Translesion Synthesis Inhibitors: A New Class of Cancer Chemotherapeutics

Overall Statement of Interests:

Taking AP Biology in junior year of high school sparked my interest in ion channels prompting me to contact Dr. Chung from the Yale School of Medicine, Department of Cellular & Molecular Physiology. For the next six months, I had the opportunity to learn new techniques to elucidate the malfunction of CatSper ion channels in sperm and its contribution to male infertility. I started in the lab making solutions and agar plates, eventually advancing to complex projects involving subcloning and protein purifications. This opportunity validated my interests in basic science research and inspired me to continue performing wet-lab research at UConn. For the past four semesters I have worked in the Hadden Lab in the School of Pharmacy, Department of Medicinal Chemistry researching anti-cancer drug development.

I was intrigued by pharmacology research, despite having a strong foundation in molecular and cell biology techniques, due to its multidisciplinary approach. As a pre-med student interested in the field of oncology, I wanted to perform research outside my comfort zone and directly applicable to a clinical, patient-care setting. I am interested in oncology research and drug development because modern cancer drugs are either associated with toxic side effects or deemed ineffective due to acquired cellular resistance to chemotherapy. My research in the Hadden Lab and my objective for the remaining three semesters is to continue developing cancer drugs, i.e. chemical compounds, that inhibit a cellular mechanism termed translesion synthesis (TLS) known to promote the onset and progression of aggressive tumors.

My second goal is to explore the relationship between access to quality and affordable healthcare and patients' socioeconomic condition. Alongside a Molecular and Cell Biology (MCB) major, I am also pursuing a minor in Healthcare Management and Insurance Studies (HCMI) from the School of Business. I am exploring the ways in which complex industries like

hospitals and insurance companies control the cost, quality, and access to medical care by currently taking HCMI 3240: Introduction to Healthcare Management. Often, socioeconomically disadvantaged patients either cannot afford primary care visits or are not offered the same quality of care, such as access to the latest medications, due to social biases and high costs. As an aspiring physician and pharmacology researcher, I believe engaging with the HCMI coursework offers a unique curriculum and profound understanding that social determinants of health are just as important in predicting treatment outcomes as genomic nuances among patients.

Learning Plan:

Having completed all the pre-med requirements during my first two years, I have the flexibility to select courses that not only interest me but will supplement my University Scholar project. I look forward to taking more undergraduate courses such as MCB 3211: Cancer Cell Biology and Genetics as well as graduate courses like MCB 5217: Biosynthesis of Nucleic Acids and Proteins. Enrolling in advanced MCB courses will deepen my knowledge on cellular physiology and offer the opportunity to delve into advanced discussions regarding the role of abnormal cellular processes in cancer development. I will also take classes outside of MCB, such as STAT 3005: Biostatistics for Health Professions, to enhance my data interpretation skills and excel at applying statistics to extract meaning from the data collected in the lab.

My career goals include pursuing a joint M.D./M.B.A. degree. I believe my undergraduate studies in MCB and HCMI as well as my involvement in multiple clinical research projects at Yale have shaped the way I perceive my role as a future physician-leader and inspired me to one day apply practical skills to the management and administrative aspect of medicine. To prepare for future M.B.A. coursework and to better connect my wet-lab experiences to the real-world healthcare industry, I will utilize the last three semesters to engage with the HCMI faculty and students outside of class. I will attend HCMI discussions and events

that focus on factors that make access to anti-cancer drugs, for example, more affordable and accessible to all. To explore how soaring costs of chemotherapeutics pose a challenge to the sustainability of cancer care, I will be taking three additional HCMI courses ranging from health care economics to health law and policy. Though I am not directly focusing on cost analysis research of chemotherapeutics, it is important as a researcher to develop drugs that are inexpensive, potent, and dose-dependent so that each dose promises maximal potency to diminish cancer growth. This will, in turn, affect the pricing and number of chemotherapy treatments cancer patients must undergo. (1)

Through the support of the 2020 SURF award, I was able to first-author a review paper, soon to be published in *Expert Opinion on Investigational Drugs*, that discusses the latest developments of TLS anti-cancer drugs. I will also be contributing my data, collected from past semesters, to two other TLS manuscripts where I will be listed as a co-author. Writing manuscripts has allowed me to translate experiments I routinely perform in the lab to words. I have also published abstracts at national conferences such as ASCO and PAS and am currently writing manuscripts on clinical research projects that explore an array of topics ranging from chaperone use during sensitive pediatric physical exams to the impact of the COVID-19 pandemic on pressure sore prevalence among the elderly. These opportunities have helped me to reinforce and synthesize the underlying concepts and questions of my research projects while allowing me to meaningfully contribute to the scientific literature and push the field forward.

