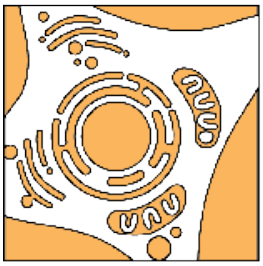
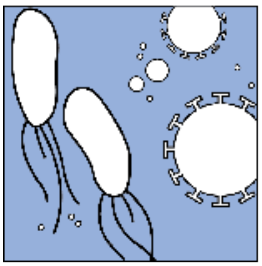




CAMB Student Newsletter

Volume 7 | Issue 5 | February 2023

In this issue

Letter from the Editors

Dear CAMB Students, Faculty, and Alumni,

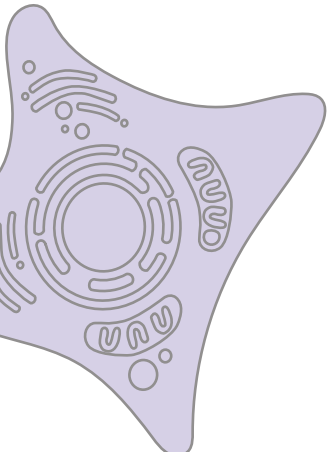
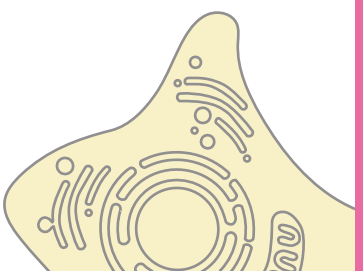
We are excited to share with you the February installment of the CAMB Student Newsletter. In our opening issue of 2023, we hear from GTV alumnus Dr. Alex Martino about his transition to an industry position at Biogen in Boston after graduation. We also sit down for a conversation with Dr. Cornelius Taabuzuing, who joined Penn's department of Biochemistry and Biophysics in early 2022. Finally, join us for an exploration of recent CAMB GTV graduate Dr. Edward Song's work on enhancing CAR T cell therapy in glioblastoma.

For additional articles, past publications, and to learn more about the CAMB student Newsletter team, visit our blog at cambnewsletter.wix.com/blog or follow us on Twitter @CambNewsletter. Current students interested in contributing to the CAMB Student Newsletter can complete our volunteer interest form (here). We hope you enjoy the February 2023 issue!

Sincerely,

James Gesualdi and Kay Labella

Editors-in-Chief

Alumni Spotlight
Dr. Alex Martino

Mara Davis

Peer Edited by Sonresa Ochoa-Vidales

Interested in pursuing a career in industry after graduate school? Read on to hear from one of CAMB's Gene Therapy and Vaccine alumni, Alex Martino, who recently graduated from the Wilson Lab and is currently at Biogen! Biogen is a biotechnology company in Boston with a focus on developing therapeutics for patients with neurological diseases.

Dr. Alex Martino

Can you tell me about your role in your current job at Biogen and how it differs from the role you had as a Ph.D. student?

The main difference is focusing on capsid development for humans for therapeutics versus during my Ph.D. I was focusing on principles necessary for the field at large.

Here, there's less focus on publishing and more on developing specific products, but largely it's pretty much the same - I'm trying to engineer blood brain barrier-penetrant AAV capsids.

What drove you to pursue industry?

In high school, I loved science. I shadowed M.D.'s early on and I realized I'd rather be the person making therapies than delivering them. Before starting grad school, I worked in drug discovery… and it was always the plan to go back into a scientific management kind of position.

I got a taste for academic life during my Ph.D. and what goes into making a paper - It's very important work and I think a lot of us in industry are waiting for these papers to come out to find where to take research next, but there's a lot of competitiveness for grants and getting publications out before others in the field.

I decided that I wanted to get back to making therapeutics. Academia was tempting, but I wanted to work on trying to make a pharmaceutical product over publishing papers.

How did your time in graduate school help set you up for your current position?

Here, there's check-ins every quarter to monitor projects and determine if they're going to continue or not. I had a lot of training for that; my lab had regular meetings and lab weekly check-ins. That made the transition a little easier for me.

