Chronic viral infection (Hepatitis B/C) and fatty liver disease caused by chronic alcohol use and/or metabolic disease are the major causes of inflammation in the liver. Chronic liver inflammation, or hepatitis, leads to cirrhosis which is characterized by immune cell infiltration, liver injury, and fibrotic changes ultimately leading to liver failure. The constant cycle of inflammation, liver injury, and repair during cirrhosis dramatically increase the risk of developing liver cancer, or hepatocellular carcinoma (HCC). If diagnosed at an early stage, liver transplantation is the best treatment option for cirrhotic patients with HCC, as transplantation removes not only the cancer but also underlying liver disease which led to HCC. Unfortunately, HCC is a very aggressive tumor which can rapidly metastasize to other tissues if left untreated. Many patients who receive liver transplants for HCC experience tumor recurrence within 3 to 5 years after transplantation. HCC recurs because of tumor cells which escape the primary tumor site, where they can survive undetectable in the circulation or as small micrometastases outside the liver.

Our laboratory uses a combination of clinical data, patient-derived specimens, and animal models to study how HCC and cirrhosis influence key immune populations which can block anti-tumor immune response. Projects available to program students this year are listed below:

The immunomodulatory function of bilirubin during T cell activation. [laboratory study]
We will study how bilirubin influences T cell activation and determine elevated bilirubin (hyperbilirubinemia) causes T cells to shift the inflammatory factors produced following activation. We will examine the hypothesis that naïve T cells are more sensitive to bilirubin compared to their regulatory counterparts and finally determine whether bilirubin has any effect on regulatory T cell function. In this study, the student will learn how specific cell populations are isolated from peripheral blood and tissue as well as several strategies to study T cell behavior in cell culture.

Impaired activation of monocytes and macrophages under conditions of hyperbilirubinemia. [laboratory study]
We will examine the influence of bilirubin on monocyte/macrophage proinflammatory signaling pathways and determine whether the effects of bilirubin are due to cell stress or altered signaling response. We will test whether bilirubin alters monocyte/macrophage programming by measuring changes in key enzymes produced following activation. In this project, the student will learn how to mature monocytes into mature macrophages as well as common molecular techniques used to study inflammation in macrophages.

Synergy between IL-6 and bilirubin in promoting tolerogenic activity in myeloid-derived suppressor cells. [laboratory study]
This project will focus a transient myeloid population which has a higher resistance to hyperbilirubinemia. We will examine whether stress caused by increased bilirubin modulates the production of suppressive mediators and suppressive function in these cells. This study will help clarify whether the tolerance-promoting effects of elevated bilirubin in cirrhotic patients are direct or indirect through promoting the development of anti-inflammatory immune populations. The student will learn how myeloid-derived suppressor cells develop during cancer, and what key stress-promoting responses trigger their suppressive programs.
Intention-to-treat analysis of HCC patients receiving locoregional therapy prior to waitlist for liver transplantation. *Clinical study*

Using a database developed by our laboratory in conjunction with several clinicians, the student will assemble a panel of several immune and metabolic parameters used by our group to identify patients with aggressive tumor biology. The student will learn how we identified these panel factors are linked with patient outcomes, and apply these strategies to a broader group of patients to determine the extent to which we can apply this panel other HCC patients whose tumors were too aggressive to attempt liver transplantation.

Longitudinal immune inflammatory indices after primary treatment in early stage HCC patients: Associations with treatment and waitlist outcomes. *Clinical study*

Using a database assembled by our lab, the student will calculate a panel of risk indices at defined time intervals after HCC patients have undergone tumor-directed treatments. Using baseline and longitudinal trends in these parameters, we will determine which index(es) most closely associate the response of the patient to therapy and whether these same measures are associated with likelihood of receiving a liver transplant.
Project 2: The Translational Cancer Research Laboratory College Student Intern Requirements:

The research interest focus of our laboratory are cancer stem cells and cancer microenvironment for B cell lymphoma, colorectal, bladder, renal cell, pancreatic, gastric cancers. It involves a lot of planning and hands on techniques to execute experiment precisely, such as media preparation, tissue culture, slides staining, and whole body live animal imaging, etc.

The research interns are required to be available 40 hours per week. They will become a part of the team in our research projects. They are required to master background knowledge and techniques including mammalian cell culture, assisting in human tumorigenesis in mouse model studies, and immunohistochemistry staining with frozen and paraffin embedded tissue sections. They will be assigned with lab projects, and they then execute as instructed, as well as independently. We encourage their enthusiasm and contribution to our projects. We will help them to get a first-class research experience and hope the research experience will make a strong positive impact on their life.
In vitro Evaluation of the Interaction of Fosfomycin and Doxycycline against Linezolid- and Vancomycin-resistant Enterococcus faecium

Enterococcus species are Gram-positive cocci related to streptococci. Enterococci live in the intestines, female genital tract, and in the environment. They can become resistant to vancomycin (the antibiotic often used to treat infections caused by enterococci) and cause serious infections, especially in immunocompromised patients. Most vancomycin-resistant enterococci (VRE) infections occur in hospitals, are spread by contact or contaminated objects, and cause infections in the urinary tract, bloodstream or wounds associated with catheters or surgical procedures. VRE have adapted and are now becoming resistant to linezolid, a second antibiotic often used to treat enterococcal infections.

The treatment of these multidrug-resistant VRE can be problematic, since there is a lack of development of new antibiotics. As a result, older antibiotics, such as fosfomycin, have gained attention since it has remained active against many multidrug-resistant Gram-positive and Gram-negative bacteria. Fosfomycin has a unique mechanism of action which may provide a synergistic effect to other antibiotics. The aim of our study is to evaluate the combination of fosfomycin and doxycycline (a tetracycline antibiotic) against a collection of 24 linezolid-resistant VRE. faecium unique clinical isolates.

Initial studies were performed using an Etest method. The Etest is a predefined stable gradient of antimicrobial concentrations on a plastic strip that is used in vitro to determine the minimal inhibitory concentration (MIC) of antibiotic for the organism. An Etest MIC:MIC synergy method was used to test the 24 isolates for synergy with fosfomycin plus doxycycline. This method showed significant synergy in 11/24 (46%) isolates and additivity in 13/24 (54%) of isolates.

In vitro determination of synergy by Time-kill assay. (Laboratory study)
We will perform synergy testing to further evaluate the interaction of the combination of fosfomycin and doxycycline in various concentrations to assess the activities of the two drugs in combination in order to determine any synergy, indifference, or antagonistic effects. Results in the time-kill studies will be compared to results from the previous Etest synergy method. The student will be introduced to sterile techniques including normal collection, subculture, freezer storage, and processing of bacterial isolates. The student will learn how to perform time-kill studies to evaluate combinations of antimicrobials for killing effectiveness.