Another learning opportunity that broadly offers relevant skills to my University Scholar project would include serving as the President of STEMTalk Magazine, UConn's only student-run STEM magazine funded by the 2016 IDEA Grant. I joined the club as a freshman and have written articles and served as an editor over the years. Partaking in this club has taught me how

to convey complex scientific information in a way that is easily digestible by an educated but non-expert audience. We recruit students from all disciplines ranging from STEM to the creative arts to produce a reader-friendly scientific magazine that investigates the latest news and development in STEM. Besides strengthening my soft skills such as time management, taking responsibility, and efficient communication, STEMTalk also offers a creative outlet where I can combine my passions for photography and graphic design to produce a tangible product.

Project Plan:

Field of Interest, Statement of Problem, Deliverables:

My research in the Hadden Lab has involved using computational studies and an in vitro assay to identify small molecule inhibitors (also referred to as compounds) that target a specific protein-protein interaction (PPI), Rev7/Rev3, involved in the DNA damage tolerance mechanism translesion synthesis. My proposed University Scholar project will extend my prior research and encompass three robust experiments that will validate whether inhibitors of Rev7/Rev3 PPIs: 7291176, 7226654, and 7253388 can enhance tumor sensitivity to first-line genotoxic drugs such as cisplatin by preventing TLS activity. I will aid in writing another manuscript where I will synthesize past data with what will be collected from my University Scholar project. Additionally, I will present the data collected from these experiments at pharmacology conferences and events such as the UConn Spring Frontiers poster exhibition.

Review of Scholarly Literature:

A diverse set of DNA damaging agents, including platinum-based drugs like cisplatin, are commonly used as cancer drugs because they directly modify the chemical structure of DNA which halts replication and triggers cancer cell apoptosis (programmed cellular death). (1) Cancer cells can avoid apoptosis by utilizing the TLS mechanism which replaces the replicative DNA polymerase with a specialized, low-fidelity TLS DNA polymerase that can copy past cisplatin-induced DNA lesions during active replication. Overexpression of TLS in cancer cells

ultimately leads to increased mutation rates, cancer cell longevity, and acquired resistance to the first-line genotoxic therapies like cisplatin. (2)

Mammalian cells employ five major TLS DNA polymerases, Rev1, Polη, Polι, Polκ, and Polζ, to carry out replication past sites of DNA damage. My research focuses on the PPIs between the accessory Rev7 and catalytic Rev3 subunits of Polζ, a promising drug target in need of further investigation. Suppressing Rev3 transcription increases cisplatin sensitivity in lung cancer cells. (3) A 2020 study by Sakurai et al. demonstrated that decreased expression of Rev7 enhances chemosensitivity, restores cisplatin resistance, and upregulates apoptosis-associated genes in testicular germ cell tumors. (4) This shows how inhibiting TLS assembly and function can significantly increase cancer cell death as TLS can no longer rescue cancer cells from cisplatin-induced DNA damage. These results underscore the therapeutic potential of targeting the Rev7/Rev3 PPI, but there are currently no effective inhibitors of the Rev7/Rev3 PPI experimentally determined. From computational studies, support from the Hadden Lab, and from our knowledge, I am the first to successfully identify the prototype of three potent inhibitors of Rev7/Rev3 and test their inhibitory effects using the first optimized cellular assay. My research is promising and novel because it provides the possibility of targeting a new molecular pocket of the multi-protein TLS machinery with inhibitors that show high specificity towards Rev7/Rev3.