The thing I think will help the most is being on top of the literature. In order to write a paper, you have to know about everything that is going on in your field in order to make sure you don't miss anything relevant to your paper. I think going into industry, it's easy for people who have been here for a while to focus on their project and not pause to look around. The biggest thing that helps from grad school is forcing yourself to be on top of the literature and what is going on in the field - that

helps in industry when projects are initiated and you're deciding the next best way to go.

What was it like applying for jobs while preparing for your defense? That sounds stressful.

Short answer - It was.

For Ph.D. positions, they have a one-hour seminar that is pretty comparable to your thesis defense, so some of that aligned.

People were reaching out to me months before graduation. You make friends that go off into the working world and they are always looking for more people to hire - biotech and gene therapy are particularly popular right now

For me, it wasn't too much sending out applications and resumes as it was using my network from the GTV program and talking to people from different companies and seeing what was available.

What is your best advice for a Ph.D. student looking to go into industry?

My advice to a student wanting to go into industry is: keep in touch with your friends and leverage that network to get yourself a job on the other side.

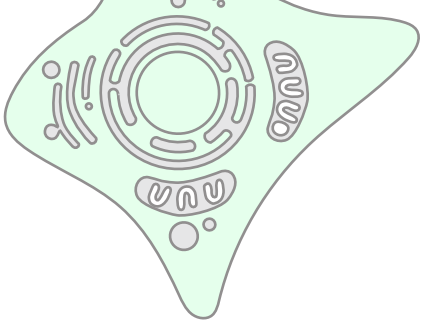
Keep up the connections with your PI and your other advisors. Reaching out to people while in grad school is going to help you down the line, and that's going to be for the remainder of your career.

So go to the conferences and make friends!

To ask Alex more about his time in industry or the program, feel free to contact him at r.alexander.martino@gmail.com or on linked in! (linkedin.com/in/ramartino)

Faculty Spotlight

Dr. Cornelius Y. Taabazuing



Sonresa Ochoa-Vidales

Peer Edited by Mara Davis

Happy Spring, readers! Please, keep reading to get to know one of our newest faculty members at Penn, Dr. Cornelius Taabazuing, who just celebrated his one-year lab-iversary! In a recent interview with the CAMB newsletter, he shared advice for incoming graduate students, his fun experiences, and a great activity for lab members to do in Philly.

What does your lab study?

My lab is interested in the molecular mechanisms of cell death. We work on two cell death pathways, 1) understanding apoptosis – a well-known cell death mechanism thought of as "silent" cell death – and 2) pyroptosis–an inflammatory defense mechanism for pathogens and host derived danger signals.

What excites you about your research?

Our immune system is essential for many functions. There are trillions of microbes out there, yet we are not constantly sick, and it is really, really fun to figure out how our immune system keeps us alive.

We are excited about understanding apoptosis – a classical form of cell death–which still has some mysteries to be learned. Most of the chemotherapeutic drugs we have function via inducing apoptosis, and it is not clear how resistance happens, so we are excited to decipher those mechanisms.

Cancer immunotherapy is another promising avenue of interest. A fun project my lab works on is killing cells in a way that wakes up the immune system, to try and develop those methods into a therapeutic.

Are you looking for rotation students?

Yes, all students are welcome. We have been fortunate to have a few rotation students already and are looking for some this spring. We are also accepting undergraduates because there is plenty of space. My ideal team consists of cancer biologists, immunologists, and biochemists; all the different disciplines are welcome.

Interested students can send me a direct email. My email, lab information, and the link to my external lab website can be found on Penn's BMB department website. When you reach out, tell me what you are interested in, and we can schedule a meeting.

What did your career path look like?

After undergrad, I took a year off and interned with Dr. Tom Cech, a Nobel Laureate, at the University of Colorado. Then, I attended the University of Massachusetts-Amherst. As a graduate student, I was a mechanistic enzymologist, spectroscopist, and crystallographer, under my advisor Dr. Micheal Knapp. My graduate work was on understanding the chemistry of the HIF hydroxylases, which regulate the transcription activator, HIF. These enzymes control hundreds of genes, which are important for different biological processes. My interest was to determine how these enzymes sense molecular oxygen, and how they then drive the chemistry to hydroxylate HIF, which then either prevents

transcriptional activity or leads to degradation of HIF, depending on which hydroxylase does the chemistry. HIF is very prominent in cancer, which planted a seed in the back of my head, so I transitioned to the Memorial Sloan Kettering Cancer Center for my postdoc in 2016.

