, Dr. Good and his lab are members of the Bioengineering graduate group and the Pennsylvania Muscle Institute (PMI).

Dr. Good completed his Ph.D at the University of California, San Francisco as a member of Dr. Wendell Lim's lab. There, Dr. Good utilized *in vitro* reconstitution of MAPK signaling to study how information is faithfully transmitted through intracellular kinases networks that share many components. His work uncovered a new role for scaffold proteins as critical 'hubs' for dictating the flow of signaling information. Furthermore, he was inspired to pursue techniques that move beyond a test tube and mirror the spatial and boundary conditions of a cell.

For his postdoctoral fellowship at the University of California, Berkeley, Dr. Good worked with Drs. Daniel Fletcher and Rebecca Heald to pursue his fascination with the plasticity of subcellular structure size. Dr. Good combined the approaches of his co-advisors: *in vitro* reconstitution of the mitotic spindle from *Xenopus* egg extracts (Heald Lab), but in cell-like compartments to modulate physical constraints (Fletcher Lab). In Dr. Good's own words, he wanted to "marry cytoplasmic extracts with microfluidic encapsulation to test the hypothesis that cell size and shape directly regulate the assembly, scaling, and function of subcellular structures." He found that cytoplasmic volume, as opposed to a hard-wired developmental program, regulates spindle size during embryogenesis.

Currently, the Good Lab continues to focus on the role of cell size (volume) and shape (geometry) as master regulators of cell function utilizing *Xenopus* egg extracts and synthetic cells. The lab also uses *in vitro* fertilization of *Xenopus* eggs to study how the rapid reduction of cell size during early embryogenesis regulates the size of organelles and other subcellular structures. When asked what excites him about his research, Dr. Good responded, "I love exploring new areas of science and creating a fuller or more detailed description of nature… I am thrilled … to be working in an era in which researchers are able to engineer new molecular and cellular functions….we can now…tease apart cause and effect."

UPenn welcomed Dr. Good in January 2015 and when asked about the biggest differences between being a faculty member, postdoc, and graduate student, Dr. Good replied, "Time and freedom. Much less time, much more freedom and manpower to explore ideas. Honestly, though, I still feel like a graduate student in professors' clothing." However, between his candor and success both in and outside of the laboratory (a full list of awards can be found on the Good Lab's website), Dr. Good is an inspiring role model and keen advisor for current graduate students. For students who are unsure of staying in science, Dr. Good astutely observes that "problems in science are solved on a timescale of months to years. This…is not a great fit for everyone."

For students who are looking to continue in science and are looking for postdoc labs, Dr. Good advises, "Be ambitious – stretch yourself and do not look for a position that is comfortable. Bring a unique skill to a postdoc lab so you can address an outstanding question in a new way. Pick an up-and-coming mentor so that you can be part of their scientific growth process. Make sure the lab is a place where you will want to come to work everyday. Push yourself, be fearless."

For more information on Dr. Good and his lab, please visit his lab's website (http://www.buenoscience.org/research.html). For inquiries into potential rotations, please contact Dr. Good directly (mattgood@upenn.edu).

WHERE ARE THEY NOW?

Amy DeMicco and Matthew Harms

Hayley Hanby

Amy DeMicco and Matthew Harms—CB and DSRB alumni, respectively—both work for AstraZeneca in Sweden. Amy and Matthew bring interesting perspectives about the transition from academia to industry, finding jobs with a significant other, and living and working abroad.

Amy completed her PhD in Craig Bassing's lab (July 2015), studying how specific gene expression programs promote both normal lymphocyte function and suppression of malignant transformation. Matt completed his thesis and a brief post-doctoral fellowship in Patrick Seale's lab. There, he investigated the role of a transcription factor, PRDM16, in the identity and function of brown adipose tissue. During his job search, Matt became aware of open postdoctoral positions at AstraZeneca. The metabolism group of AstraZeneca is based in Gothenburg, Sweden, so Matt knew he would have to relocate if he was offered the job. Big companies sometimes help place significant others, so since Amy was close to graduating, AstraZeneca passed around Amy's application to their various research divisions. At the time, Amy hoped that she would get the job at AstraZeneca, but she made sure to set up a few back-up plans, which included interviews for academic postdocs at local universities in Gothenburg. Someone in the department of respiratory and autoimmune diseases at AstraZeneca saw

Amy's experience with B cells and flow cytometry and asked her to interview for a senior research scientist position. Matt ended up accepting a postdoc position at AstraZeneca, and Amy joined as a senior research scientist.