Preliminary Studies, Purpose, and Hypothesis:

Using a computational method, known as virtual screening, I aided my lab in screening 150,000 compounds that can disrupt the Rev7/Rev3 PPIs. The top ten compounds were chosen based on the XP GScore, a quantity that predicts the binding affinity of the inhibitor to the protein target of interest, Rev7. I performed an in vitro fluorescence polarization (FP) assay to quantify the displacement of fluorescently labeled RBM2, a short peptide sequence from Rev3 containing the primary amino acids that bind to Rev7, at varying concentrations of the ten

inhibitors. I observed a clear drop in polarization for all ten inhibitors, which indicates they bind to Rev7 and have the potential to disrupt the Rev7/Rev3 PPIs. From the FP assay data, I determined the IC_{50} value— the concentration of compound required for 50% inhibition —for each inhibitor. The lower the IC_{50} value, the more potent the inhibitor as this indicates a lower concentration of the inhibitor is required to disrupt the Rev7/Rev3 PPIs and hinder TLS assembly and function. Out of the ten inhibitors, Compound 7291176 was shown to have the most inhibitory potential (IC_{50} 11.32±2.3 μM).

An additional ten compounds structurally like compound 7291176 were screened and tested using the FP assay. From this batch, I found compounds 7226654 (IC_{50} 19.8±2.7 μM) and 7253388 (IC_{50} 10.5±0.3 μM) have the most potential at inhibiting the Rev7/Rev3 PPIs. Based on my past research and FP assay data, I hypothesize that Compound 7253388 will sensitize A2780 human ovarian cancer cells the most, a clinically relevant model commonly used in the Hadden Lab and by others, to cisplatin and show synergistic effects when co-administered with higher concentrations of cisplatin by firmly binding to and disrupting Rev7/Rev3 PPIs.

Research Question, Methods, and Theoretical Approach:

When the combined effect of two compounds (TLS inhibitor and cisplatin) is greater than that predicted by their individual potencies, the combination is synergistic. (6) I will test whether my Rev7/Rev3 TLS inhibitors synergize with cisplatin as this will determine whether co-administration of both can enhance cisplatin sensitivity and cancer cell death. Synergistic interactions are ideal because they allow the use of lower doses of the combination constituents, a situation that may reduce toxic side effects commonly associated with chemotherapy. (6)

I. *CellTiter-Glo (CTG) Assay with A2780 Ovarian Cancer Cell Line:*

The CTG assay determines the number of viable A2780 cells by quantifying the amount of ATP present, an indicator of metabolically active cells, using the luciferase reaction. The

reaction requires ATP so the luminescent signal produced is directly proportional to cellular ATP concentration. I will be treating cells with only cisplatin, only inhibitor, and then a combination of both to compare differences in cell viability across all treatment groups. The controls will be treated with 1% and 2% DMSO (dimethyl sulfoxide) to account for the DMSO used to prepare cisplatin stock and co-treatment with cisplatin and compound, respectively. Ideally, I want to show that the TLS inhibitors alone are not lethal to the cells but in conjunction with cisplatin lead to a lower cell viability. If the compound is inhibiting TLS, then I should observe a decrease in cell viability when higher concentration of cisplatin is administered to A2780 cells. The data collected from the CTG assay will be plotted as % cell viability vs. log concentration of either cisplatin or the inhibitor administered to create a negative sigmoidal curve. The data will be adjusted for background noise and an IC_{50} value will be determined using a statistical analysis software called GraphPad Prism. Lower IC_{50} values with co-administration will denote cisplatin is more effective at lower concentrations when administered with a Rev7/Rev3 inhibitor.

II. *CellTiter-Glo Assay Using A2780cisR (Cisplatin Resistant) Cells:*

I will repeat the CTG assay and data analysis using A2780cisR cells, A2780 mutants that can survive at low concentrations of cisplatin. Using A2780cisR cells is important to determine if the compounds can re-sensitize the cells to cisplatin by disrupting the Rev7/Rev3 PPI. Use of A2780 cells allows us to determine whether our compounds *enhanced* cancer cell death in cells that are acutely damaged by cisplatin. However, using A2780cisR cells provides a new perspective by allowing us to test whether inhibition of TLS function can *promote* cisplatin resistant cells to regain their sensitivity to cisplatin. This is important because most cancer patients develop cellular resistance towards cisplatin after a short period of time. If I can develop an inhibitor that helps to regain cisplatin sensitivity, then this can prevent using higher doses of cisplatin, which can improve quality of life, lower costs, and increase accessibility.