During my postdoc, the idea was to transition to cell biology and do more cancer biology related work. I ended up joining Dr. Daniel Bachovchin's lab, studying the innate immune system. Dr. Bachovchin's group was small, and I was their first postdoc, which was lots of fun. I thought I would be working more on enzymology, but I ended up trying to understand how a small molecule activated the innate immune system. It turns out the molecule inhibits some proteins, which creates an endogenous danger signal that kick-starts the innate immune system. I fell in love with this powerful way of using a chemical to turn on the immune system, and it formed the base for that area of research in my lab. COVID delayed my transition a little bit, but afterwards I started my lab here at Penn in January 2022, and this January was our one-year anniversary.

What is the biggest difference between being a student, postdoc, and faculty member?

The biggest difference between transitioning from a graduate student to a postdoc is you now know how to think about science. First and second year grad students are still early in the process of learning this skill set. Some are great, but I do not think you are quite there as far as your broader knowledge of things of being able to connect the dots and get the bigger picture. Many PhDs are done after 2-3 years because you are first learning how to think about science versus when you get to a postdoc, you might spend 5-6 months learning a new topic and then you are off to the races. Meaning that you can design experiments to test what you are testing and not get inconclusive results, and sort of piece together the bigger picture of what is missing in the field better.

Dr. Cornelius Y. Taabazuing

The shift from postdoc to faculty is not that different, at least for junior faculty positions, except that I have way more administrative things to do. Now, it is more of managing people than doing my own thing, which is a huge change. In your postdoc, you may have a tech you work with, but it is not to the same degree. When you start out as a faculty member, nobody else is as experienced as you in that particular field or research topic or even the techniques, so you spend a lot of time teaching people, having meetings with them, and driving projects forward. It might change as the job changes, when it becomes purely a writing and people managing job, but right now, 80-90% of my time is spent in the lab.

What changes do you anticipate happening in academia?

There are lots of new, young faculty, at least in my department, so the old way of mentorship might be getting phased out for newer, younger, fresher ideas.

The currency in academia, such as the number of papers, those things that we typically think of as our measure of success might change in the long run. I recently applied for a grant where instead of a paper you can put a software as your contribution. With the advent of preprints and journals like eLife changing their model, I think how we count the currency of academia or what it means to be successful might be redefined a little bit.

In general, technology keeps getting better and better. Within the research fields themselves, I think we are going to be able to make significantly more progress than we have ever been able to. For example, cryo-EM, mRNA technology, those sorts of things will allow us to explore new avenues of research that we have not explored before. Yi-Wei, one of my colleagues, was recently talking about a FIB machine where you are literally looking at proteins inside a whole living cell, which is amazing. It is incredible.

What is your best/funniest memory of graduate school?

I distinctly remember the day I got crystals of my protein. One of the most challenging things to do is to look at the active site geometry of my protein with oxygen bound because it is going to get oxidized. To solve a crystal structure with this and try to understand how molecules might be positioned for chemistry is not a simple task. My lab was not a crystallography lab at all, so I took a two day course and thought, "You know what, I really want to do this," because when you look at the structure of it, it is what it is. It was a humbling experience because I realized afterwards it was up to my interpretation of how I wanted things to fit into the density. I tried to solve a crystal structure with an oxygen memetic, nitric oxide, bound to the active site of our enzyme using hanging drop vapor diffusion crystallization. I put a molecule inside the media in the bottom that released NO gas, we called it DEANO, so if our enzyme is coordinated by NO you get a yellow color spectroscopically. I grew crystals that were yellow, and my advisor saw it for the first time and went, "Wow!" I was super independent by this time, and I explained to him what was going on, and we were both like, "Oh yeah!" He gave me a hug and said, "Wow, you got this really challenging technical thing to work!" We got a crystal structure out of that. It was a good, sweet time.

My funniest memory would be the time I was black Santa, and all the families brought their kids and their kids were like something is up with this Santa. A few of them were confused, I think. Yeah, Santa was different that year.

Hanging drop vapor diffusion crystallization: A common method of protein crystallization. Droplets with purified protein, buffer, and precipitant equilibrate with a larger reservoir containing similar buffers and precipitants in higher concentrations.