Many people who move to a foreign country for work go through two phases of emotions: initial excitement and interest, then a realization of being a bit out of place. Amy and Matt were no exception, especially during this past holiday season since they stayed in Sweden for Christmas. But things are getting better. To combat the "fish out of water" feeling, Amy and Matt started taking Swedish lessons. Amy mentions that all Swedes speak English well, but only if they need to; most Swedes assume you speak Swedish since you're living in Sweden. Matt echoed these sentiments, claiming, "Going to the doctor is slightly different. Understanding pension is slightly different." One advantage of living in Europe, which perhaps alleviates the feeling of being isolated in a single place, is the accessibility of other countries. Matt said that a recent trip to London only cost them 30-50 USD roundtrip. Ultimately, Amy and Matt see this as a positive experience—as an opportunity for character development and as a chance to explore Europe.

If you're wondering what day-to-day life is like for a post-doc and staff scientist at AstraZeneca, both Amy and Matt admit that it's remarkably similar to what they did in graduate school, which isn't surprising. What is quite different to Matt is the work environment. As a post-doc, Matt is one of 30 on the AstraZeneca campus out of a total of 2,000 people on site. Aside from being in the exceptional minority, Matt says that the mindset of a pharmaceutical company is a paradigm shift for him: everything is on a massive scale—tens of thousands of compounds are screened per project—and the questions and priorities that pharmaceutical research have are very different from academic research. As a small molecule company, AstraZeneca is earnestly trying to develop the best, safest drugs for the market. Amy's position as a senior research scientist is more product-driven. She has less freedom, compared to Matt, because, as she says, "You do what the project wants you to do." While Matt is trying to publish, Amy is contributing to a team that's trying to get a product out into the market. As for long-term career goals, before moving to Sweden, Amy was interested in intellectual property and patent law. She hopes to transition to a position within AstraZeneca that would give her more relevant experience in perusing those original goals. Matt's end goal is to move away from the bench and gain more of a leadership role to direct projects himself. Disclaimer: This goes without saying, but what is true at one company may not be true at another. Amy and Matt's only experience with pharma is in Sweden, so they didn't want to generalize about this situation in other countries like the US.

Amy DeMicco, CB and Matthew Harms, DSRB

To find out what Amy and Matt's advice to current students looking for post-docs in Europe or positions in industry is and more, visit the CAMB Student Newsletter blog at http://cambnewsletter.wix.com/blog

Aleksandra Nall

Katherine Palozola

Aleksandra Nall, GGR

Aleksandra (Leksa) Nall was a GGR student in Amita Sehgal's lab, where she used small molecules to study sleep regulation in *Drosophila*. She defended her thesis in July 2014 and was hired as a medical writer at Articulate Science the following October.

Located in Hamilton, New Jersey, Articulate Science is a premier medical communications agency division of Nucleus Global. Medical communications agencies provide a variety of services to a diverse range of clients, but are most often utilized by pharmaceutical, biotech, and medical device companies. The agencies design and deliver customized products to be used for patient education, sales representative training, marketing, consulting, and continuing medical education. Medical writers at most agencies, including Articulate Science, work in teams under a project manager. As a medical writer, Leksa's responsibilities include communicating the basic science of a specific disease state, a drug mechanism of action, or interpretations of clinical data in lay language. After a year as a medical writer, Leksa was promoted to a senior medical writer, and her responsibilities have grown to include more responsibility and mentorship roles.

Her immediate transition from the bench upon completion of her degree naturally involved many changes in her day-to-day life. Since leaving academia, she notes that she is still surrounded by a great group of fellow PhDs and gets to attend the occasional scientific conference, allowing her to remain connected to science However, she no longer has the flexible schedule that she enjoyed so much in graduate school. Leksa admits the main downside to her new life is the 7AM wakeup call and the commute from her row home in Center City. However, the transition from academia to agency has been accompanied by a welcome increase in compensation to temper this hardship.

Leksa's advice to current students considering a job outside of academia is to take breaks from lab work and pursue activities that develop other skills. For example, do something in which you can interact with other people, develop leadership skills, or cultivate a passion. Having hobbies doesn't necessarily mean decreased productivity — Leksa completed her degree in 5 years and published two first author papers and a review article. Despite the pressure that students face to spend all of their time at the bench, they will be well-served by having experiences and accomplishments outside of science. To this end, Leksa's leadership experience included being the captain of her ultimate frisbee team and organizing the Penn Med Art Show. Being able to speak to these types of experiences during interviews was essential to successfully landing a job. Leksa also emphasized the benefit of the many opportunities within CAMB to give research talks and learn to clearly and concisely communicate the significance of research, the logic of experimental design, interpretation of the results, and the implications for the field. These skills will be important no matter what career you pursue.