Human Immunodeficiency Virus, or HIV, is a retrovirus that causes Acquired Immunodeficiency Syndrome (AIDS). More than 3 million people die from AIDS related deaths each year, making HIV/AIDS a top area for research. HIV infects white blood cells called CD4+ T-cells. It does so by binding to the surface of the T-cells, inserting its genetic material (RNA), converting this RNA into DNA and then integrating the DNA into the host cells genome. The HIV makes use of the T-cell’s resources to replicate and grow more HIV. HIV can more easily infect T-cells that are activated; T-cells that are responding to an immune stimulus. Previous research in the field indicates that activated T-cells are infected all at once soon after contact with the virus, while resting T-cells are slow to infect and only infect in low levels. Dr. Shen wanted to explore the idea of the timeline of infection for activated, resting and endothelial cell co-cultured t-cells. From previous research she knew that co-culture with endothelial cells allows for greater infection of resting T-cells. So the question I pursued this summer was this: When do reverse transcription (viral RNA to DNA) and integration (insertion of DNA into the host genome) begin and end in the various type of t-cells?

In order to have T-cells to work with blood was drawn from HIV negative blood donors. This blood was then separated into cell types using a ficoll sugar gradient. The white blood cells were taken from this gradient and run though a negative bead depletion to isolate the types of cells we were after (either resting or total CD4+ T-cells). These cells were then plated into wells with the appropriate conditions (activated alone, resting alone, resting with EC+ and resting with EC-). In order to observe when reverse transcription and integration were occurring I used the drugs Efavirenz (reverse transcriptase inhibitor) and Raltegravir (integrase inhibitor) to block the respective activity after a certain day. For example Day 0 wells would have drug added before infection in order to block all possible RT or integrase activity. Day 1 wells would allow these to occur only in the first 24 hours of infection and so on. I carried out the experiment for 6 days. The virus we use for our research has a green florescence protein (GFP) inserted into it so that cells that are infected will glow green when run through a flow cytometer. On day 6 of my experiment I run my cells through the flow cytometer and see on what day the amount of GFP is significantly different from day 0 (the first appearance of integration or RT - depending on the drug used) and on which day the GFP stops being significantly different from day 6 (the peak of integration or RT).

While I have not at this point completed my data collection it appears that reverse transcription begins on day 0.5 for activated, day 2 for EC+,EC- and resting cells. RT peaks on day 4 for all cell types except resting which peaks on day 5. Integration begins on day 1 for activated, day 1 to day 2 for resting and day 2 for EC+ and EC-. It peaks on day 4 for resting and EC+ and day 5 for EC- and activated. Longer-term experiments seeing what happens past day 6 are still being carried out.

My summer researching under Dr. Shen has allowed me to get a taste of what true scientific research is really like. I am now contemplating pursing research during my time at medical school, or at least I will be more open to research opportunities that present themselves. I have learned so much and am so grateful for my summer experiences, my co-workers, my faculty mentor and the donors who made it all possible.