Who is your scientific role model?

I have been fortunate enough to have good mentors throughout my entire career. Starting from undergraduate research, I worked with Dr. Nathan Schnnar, who was a phenomenal educator at UMass. I appreciated how involved he was and how he taught people well. In graduate school, I had Dr. Michael Knapp who was also a great educator. He trained you well in how to write and think about science, which I appreciated. In between there, I interned with Dr. Tom Cech, who was a Nobel laureate and super famous, but he would sit down and do weekly journal clubs with us to make sure we understood the literature. After grad school, I transitioned to Dr. Dan's lab at Memorial Sloan Kettering Cancer Center. Dan is super brilliant and super involved in the research, and he would always come up with new ideas. You would not be done with your experiment, and he would go back to the office, read a bunch of things, come back, and go, "What if this, this, and that?" Each mentor was slightly different, and they were all role models for me.
There are also people you have not met, people you meet at conferences, and people in your

field that inspire you, like Drs. Feng Shao, Judy Lieberman, and Hao Wu in my field. All these people are doing phenomenal work in your field, and you aspire to be that good.

What advice can you share for students starting out in their graduate programs?

There are a couple of things I say all the time. My lab even dressed up as me on Halloween with a bunch of quotes I would say. My advice to students, which may sound cliché, would be:

The devil is in the details. Meaning, you must be extremely self-aware when you are doing experiments because one little difference in how you do things can drastically change the results, such as loading the buffer past the fill line or not looking at the pipette tip to confirm the volume was dispensed.

Haste makes waste. From my personal experience, when you are trying to go too fast you end up making mistakes and must repeat it over or you are not sure if you added the thing that needed to be added.

Organization is your best friend in grad school. If you plan ahead and are well organized, it will streamline a bunch of things. You will not waste time looking for things or spending so long on an experiment because you planned ahead.

My last piece of advice, and there are a lot more, but then we would be here all day, would be:

Learn to fail fast and learn to get rid of fear. Fear is meaningless. In the business of science, we are so consumed with the idea that, "This is the definition of success. I have to do this; I have to do that. I must climb the career ladder in this way." and sometimes that takes a big mental toll on people. It is easier said than done, but if you understand that the worst-case scenario is not as bad as you think it is and that there are different definitions of success, then you might have better mental health during the process because you will just do your thing. The outcome will be the outcome, and you will do fine at the end of the day.

What excites you about Philadelphia?

Well, it is the city of brotherly love. I love where we live, I am in Media, Pennsylvania and we love the neighborhood. We are excited to be here.

The food has been amazing, even the food carts. I am excited because I am a big eater, so I have not had a bad meal in a while, except for the other day where I wanted to try cactus and the texture was not bad; it just was not what I like. My favorite food cart is the Vietnamese one, right in the middle of Spruce, across from the Wistar Institute. Rafa's is good, the Jamaican cart is pretty good too, and the one right at the corner of Spruce and University St., though I cannot remember the name. Coming from New York where there is a restaurant on every block, it is good to have options.

Philly fans are obsessed with their sports, which are not bad. I have been to two Eagles games and those are fun. The downtown area is really nice with good food there, too. I know there are lots of historical parts of Philly, and as a lab we have done the Dark History of Philadelphia tour. There is good weather here because it is more inland and less cold, not as cold as Boston and New York, so I appreciate that.

Is there anything else you would like to share?

To the students of Penn, you are in a very privileged position. Penn is a fantastic institution, without some of the uptightness of other Ivy's. It is a relaxed atmosphere with phenomenal science. If you do not understand this already, you should understand it now. All of you are special in your own ways and all of you are going to go on and do amazing things. Do not let your own fear hold you back.

