This summer, I was blessed with the amazing opportunity to work as a research assistant in the lab of pediatric oncologist Dr. Emmanuel Volanakis at the Vanderbilt Ingram Cancer Center in Nashville, Tennessee. Dr. Volanakis specializes in investigating T cell acute lymphoblastic leukemia (T-ALL), which is an aggressive cancer of white blood cells affecting children and adults alike. Many children with T-ALL can be cured with extremely intensive chemotherapy, but there are significant long-term health problems associated with this treatment. A major goal for T-ALL is to develop new therapies that can help reduce the burden of treatment and offer cures to the approximately 20% of pediatric patients who succumb to the disease. Identifying certain genes that are damaged in many cases of T-ALL cell has helped scientists develop mouse models of the human disease. These models provide a useful tool for testing our ideas about the causes of T-ALL, and also for testing whether treating those causes may provide promising new therapeutic strategies.

This summer my research project focused on the genes NOTCH1 and myr-AKT. Gain-of-function NOTCH1 mutations are found in 50%–70% of human T-ALL cases. Gain-of-function NOTCH1 alleles that initiate strong downstream signals induce leukemia in mice. More commonly, however, these gain-of-function alleles initiate only weak downstream signals and although they induce ectopic T cell development, these more common alleles fail to initiate leukemia development on their own. I aimed to answer the question that these gain-of-

function NOTCH1 alleles that initiate only weak downstream signals, when co-expressed with strong gain of function myr-Akt alleles (another mutation common to T-ALL), will compliment one another to induce T-ALL.

In order to express these alleles, I first created a N1(HDmut)T2Amyr-AKT plasmid from which I isolated myr-AKT by performing a restriction digest and imaging it using a procedure called agarose gel electrophoresis in which DNA fragments are run out on a gel and are organized by size. These isolated DNA samples were sent off for sequencing and returned with 100% match to AKT. These plasmid DNA samples then underwent a double restriction digest of BSPE1 and XHO1. This digest was then repeated in parallel with a NOTCH1 plasmid, and imaged. Knowing the correct size DNA fragments for both NOTCH1 and myr-AKT, these fragments removed and purified from the gel. These purified DNA fragments were then bound together in a ligation reaction, and were once again analyzed by restriction digest. The gel showed the correct size DNA fragments, showing that both activated NOTCH1 and myr-AKT pathways were present in the same plasmid vector (NTMA).

Through a triple transduction of human kidney 293T cells with my constructed NTMA vector and helper vectors, I was able to create a retrovirus that encoded our vector DNA into RNA. 3T3 mouse cells were then infected with this virus through a retroviral transduction, causing the genes of the retrovirus to be incorporated into the genome of mouse cells. Through a western blot, a procedure that measures protein expression in cells, I was able to show that

both activated ligand independent NOTCH1 and activated Phospho-Akt can be co-expressed in the same retrovirus. I got these results in the last week of my internship, and it is extremely exciting, but I wish I had had more time to continue with my research. The next step for my project is to infect murine bone marrow cells with the retrovirus I created to see if these two activated protein pathways when co-expressed induce T-ALL, but sadly I will no longer be the researcher spearheading it.

Before this summer, I was not very experienced in the lab and this has changed my whole view of the scientific experience. The active work in the lab, as well as the anticipation for experimental results, were the major highlights for me. I was especially interested/invested in my research on T-ALL because I am a former leukemia patient myself. In the 1960s, the 5-year survival rate for my particular type of leukemia was less than 10%. Now thanks to extensive research, the survival rate is over 90%. Knowing that my work in the lab this summer may increase survival rates for children with a disease similar to my own has fueled me to continue pursuing my major in Biology as well as help in my applications to medical school so that one day I can be an even greater part in the fight against cancer.