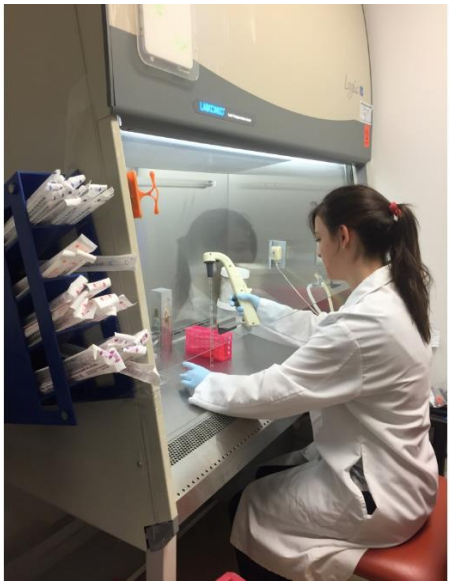

Targeting Replicative Stress in Pediatric High Grade Gliomas
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Advisor: Kristina Cole

Thanks to a generous grant from the Alex's Lemonade Stand Foundation, I worked on a project targeting replicative stress in a class of pediatric cancer called DIPGs. I conducted my research under the mentorship of Dr. Kristina Cole, a neuro-oncologist at the Children's Hospital of Philadelphia.

Diffuse intrinsic pontine gliomas (DIPG) are a subset of high-grade gliomas (HGG) found in pediatric patients; they are the most common brainstem tumor in children, affecting 200-400 children in the U.S. each year. These tumors' location makes them inoperable, yielding a tragic five-year survival rate of less than one percent.

One potential drug target in these cancers involves stress associated with DNA replication. All cells have regions of repeated DNA segments at the end of their chromosomes called telomeres, which are shortened with each replication cycle and re-synthesized by the enzyme telomerase. However, a small fraction of cancers (which seem to show a loss of the chromatin-remodeling protein ATRX) instead must rely on a process called alternative lengthening of telomeres (ALT), which requires the protein kinase ATR. Inhibition of ATR in these types of cancer cells supposedly disrupts ALT, preventing them from mediating DNA damage and ultimately leading to their death. My project investigated the connection between ALT dependence and sensitivity to ATR inhibition.

To examine this correlation, I utilized sterile cell culture technique to propagate three human HGG cell lines and treat them with varying drug concentrations. I then conducted cell viability assays to quantify the number of living cells in each condition and graphed this data in the form of dose response curves. These graphs allowed me to find the IC50's, the drug dose at which growth is inhibited by 50%. Finally, I utilized protein extractions, BCA assays, and western blotting to measure the amount of specific proteins present in different samples.

My findings showed that the ATR inhibitor VE-822 is effective in inhibiting cell growth, and can inhibit growth by 50% at concentrations as small as 1-2 micromolar. Furthermore, there appears to be an inverse relationship between cancers' levels of the chromatin-remodeling protein ATRX and their sensitivity to ATR inhibition-- the less ATRX, the more dependent a cancer is on ALT, and the more sensitive it is to ATR inhibitors. More research is needed to further illuminate the ALT pathway, but ATR inhibitors are already in clinical trials throughout the country.

This mentorship was indispensable to my educational experience. Dr. Cole provided knowledge and experience not only as a researcher but also as a physician in a top children's hospital. I was also able to attend weekly seminars from the Center for Childhood Cancer Research, which ranged from presentations by physicians to discussions with families of cancer patients. These sessions provided insight into a side of research I hadn't yet been able to see-- the benefits conferred onto patients by bench research. Finally, I was able to work with the ALSF foundation; I volunteered for their annual telethon, attended Lemonade Day at CHOP, toured their headquarters, and met with their founders and chairs. The opportunity to work with such an amazing foundation and hear from the families impacted by our research was an invaluable supplement to my research experience, and is one that will resonate with me for years to come.