Research Spotlight:

Dr. Edward Song

Kay Labella

Peer Edited by Lauren Elizabeth Lee

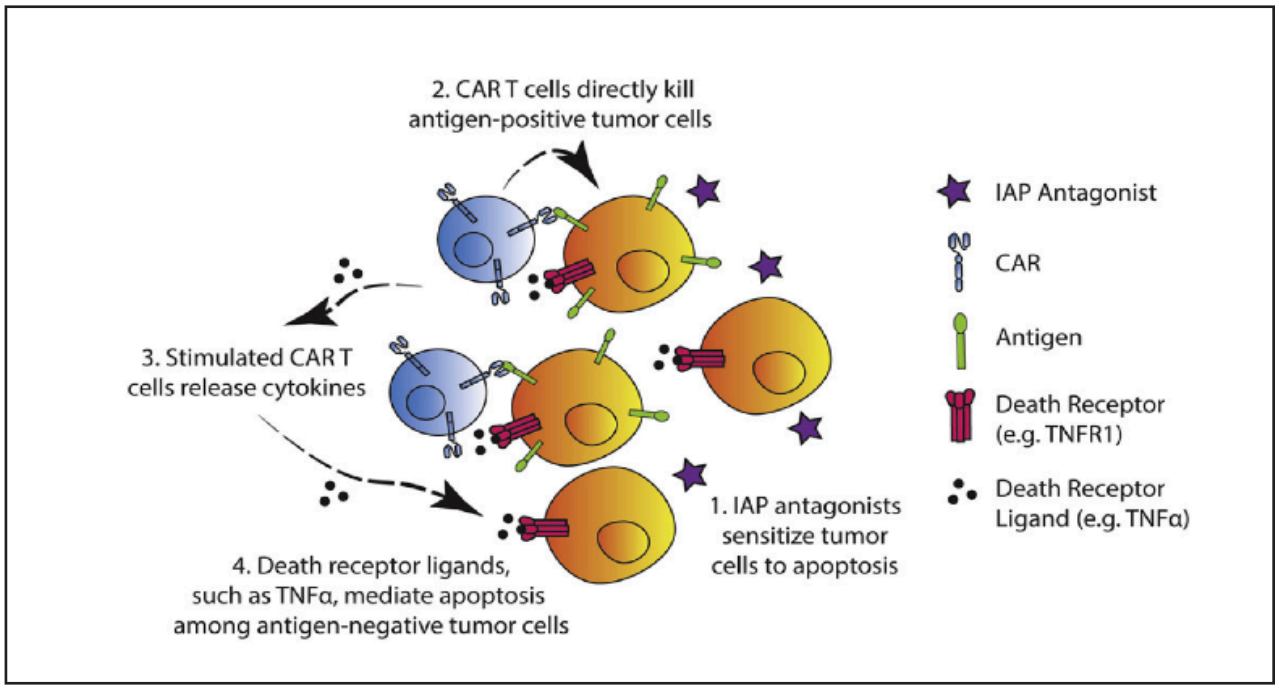
While chimeric antigen receptor (CAR) T cell therapy has seen major success as a durable treatment for hematologic malignancies, its efficacy as a treatment for solid tumors has been much more modest. In particular, CAR T cell therapy has proven minimally effective in treating glioblastoma multiforme (GBM). GBM has both a high prevalence among other malignant brain and central nervous system tumors and is extremely aggressive. To date, there are no curative therapies; with current standards of care, median patient survival is limited, at 16.9 months. A primary damper of CAR T efficacy in GBM is the high degree of tumoral heterogeneity. Not all GBM cells within a tumor would express the tumor antigen targeted by the CAR T cells, such as epidermal growth factor receptor variant III (EGFRvIII). As a result, despite active and functioning CAR T cells, some cancerous cells are able to persist via this method of antigenic escape.

Recent CAMB graduate Dr. Edward Song (GTV, Milone Lab) aimed to address this issue of antigen heterogeneity in his thesis work "The IAP antagonist birinapant enhances chimeric antigen receptor T cell therapy for glioblastoma by overcoming antigen heterogeneity." GBMs often display upregulation of inhibitor of apoptosis protein (IAP) family members; as may follow, this results in the inhibition of death receptor-mediated apoptosis, preventing cell death via this pathway. However, small molecule IAP inhibitors have been developed, and several have shown promise as a candidate for treating other cancers. Dr. Song hypothesized that combining these IAP antagonists with CAR T therapy would assist in limiting antigen escape of bystander tumor cells. Though these cells would avoid direct CAR T cell killing, they would theoretically succumb to apoptosis upon IAP inhibition, as CAR T cells produce apoptosis-inducing cytokines.