III. *Clonogenic Assay with A2780 Cells and A2780cisR Cells:*

The clonogenic assay is a more robust, in vitro cell survival assay that tests a cell's ability to replicate and divide to form colonies, defined to contain at least 50 cells in diameter. (7) It will determine if the inhibitors can enhance the anti-cancer activity of cisplatin. Disrupted binding of the Rev7/Rev3 via activity of the inhibitor should lead to TLS inactivation, triggering an apoptosis response via recognition of DNA damage, resulting in the formation of fewer colonies. I will use a light microscope to calculate the number of Coomassie Brilliant Blue R-250 stained colonies after treatment using surviving fraction = (no. of colonies formed after treatment ÷ no. of cells seeded) x (no. of colonies formed before treatment ÷ no. of cells seeded) x 100%. (7)

IV. *Cellular TLS Efficiency Assay*

The most potent inhibitor, lowest IC_{50} value, confirmed by the above experiments will be tested using an assay described in a 2020 paper published by Dr. Basu in *DNA Repair*. (8) With the help of a postdoctoral chemist from the Hadden Lab, I will create and transfect plasmids with a single specific DNA mutation called *N*-(dA-8-yl)-6-AC adduct in human embryonic kidney (HEK) cells because it is well-established that TLS is essential to replicate past this specific DNA adduct. (9) I will not be using A2780 cells for this experiment because performing transfection in cancer cells is very difficult and time consuming since there is not a robust transfection protocol in place for TLS. HEK cells will provide the same conclusions since TLS is active in all dividing cells. TLS efficiency is determined as the number of the colonies originating from the *N*-(dA-8-yl)-6-A containing plasmid relative to the control cells that did not contain the plasmid. (8) Ideally, I should observe low levels of transcription of the plasmid vector and fewer colony formation when TLS inhibitors are administered because if TLS cannot function then cells would be subjected to cisplatin and therefore undergo apoptosis.

Citations:

1) Truong, Judy et al. “The impact of pricing strategy on the costs of oral anti-cancer drugs.” *Cancer medicine* vol. 8,8 (2019): 3770-3781. doi:10.1002/cam4.2269
2) Cheung-Ong K, Giaever G, Nislow C. DNA-Damaging agents in cancer chemotherapy: Serendipity and chemical biology. Cell Chem. Biol. 2013;20:648-659.
3) Korzhnev DM, Hadden MK. Targeting the translesion synthesis pathway for the development of anti-cancer chemotherapeutics. J Med Chem. 2016;59:9321-9336.
4) Doles J, Oliver TG, Cameron ER, et al. Suppression of Rev3, the catalytic subunit of Polζ, sensitizes drug-resistant lung tumors to chemotherapy. Proc Natl Acad Sci U S A. 2010;107:20786-20791.
5) Sakurai Y, Ichinoe M, Yoshida K, et al. Inactivation of REV7 enhances chemosensitivity and overcomes acquired chemoresistance in testicular germ cell tumors. Cancer Lett. 2020;489:100-110.
6) Tallarida, Ronald J. “Quantitative methods for assessing drug synergism.” *Genes & cancer* vol. 2,11 (2011): 1003-8. doi:10.1177/1947601912440575.
7) Franken, N., Rodermond, H., Stap, J. et al. Clonogenic assay of cells in vitro. Nat Protoc 1, 2315–2319 (2006) doi:10.1038/nprot.2006.339.
8) Powell BV, Bacurio JHT, Basu AK. Translesion synthesis of 6-nitrochrysene-derived 2'-deoxyadenosine adduct in human cells. DNA Repair. 2020 Jul;95:102935. DOI: 10.1016/j.dnarep.2020.102935.
9) Pande, Paritosh et al. “Site-Specific Incorporation of *N*-(2'-Deoxyguanosine-8-yl)-6-aminochrysene Adduct in DNA and Its Replication in Human Cells.” *Chemical research in toxicology* vol. 33,7 (2020): 1997-2005. doi:10.1021/acs.chemrestox.0c00197.

