Analyzing the Effectiveness of a Function-Blocking Antibody Treatment in Zebrafish Models of Fibrodysplasia Ossificans Progressiva

Zebrafish are an incredibly valuable model for studying human genetics and disease, sharing 70% of their genes with humans and exhibiting highly conserved gene function. These model organisms have the potential to help develop treatments and preventions for some of debilitating human diseases. The research I would be conducting as part of the Laidlaw Scholars Program involves characterizing novel zebrafish models for the rare human genetic disease fibrodysplasia ossificans progressiva (FOP). FOP is often the result of a missense mutation of the activin receptor type-1 (ACVR1) that results in the substitution of arginine by histidine in the codon encoding amino acid 206 (R206H), imposing neo-function of this receptor to being activated by actin A rather than inhibited by it. This neo-function results in heterotopic bone formation in soft tissues including muscle, tendon, and ligaments. The key research question that I will be seeking to answer is whether a function-blocking Activin A antibody treatment can successfully block the erroneous activin A signaling in humanized ACVR1-R206H expressing zebrafish. Putative function blocking Activin A antibodies have been developed by Regeneron Pharmaceuticals (Tarrytown, NY), with whom the Yelick Lab has a Material Transfer Agreement.

In order to answer this question, I will use three different lines of zebrafish recently created in the Yelick Laboratory. The first line expresses the zebrafish *acvr1l* gene, homologous to human *ACVR1*, containing the analogous R203H mutation. A second line expresses the human *ACVR1* containing the R206H mutation. Both of these zebrafish lines need to be heat shocked in order to express the FOP associated genes, as they are under the direction of the heat-shock promoter. The third line contains the R203H mutation introduced into the endogenous zebrafish *acvr1l* gene via CRISPR-Cas9 genome editing technology. The first task I will complete is to create the next generations of each of these three lines, which is necessary after the COVID research hiatus. Next, I will identify zebrafish who express the mutation by screening them under fluorescent microscopy after heat-shock, taking advantage of their mCherry tags. PCR and nucleotide sequencing or restriction digestion will be used to identify zebrafish harboring the endogenous R203H *acvr1l* mutation. Once I have identified zebrafish for each line that express the FOP-related mutations, I will use them to generate embryos that will be injected at the single cell stage with each activin A putative function blocking antibody to determine if they can block the neo-functional signaling of activin A in each of the respective lines. After I have performed function blocking antibody validation, I will then work to understand how this treatment is working at a molecular level, using techniques such as mRNAseq, mRNA injections, and Western blot analyses of downstream signaling partner expression. A robust understanding of FOP associated activin A signaling in an in vivo zebrafish model will not only help to inform human clinical research for FOP patients but will also be useful for future analysis of critical activin and bone morphogenetic protein signaling pathways in other related bone diseases

characterized by heterotopic bone formation. If this antibody treatment is proven effective in the zebrafish model, it could have tremendous implications for its effectiveness in humans, as well as its potential effectiveness in treating other diseases characterized by erroneous activin A signaling.

Through this project I hope to learn more about the general process of testing antibody treatments and analyzing their effectiveness on a molecular level. I also hope to learn more about the development of zebrafish models for human disease, and to define similarities and differences in the physical and molecular processes in humans and zebrafish FOP. I believe I am qualified to successfully conduct these studies for a few key reasons. In High School, I had the very enriching opportunity to conduct an introductory research project in the Yelick Lab involving the analysis of zebrafish mutants displaying an unknown mutation that resulted in unexplained brittle and/or abnormal fin phenotypes. During this time, I became familiar with zebrafish husbandry, including setting up breeding crosses, and performing bone staining to identify zebrafish mutants. This experience also gave me the chance to work collaboratively with Dr. Yelick and the rest of the Yelick Lab members, and to learn valuable lessons about working in a lab environment, and more specifically the Yelick Lab environment. Additionally, this past semester I engaged in an online research experience with the Bing Lab of Waldenström's Macroglobulinemia (WM) at the Dana Farber Cancer Center where I identified potential pathogens involved in WM peripheral neuropathies via a mechanism of molecular mimicry. During this time, I conducted an extensive literature review of the project, which gave me a strong understanding of introductory immunology that I can apply to better understand the mechanisms of the function-blocking antibody treatments I will be analyzing as part of my FOP project at the Yelick Lab. I also composed a cumulative paper and learned how to effectively present my research; I can apply these skills to the paper, poster, and presentation I would create throughout the Laidlaw Program. Also, the knowledge I gained from taking General Genetics, and the Cell and Developmental Biology course I will be taking this semester at Tufts University will greatly aid me in better understanding the genetic and molecular basis of zebrafish and human FOP mutations, and of the antibody treatment effectiveness. I am extremely motivated to perform well on this project using the technical and academic skills I have acquired through my previous experiences, as well as passionate about furthering my knowledge of disease development and treatment in zebrafish and my ideas for future research directions.

Through my previous research experiences, I have learned valuable laboratory techniques that can be used both specifically with the proposed zebrafish models, as well as in general with the molecular analysis involved in this project. I have also engaged in the extensive academic analysis/literature reviews that need to occur before beginning a new research project. I believe these skills will prove useful throughout the proposed project. Additionally, I have learned how to effectively work and communicate with a mentor in a laboratory setting, and the importance of engaging in constant analysis and questioning of the research in order to perform effective

investigation of the project and come up with directions for future research. Through this experience, I am looking forward to learning how to work more independently on research, and to take the initiative to make decisions regarding the most effective steps to take throughout the process. I am also looking forward to analyzing the proposed antibody treatment to the point of being able to come up with some of my own suggestions for potential improvements using what I determined through the genetic and molecular analyses I will perform. I really enjoyed my previous research experience working in Dr. Yelick's lab, and under her mentoring I look forward to engaging in the proposed project and validating the effectiveness of this function-blocking antibody treatment on FOP zebrafish models, potentially opening new doors for zebrafish models for human FOP research.