Antigenic escape: When immune cells do not recognize and kill tumor cells or pathogens due to lack of antigen.
Epidermal growth factor receptor variant III (EGFRvIII): a common tumor cell-specific antigen found on GBM cells that is often used to target the tumor in CAR T cell therapy.
Birinipant: small molecule antagonist for IAPs. Treating cells with birinapant can restore and promote apoptosis by inhibiting IAP activity.
Inhibitor of Apoptosis Protein (IAP): Family of proteins such as X chromosome-linked IAP (XIAP) and cellular IAPs 1 and 2. They are often over expressed in tumors, preventing cell death via apoptosis.

To test his hypothesis, Dr. Song treated GBM cell lines and patient-derived organoids (GBOs) with birinapant, a well-characterized IAP antagonist. He was able to show that treatment with birinapant downregulated cellular IAP1 (c-IAP1), a factor which blocks the apoptotic pathway. Additionally, he demonstrated that combinatorial treatment of birinapant and tumor necrosis factor-α (TNF-α), an apoptosis-inducing factor, leads to reduced cellular viability and increased active caspase 3/7 in several GBM cell lines and GBOs. Together, these data suggest that birinapant works as intended to restore the apoptotic pathway, and might prove a viable candidate to combine with CAR T therapy for GBMs.

To evaluate the efficacy of this combinatorial strategy, Dr. Song developed a coculture system combining luciferase-



Caption: Graphical abstract of Dr. Song's work, "The IAP antagonist birinapant enhances chimeric antigen receptor T cell therapy for glioblastoma by overcoming antigen heterogeneity." (Song, E., et al, 2022)

labeled EGFRvIII-null GBM cells ("bystander cells") with unlabeled, EGFRvIII-positive cells from the same line. This system allowed for monitoring of an antigen heterogeneous population upon treatment with CAR T cells alone or with birinapant. After 48 hours of treatment, bystander cells were completely eliminated.

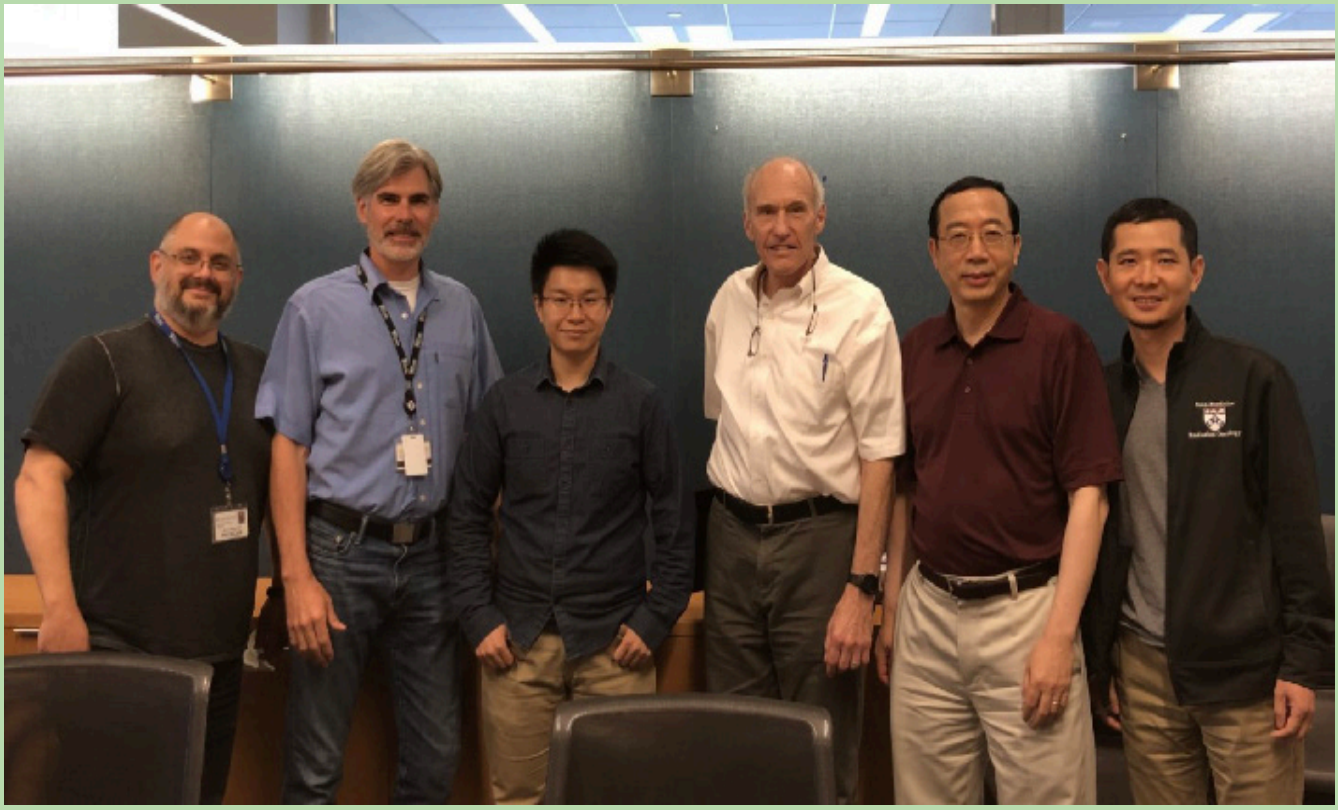
Dr. Song further went on to confirm that this increase in cell death was the result of soluble factors released by the CAR T cells. In this experiment, bystander cells were cultured with conditioned media from stimulated CAR T cells with or without birinapant. Again, he found that these cells experienced a significant decrease in viability, and that treatment with an inhibitor of TNF-α was able to partially rescue this effect. Subsequent experiments in GBOs showed similar results. Overall, this suggested that soluble factors produced by CAR T cells, especially though not solely TNF-α, sensitized antigen-null GBM cells to death by apoptosis.