Learning and Project Plan

Student Name:

Note on the Experiment Significance and Timeline:

Each experiment/assay builds from the other and is carefully chosen with the three semester timeline in mind. Each assay merely adds a layer of complexity to test inhibitor efficiency and potency in a cancer cell model. The CTG assay simply quantifies ATP present in cancer cells to measure cytotoxicity (% of viable cancer cells) in the presence and absence of cisplatin and Rev7/Rev3 TLS inhibitor. The clonogenic assay takes this a step forward by quantifying the number of cancer cells that were able to replicate, divide, and form colonies in presence and absence of cisplatin and TLS inhibitor. Lastly, the cellular TLS efficiency assay is a transfection experiment where I will deliberately introduce a DNA-damaged plasmid vector in eukaryotic HEK cells and quantify HEK cell colony formation. It is well-known that TLS is the only mechanism that allows cells to bypass this specific plasmid DNA adduct and avert apoptosis. Therefore, successful inhibition of TLS activity will be seen through increased apoptosis and fewer HEK colonies under cisplatin and TLS inhibitor co-treatment.

The most potent inhibitor (lowest IC_{50} value) identified from the CTG assay and confirmed by clonogenic assay will be tested in the final experiment, cellular TLS efficiency assay. There is no IC_{50} value cut-off. The lower the IC_{50} value, the more potent the inhibitor.

Spring 2021

Courses

Dept &Course#	*Course Title*	*Credits*
MCB 3413	Concepts of Genetic Analysis	4
STAT 3005	Biostatistics for Health Professions	3
HCMI 3243	Health Care Economics – Honors Conversion	3
UNIV 3784	The Health of Communities – required class for Rowe Scholars	3
PHAR 3099	Honors Undergraduate Research	3

Honors Conversion courses are to fulfill the University Honors Laureate Award requirements.

Other Learning Opportunities

Opportunity	*Location/Date*
Poster Presentation: Present 2020 SURF Research at Spring Frontiers	UConn
Academic Writing: Read and learn how to write a clinical research systematic review paper for the pressure sore and social determinants of health project with Dr. Hsia (Yale New Haven Hospital) Skills: Learn how to use the PRISMA software to perform meta-analyses	Remote/Present-August 2021
MCAT – finished content review, start taking practice exams	Late May 2021
Prepare medical school application – attend pre-med advising events at UConn and start drafting the personal statement essay	March 2021-May 2021

Project Milestones

Key Tasks
Start and maintain cell cultures of A2780 and A2780cisR cells stored in the Hadden Lab
Perform at least three CTG experiments (3 Trials) for each inhibitor and control (DMSO) in both cell lines.* After the first trial, I will confirm that the TLS inhibitor is showing signs of enhanced cancer cell death when co-administered with cisplatin. Data should show a negative sigmoidal curve and low IC_{50} value. I will modify experimental errors as needed before proceeding to subsequent trials.
Synthesize data and assess data outcomes (IC_{50}) using the GraphPad Prism software.
Luminescent signal, IC_{50} value, and cell viability should decrease significantly more with TLS inhibitor and cisplatin co-treatment.
Repeat any CTG experiments as needed.
In the meantime, start optimizing the clonogenic assay protocol.

*Performing one CTG assay takes 5 days from treating the cells with the inhibitor, allowing cells to proliferate, and collecting the % cell viability data. I will be performing multiple CTG assays simultaneously as there is a 2 day incubation period set aside for cells to proliferate. This will allow me to use my time efficiently.

Summer 2021

Other Learning Opportunities

Opportunity	*Location/Date*
Apply to Medical Schools: involves preparing the primary and secondary application and going on interviews	May 2021-March 2022
Academic Writing: Make revisions suggested by Dr. Hsia as necessary and submit 2 pressure sore manuscripts to peer reviewed journals	Remote
Draft TLS Manuscript: With the approval of Dr. Hadden, I will be writing another manuscript that synthesizes all my Rev7/Rev3 data collected since freshman year. Read more TLS papers to understand how to organize a technical and scientific manuscript.	Remote – throughout senior year

Fall 2021

Courses

Dept &Course#	*Course Title*	*Credits*
GEOG 1200	The City in the Western Tradition	3
HCMI 4243	Health Law & Policy	3
LING 1010	Language and Mind	3
MCB 5217	Biosynthesis of Nucleic Acids and Proteins	3
PHAR 3099	Honors Undergraduate Research	3

Other Learning Opportunities

Opportunity	*Location/Date*
Continue medical school application process	August 2021-January 2022

Read papers and protocols on cellular TLS efficiency assay to prepare myself for the next experiment	August 2021-December 2021
Join and attend events hosted by the UConn student organization, Healthcare Management Society, and the School of Business to network with faculty, students, UConn alumni, and industry companies like Cigna and Aetna	UConn/This will be done all 3 semesters.

Project Milestones

Key Tasks
Start and maintain cell cultures of A2780 and A2780cisR cells
Perform clonogenic assays (2 trials) in both cell lines using all 3 inhibitors, cisplatin, and DMSO control.* After the first trial, assess data outcomes by confirming fewer colony formation in cancer cells co-treated with TLS inhibitor and cisplatin. Modify any experimental errors as needed before proceeding with subsequent trials.
Repeat any clonogenic experiment as needed.
During the incubation periods of the clonogenic assay, I will work with a postdoctoral chemist in the Hadden Lab to create the plasmid vectors containing the *N*-(dA-8-yl)-6-AC adducts. I will use this time for trial-and-error and optimize the cellular TLS efficiency protocol.

*Performing one clonogenic assay and collecting the colony formation data takes approximately 11-12 days (with 2 incubation periods of 3 days and 7 days). For efficiency and time management, I will be performing multiple clonogenic assays simultaneously.

Winter Intersession 2022

Other Learning Opportunities

Opportunity	*Location/Date*
Honors Thesis: create a general outline using the data collected from past semesters	December 2021-January 2022
HCMI Industry Exposure: Take a couple of free Coursera classes. I am looking forward to taking "Drug Development and Product Management" taught by Dr. Ettouati and "The Business of Health Care" taught by Wharton School of Business and Penn Medicine. This is an excellent opportunity to prepare for future M.D./M.B.A. coursework and gain exposure to challenges facing the healthcare industry.	Remote/December 2021-January 2022

Spring 2022

Courses

Dept &Course#	*Course Title*	*Credits*
MCB 3211	Cancer Cell Biology and Genetics – Honors Conversion	3
MCB 3412	Genetic Engineering and Functional Genomics – Honors Conversion	3
HCMI 4225	Health and Social Insurance	3
MCB 4997W	Honors Research Thesis in Molecular and Cell Biology	3
PHAR 3099	Honors Undergraduate Research	3

Other Learning Opportunities

Opportunity	*Location/Date*
University Scholar Poster Presentation at Spring Frontiers	UConn/date to be determined
Honors Thesis: continue adding and discussing University Scholar project experimental data	December 2021-May 2022
Rev7/Rev3 TLS Manuscript: Continue writing and adding University Scholar project data. Prepare for submission to a peer reviewed journal.	Remote – Summer 2021 to senior year

Project Milestones

Key Tasks
Start and maintain cell cultures of HEK cells provided by the Basu Lab
Perform at least two transfection experiments using the most potent (lowest IC_{50} value) TLS Rev7/Rev3 inhibitor confirmed by the CTG and clonogenic assays.
Recreate fresh plasmid vectors using the optimized protocol developed in Fall 2021 and perform transfections of HEK cells simultaneously as the plasmid vectors do not have a long shelf life.
Correct any experimental errors as needed.
Assess data outcomes by observing fewer HEK colonies containing the DNA adduct in the presence of TLS inhibitor and cisplatin.

*Transfections typically take 2-3 days. However, this is a new experiment that I am performing so I will be allotting ample time to account for any experimental errors and protocol revisions.