To validate the efficacy of birinapant and CAR T in vivo, Dr. Song generated two GBM cell populations from the same parental line: an EGFRvIII-positive "antigen" GBM line expressing click beetle green (CBG) luciferase and a EGFRvIII-negative bystander GBM line expressing click beetle red (CBR) luciferase. He subcutaneously implanted a heterogenous population of these cells in NSG mice to mimic the heterogeneity of antigens found in GBM. These tumors were treated with CAR T therapy with vehicle or birinapant every three days. Though the tumor size manually showed regression and CAR T therapy effectively eliminated "antigen" cells in both groups, vehicle-treated mice showed a significant bioluminescent signal from bystander cells, while birinapant-treated mice showed a greatly reduced signal from these same cells. Subsequent experiments in an orthotopic model of GBM, in which these cells were implanted into NSG mice intracranially, showed similar results to the subcutaneous model. Furthermore, additional experiments demonstrated that birinapant did not negatively alter the proliferative ability of CAR T cells or impair their efficacy in any manner. Given these robust in vivo and in vitro data, Dr. Song concluded that "combining birinapant with CAR T cells enhances bystander death of antigen-

negative tumor cells and prevents antigenic escape in antigen-heterogenous tumors." Overall, he has demonstrated that resensitizing antigen-negative GBM cells to apoptosis-inducing cytokines via birinapant treatment allows CAR T cells to clear antigen-heterogenous GBM tumors more completely.

While antigenic escape remains an area of pressing concern, this combinatorial strategy shows promise as a means of enhancing the overall efficacy of CAR T therapy. Going forward, additional avenues to explore may include advancing this treatment to a phase I clinical trial and examining why some GBM cell lines and GBOs are resistant to birinapant treatment. Dr. Song also notes that the patient-derived GBO model system used in this study, if optimized, could serve as a means by which to identify potential biomarkers and therapeutic resistances and sensitivities. And though Dr. Song's focus was on GBM, the utilization of IAP antagonists to improve the cytotoxicity of CAR T cell therapy towards bystander cells could prove broadly applicable; with a variety of IAP antagonists besides birinapant to interrogate, this may open an entire new area of future study for treating other solid tumor types.

References:
Song, E. Z. et al. The IAP antagonist birinapant enhances chimeric antigen receptor T cell therapy for glioblastoma by overcoming antigen heterogeneity. Molecular Therapy - Oncolytics 27, 288–304 (2022).



Dr. Edward Song and his thesis committee

Thank you for reading.

For any questions, comments, concerns, or if you're interested in joining our team, please feel free to contact us at:
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Check out our blog and twitter page!

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